RADIOLOGY AND ONCOLOGY

vol.58 no.2
june 2024

RADIOLOGY AND ONCOLOGY

June 2024
Vol. 58 No. 2
Pages 153-312
ISSN 1318-2099
UDC 616-006
CODEN: RONCEM
eISSN: 1581-3207

Publisher
Association of Radiology and Oncology

Aims and Scope
Radiology and Oncology (ISSN 1318-2099) is a multidisciplinary journal devoted to the publishing original and high-quality scientific papers and review articles, pertinent to oncologic imaging, interventional radiology, nuclear medicine, radiotherapy, clinical and experimental oncology, radiobiology, medical physics, and radiation protection. Papers on more general aspects of interest to the radiologists and oncologists are also published (no case reports).

Editorial office

Radiology and Oncology
Zaloška cesta 2
P. O. Box 2217
SI-1000 Ljubljana
Slovenia
Phone: +386 1 5879 369
Phone/Fax: +386 1 5879 434
E-mail: gsersa@onko-i.si

Reader for English
Vida Kološa

Secretary
Mira Klemenčič, Zvezdana Vukmirović, Vijoleta Kaluža, Uroš Kuhar

Design
Monika Fink-Serša, Samo Rovan, Ivana Ljubanović

Layout
Matjaž Lužar

Printed by
Tiskarna Ozimek, Slovenia

Published quarterly in 300 copies

Beneficiary name: DRUŠTVO RADIOLOGIJE IN ONKOLOGIJE
Zaloška cesta 2
1000 Ljubljana
Slovenia

Beneficiary bank account number: SI56 02010-0090006751
IBAN: SI56 0201 0009 0006 751
Our bank name: Nova Ljubljanska banka, d.d.,
Ljubljana, Trg republike 2,
1520 Ljubljana; Slovenia

SWIFT: LJBASI2X

Subscription fee for institutions EUR 100, individuals EUR 50

The publication of this journal is subsidized by the Slovenian Research Agency.

Indexed and abstracted by:

- *Baidu Scholar*
- *Case*
- *Chemical Abstracts Service (CAS) - CAplus*
- *Chemical Abstracts Service (CAS) - SciFinder*
- *CNKI Scholar (China National Knowledge Infrastructure)*
- *CNPIEC - cnpLINKer*
- *Dimensions*
- *DOAJ (Directory of Open Access Journals)*
- *EBSCO (relevant databases)*
- *EBSCO Discovery Service*
- *Embase*
- *Genamics JournalSeek*
- *Google Scholar*
- *Japan Science and Technology Agency (JST)*
- *J-Gate*
- *Journal Citation Reports/Science Edition*
- *JournalGuide*
- *JournalTOCs*
- *KESLI-NDSL (Korean National Discovery for Science Leaders)*
- *Medline*
- *Meta*
- *Microsoft Academic*
- *Naviga (Softweco)*
- *Primo Central (ExLibris)*
- *ProQuest (relevant databases)*
- *Publons*
- *PubMed*
- *PubMed Central*
- *PubsHub*
- *QOAM (Quality Open Access Market)*
- *ReadCube*
- *Reaxys*
- *SCImago (SJR)*
- *SCOPUS*
- *Sherpa/RoMEO*
- *Summon (Serials Solutions/ProQuest)*
- *TDNet*
- *Ulrich's Periodicals Directory/ulrichsweb*
- *WanFang Data*
- *Web of Science - Current Contents/Clinical Medicine*
- *Web of Science - Science Citation Index Expanded*
- *WorldCat (OCLC)*

This journal is printed on acid- free paper

On the web: ISSN 1581-3207
https://content.sciendo.com/raon
http://www.radioloncol.com

contents

clinical oncology

medical physics

study protocol

correspondence

slovenian abstracts

review

Endoscopic management of patients with familial adenomatous polyposis after prophylactic colectomy or restorative proctocolectomy - systematic review of the literature

Aleksandar Gavric[1], Liseth Rivero Sanchez[2,3,4], Angelo Brunori[2,3,4], Raquel Bravo[3,4,5], Francesc Balaguer[2,3,4], Maria Pellisé[2,3,4]

[1] Department of Gastroenterology and Hepatology, University Medical Centre Ljubljana, Slovenia
[2] Department of Gastroenterology, Hospital Clinic de Barcelona, Barcelona, Spain
[3] Institut d'Investigacions Biomediques August Pi I Sunyer (IDIBAPS), Barcelona, Spain
[4] Center for Biomedical Research in the Hepatic and Digestive Diseases Network (CIBERehd), Barcelona, Spain
[5] Surgery Department, Hospital Clinic de Barcelona, Barcelona, Spain

Radiol Oncol 2024; 58(2): 153-169.

Received 6 March 2024
Accepted 16 April 2024

Correspondence to: Maria Pellisé, M.D., Ph.D., Department of Gastroenterology Hospital Clinic de Barcelona. Institut d'Investigacions Biomediques August Pi I Sunyer (IDIBAPS). Hospital Clinic of Barcelona. Centro de Investigación Biomédica en Red de EnfermedadesHepáticas y Digestivas (CIBERehd). Universitat de Barcelona, Barcelona, Spain. E-mail: mpellise@clinic.cat

Disclosure: Maria Pellise: consultant fees from Olympus and Fujifilm, speaker fees from Norgine, Mayoli and Casen recordati. Francesc Balaguer: consultant fees from Olympus, Nouscom and Norgine, editorial fees from Elsevier, endoscopic equipment on loan of FujiFilm and Olympus, research grant from FujiFilm, ZiuZ and Casen recordati, consultancy for FujiFilm, Olympus, and speakers' fee from Olympus, Fujifilm, Norgine, IPSEN.

Background. Patients with familial adenomatous polyposis (FAP) develop early colorectal adenomas and if left untreated, progression to cancer is an inevitable event. Prophylactic surgery does not prevent further development of cancer in the rectal remnant, rectal cuff in patients with ileal pouch anal anastomosis (IPAA) and even on the ileal mucosa of the pouch body. The aim of this review is to assess long-term rates of cancer and adenoma development in patients with FAP after prophylactic surgery and to summarise current recommendations for endoscopic management and surveillance of these patients.

Materials and methods. A systematic literature search of studies from January 1946 through to June 2023 was conducted using the PRISMA checklist. The electronic database PubMed was searched.

Results. Fifty-four papers involving 5010 patients were reviewed. Cancer rate in the rectal remnant was 8.8–16.7% in the western population and 37% in the eastern population. The cumulative risk of cancer 30 years after surgery was 24%. Mortality due to cancer in the rectal remnant is 1.1–11.1% with a 5-year survival rate of 55%. The adenoma rate after primary IPAA was 9.4–85% with a cumulative risk of 85% 20 years after surgery and a cumulative risk of 12% for advanced adenomas 10 years after surgery. Cumulative risk for adenomas after ileorectal anastomosis (IRA) was 85% after 5 and 100% after 10 years. Adenomas developed more frequently after stapled (33.9–57%) compared to hand-sewn (0–33%) anastomosis. We identified reports of 45 cancers in patients after IPAA of which 30 were in the pouch body and 15 in the rectal cuff or at the anastomosis.

Conclusions. There was a significant incidence of cancer and adenomas in the rectal remnant and ileal pouch of FAP patients during the long-term follow-up. Regular endoscopic surveillance is recommended, not only in IRA patients, but also in pouch patients after proctocolectomy.

Key words: familial adenomatous polyposis; ileorectal anastomosis; ileal pouch-anal anastomosis

Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease caused by pathogenic variants in the adenomatous polyposis coli (APC) gene[1] with reported incidence of one in 8,000 to 12,000 live births.[2] The main hallmark of the disease is the presence of multiple colorectal adenomas, leading to a 100% lifetime risk of developing cancer if the colon remains in situ.[3] To prevent the development of cancer, prophylactic colectomy or proctocolectomy is performed when the adenoma burden cannot be managed endoscopically or at the age of 18–25 years old. The following types of surgery are available[4]: total colectomy with ileorectal anastomosis (IRA) or ileosigmoid anastomosis (ISA); proctocolectomy with/without mucosectomy and stapled ileal pouch-anal anastomosis (IPAA) or hand-sewn IPAA; and total proctocolectomy with end ileostomy. Until restorative proctocolectomy with IPAA and pouch reconstruction was described in the 1970s, colectomy with IRA or end ileostomy was the only surgical prophylactic procedure available and was associated to a considerable high CRC incidence and mortality.[5] After this, proctocolectomy with pouch reconstruction (IPAA) was the technique of choice in patients with a high adenoma burden and was sought to eliminate the risk of CRC in FAP patients. However, since the first report of pouch cancer in 1994[6], there has been a substantial increase in published literature reporting rates of adenoma and cancer development after primary IPAA. The development of adenomas along life in remnant rectal mucosa is a natural phenomenon in this population. Long live periodical surveillance with rectoscopies is widely recommended in international guidelines as shown in Table 1.[4,7-10] As there are no randomised trials comparing endoscopic surveillance and management strategies for FAP patients with IRA and IPAA, we aimed to systematically evaluate adenoma and cancer development after prophylactic surgery, define potential risk factors and to summarise endoscopic practices from published series.

Materials and methods

Our review is reported according to the PRISMA guidelines.[11]

Search strategy

We searched PUBMED from inception to June 2023 to identify studies evaluating long-term adenoma and cancer development in patients with FAP after prophylactic surgery. Deduplication was performed using Zotero software.[12] Reference lists of included studies were hand-searched for additional relevant studies. The search was limited to studies, published in English. We used the following keywords: "FAP", "IRA", "IPAA", "familial adenomatous polyposis" and "proctocolectomy".

Inclusion criteria

We included single-or multicentre retrospective cohort studies, prospective cohort studies and retrospective analyses of polyposis registries. Due to

TABLE 1. Summary of recommendations from the international guidelines

First author and publication date (ref.)	Endoscopic surveillance – patients with IRA	Indications for secondary proctectomy patients with IRA	Endoscopic surveillance – patients with IPAA
Vasen et al., 2008[7]	Every 3 to 6 months	Multiple large adenomas (> 5 mm) Adenomas with dysplasia	Every 6 to 12 months
Balmaña et al., 2013, ESMO[8]	Every 12 months	No recommendations	Every 12 months
Stoffel et al., 2015, ASCO[9]	Every 6 to 12 months	No recommendations	Every 6 months to 5 years (Intervals should be determined on a case-by-case basis and may be even shorter than 1 year for some individuals)
Sygnal et al., 2015, ACG[10]	Every 12 months	No recommendations	Every 12 months
Herzig et al., 2017, ASCRS4	Every 12 months	No recommendations	Every 12 months
Van Leerdam ME et al., 2019, ESGE[53]	Every 12 to 24 months	No recommendations	Every 12 to 24 months
Yang J et al., 2020, ASGE[54]	6 months after surgery with 6 to 12 months further surveillance interval		12 months after surgery with 12 to 24 months further surveillance interval. 6 months if advance adenoma

ACG = American College of Gastroenterology; ASCO = American Society of Clinical Oncology; ASCRS = American Society of Colon and Rectal Surgeons; ASGE = American Society for Gastrointestinal Endoscopy; ESGE = European Society of Gastrointestinal Endoscopy; ESMO = European Society for Medical Oncology; IPAA = ileal pouch anal anastomosis; IRA = ileorectal anastomosis

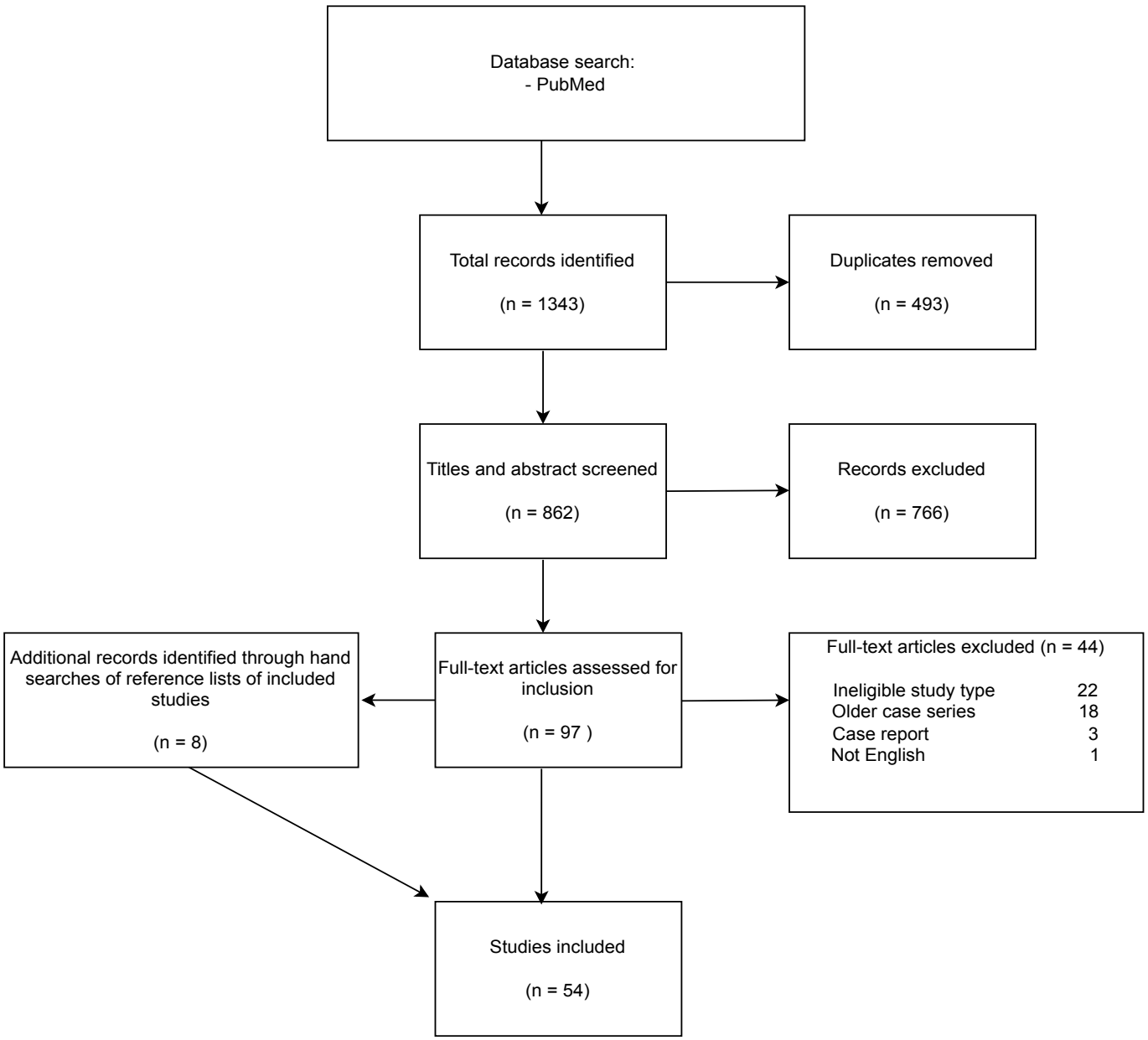


FIGURE 1. Flowchart of the systematic review according to the Preferred Reporting Items for Systematic Reviews (PRISMA) schema.

the rarity of the events, we only considered case reports for inclusion when summarising reports on cancers after primary IPAA. Only the most recent series from the same institution or polyposis registry were included in the analysis, as some research groups regularly publish retrospective analyses of their cohorts or polyposis registries. Full-text screening and data extraction were performed by a single researcher (AG). Manuscripts of three case reports could not be obtained, data were summarised from the two review articles.[13,14]

Results

Studies identified

Of 97 full-text articles screened for eligibility (Figure 1), 46 met our inclusion criteria. A further 8 articles were identified by hand searching the reference lists of the included studies (6 case reports, 1 retrospective cohort, 1 polyposis registry analysis). We included 22 retrospective analyses, 14 case reports (carcinoma development after primary IPAA), 15 retrospective analyses of prospectively

maintained polyposis registries and 3 prospective cohort studies. Only 5 studies were multicentre and 1 was bi-centre. The studies were published between 1994 and 2023. The studies included between 1 and 925 patients. A total of 5010 patients were included in the review. Summary characteristics of the included studies are shown in Table 2.

Total colectomy with ileorectal anastomosis

Adenomas

Five studies described the rate of adenoma development in the residual rectum (Supplementary Table 1). In 8 studies that analysed the frequency of secondary proctectomy due to endoscopically unmanageable polyposis, the rate of proctectomy ranged from 3.7% to 35%.[15] Five studies described adenoma evaluated in the neoterminal ileum (Table 3), with a high variance in reported rates from 0%[16] to 47.6% in patients followed-up for median of > 20 years[17] in one study including a paediatric cohort[18], 2 patients required resection of the terminal ileum and construction of a new IRA, one due to low grade dysplasia (LGD) and one due to high grade dysplasia (HGD) adenoma.

Rectal cancer

The reported rate of cancer in the rectal remnant (Table 4) after primary IRA is 8.8%[18] to 16.7%[19] with a median follow-up from surgery[19] of 91.1 months (3–557 months). However, studies from Japan report higher rates of up to 37%[20], but this is due to the inclusion of *in situ carcinoma* in the cancer definition. The same study had the longest median follow-up of 21.1 years (3–35). On the other hand, a small cohort of 21 patients from France reported zero cases of cancer during a median follow-up of 8.4 years. Jenner *et al*.[21] only included patients with a confirmed mutation. Five studies reported a cumulative incidence of rectal cancer ranging from 3%[22] to 17.2%[19] at 5 years, 7.7%[23] to 24.1%[19] at 10 years, 11%[22] to 23%[23] at 20 years, and 24%[22] at 30 years after the primary IRA. In one of the largest studies[24], which analysed data from 4 national registries and 776 patients, the 10-year cumulative risk of residual rectal cancer was 4.4% (95% CI, 2.6–6.2) for patients who underwent surgery before 1990 and only 2.5% (0–5.5) after the 1990. Only one study reported the time from surgery to cancer diagnosis (median 102 months [1–26 years])[23]; other studies reported follow-up time from surgery, but did not clearly define when follow-up started nor the surveillance regime. Five studies reported mortality ranging from 1.6%[23] to 11.1%[20] in which 3 out of 27 patients died from cancer in the rectal remnant. Only one of two studies that examined long-term survival after diagnosis of residual rectal cancer reported a 5-year survival rate of 55%.[22] In a study from Japan, 5-year survival was 94%[25], but the excellent survival was explained by the inclusion of carcinoma in situ despite the exact proportion of these was not given.

Risk factors for progressive phenotype of rectal remnant

Eleven studies reported nine risk factors predictive of the progressive rectal residual phenotype (Supplementary Table 2). Four studies analysed the genotype-phenotype relationship; The presence of a pathogenic variant between codons 1250–1464 was an independent risk factor for subsequent cancer development (HR 4.4 [1.3–15.0][23] and for the secondary proctectomy[26,27] (HR 3.91 [1.45–10.51], $P = 0.007$). In a small study of 25 patients, all patients (n = 3) with carpeting rectal remnant polyposis had a pathogenic variant in codon 1309, but this was only descriptive data.[28] An aggressive colonic phenotype with at least 500 polyps at time for surgery was identified as a risk factor in three studies (Supplementary Table 2). Two studies[15,25] have identified > 20 rectal remnant polyps at the time of surgery or during the endoscopic surveillance[26] as an independent risk factor for secondary proctectomy (HR 30.99 [9.57–100.32] $P < 0.001$), while in one study a cut-off of > 10 rectal adenomas[28] was associated with a more aggressive phenotype, as these patients developed a mean of 9.29 rectal residual adenomas per patient per year compared with 0.67 adenomas per patient per year if they had < 5 rectal polyps at the time of surgery. Other potential risk factors included patient age at diagnosis of rectal residual cancer, time since surgery, presence of congenital hypertrophy of the retinal pigment epithelium, and presence of colon cancer at the time of primary surgery. APC site mutation, preoperative colon phenotype, presence of duodenal adenomas and rectal remnant phenotype on surveillance were not identified as risk factors for progressive rectal remnant disease phenotype only in one study.[20]

Proctocolectomy with ileal-pouch anal anastomosis

Adenomas

Seventeen studies (Table 5) reported on the development of adenomas after IPAA, of which eight studies differentiated between the pouch body

TABLE 2. Characteristics of included studies

First author and publication date (ref.)	No. of patients	Country	Setting	Study design	Surgery performed (period)	Study population
Aelvoet *et al.*, 2023[55]	144 (111 IPAA, 33 ileostomy)	The Netherlands	Single	Cohort/ Retrospective	/	IPAA, ileostomy
Tatsuta *et al.*, 2023[56]	65 (22 IRA, 20 IPAA)	Japan	Single	Cohort/ Retrospective	1976–2022	IRA, IPAA
Anele *et al.*, 2022[57]	199 (199 IRA)	United Kingdom	Single	Cohort/ Retrospective	1990–2017	IRA
Colletti *et al.*, 2022[58]	715 (715 IRA)	Italy	Multicentre	Retrospective analysis of the Registry	1977–2021	IRA
Pasquer *et al.*, 2021[59]	289 (197 IRA, 92 IPAA)	France	Multicentre	Retrospective analysis of the Registry	1965–2015	IRA, IPAA
Ardoino *et al.*, 2020[60]	925 (585 IRA, 340 IPAA)	Italy	Multicenter	Retrospective analysis of the Registry	1947–2015	IRA, IPAA
Tajika *et al.*, 2019[16]	47 (14 IRA, 25 IPAA, 8 ileostomy)	Japan	Single	Cohort/ Retrospective	1965–2017	IRA, IPAA and ileostomy
Ganschow *et al.*, 2018[61]	192	Germany	Singe	Cohort/ Prospective and retrospective analysis of Polyposis Registry	Endoscopy data collected during 2010–2013	IPAA
Kariv *et al.*, 2017[62]	45	Israel	Single	Cohort/ Retrospective	1986–2013	IPAA
Patel *et al.*, 2016[42]	21 (6 IRA, 5 IPAA, 10 intact colon)	Indianapolis, USA	Single	Cohort/ Retrospective	Endoscopies performed between 2004–2016	IRA, IPAA and intact colon
Walsh *et al.*, 2016[63]	1	Ireland	Single	Case report	1987	IPAA - cancer
Maehata *et al.*, 2015[20]	27	Japan	Single	Cohort/ Retrospective	1990–2004	IRA
Ganschow *et al.*, 2015[50]	100; 50 hand-sewn and 50 stapled anastomoses	Germany	Single	Cohort/ Prospective	?	Hand-sewn vs. stapled anastomosis
Goldstein *et al.*, 2015[63]	59	Israel	Single	Cohort/ Retrospective	1986–2013	IPAA
Zahid *et al.*, 2015[64]	27	Australia	Single	Cohort/ Retrospective	1984–2011	IPAA
Kennedy *et al.*, 2014[65]	95; 85 hand-sewn and 1 stapled anastomosis	Rochester, Mayo Clinic, USA	Single	Cohort/ Retrospective	1987–2011	IPAA
Koskenvuo *et al.*, 2013[22]	140	Finland	Single	Cohort/ Retrospective	1963–2012	IRA
Pommaret *et al.*, 2013[35]	118	France	Single	Cohort/ Retrospective	/	IPAA and IRA
Boostrom *et al.*, 2013[66]	117	Rochester, Mayo Clinic, USA	Single	Cohort/ Retrospective	1972–2007	IPAA
Ozdemir *et al.*, 2013[37]	260; 86 hand-sewn and 175 stapled anastomoses	Cleveland, USA	Single	Analysis of polyposis registry	1983–2010	Hand-sewn vs. stapled anastomosis
Wasmuth *et al.*, 2013[67]	61; 39 hand-sewn with mucosectomy and 22 without of which 15 were stapled and 7 hand-sewn anastomoses	Norway	Multicenter	Analysis of polyposis registry	1986–2008	IPAA (mucosectomy vs. no-mucosectomy)
Yan *et al.*, 2012[68]	42 (33 IPAA; 6 IRA ?)	China	Single	Cohort/ Retrospective	1988–2008	IPAA and IRA

First author and publication date (ref.)	No. of patients	Country	Setting	Study design	Surgery performed (period)	Study population
Makni *et al.*, 2012[69]	1	Tunisia	Single	Case report	1996	IPAA - cancer
Tonelli *et al.*, 2012[51]	69	Italy	Single	Cohort/ Prospective data collection	1984–2008	IPAA
von Roon *et al.*, 2011[70]	140; 44 hand-sewn and 76 stapled anastomoses	UK	Single	Retrospective analysis of St. Mark's Hospital Polyposis Registry	1978–2007	Hand-sewn *vs.* stapled anastomosis
Banasiewicz *et al.*, 2011[32]	165	Poland	Bicenter	Bicenter/ Retrospective analysis	1985–2009 operated, Clinical data from endoscopy FUP between 2004–2009	IPAA
Booij *et al.*, 2010[18]	43 (34 IRA)	The Netherlands	Single	Cohort/ Retrospective	1977–2005	IRA and IPAA
Sinha *et al.*, 2010[26]	427	UK	Single	Retrospective analysis of St. Mark's Hospital Polyposis Registry	1990–2008	IRA
Ault *et al.*, 2009[71]	2	Los Angeles, USA	Single	Case series	1990, 1993	IPAA - cancer
Nieuwenhuis *et al.*, 2009[27]	475	Denmark, Finland, Sweden, Netherlands	Multicenter	Analysis of polyposis registry	/	IRA
Yamaguchi *et al.*, 2009[25]	59	Japan	Single	Cohort/ Retrospective	1962–2007	IRA
Friederich *et al.*, 2008[31]	212; 71 hand-sewn with mucosectomy and 115 stapled anastomoses	The Netherlands	Single	Analysis of National Polyposis Registry	1985–2005	IPAA
Campos *et al.*, 2008[19]	36	Brasil	Single	Cohort/ Retrospective	1977–2006	IRA and IPAA
Bullow *et al.*, 2008[24]	776; 576 operated in pre-pouch period and 200 in pouch period starting in 1990	Denmark, Finland, Sweden, Netherlands	Multicenter	Analysis of polyposis registry	1950–2006	IRA
Gleeson *et al.*, 2008[30]	16	Rochester, Mayo Clinic, USA	Single	Cohort/ Retrospective analysis	1964–2003 (Analysis of endoscopies between 1992–2006)	IPAA and IRA
Lee *et al.*, 2008[72]	1	Korea	Single	Case report	1998	IPAA - cancer
Linehan *et al.*, 2007[73]	1	Ireland	Single	Case report	1997	IPAA - cancer
Valanzano *et al.*, 2007[28]	25	Italy	Single	Cohort/ Prospective	1986–2004	IRA
Moussata *et al.*, 2007[17]	21	France	Single	Cohort/ Retrospective	/	IPAA and IRA
Ulas *et al.*, 2006[74]	1	Turkey	Single	Case report	1993	IPAA - cancer
Campos *et al.*, 2005[19]	1	Brazil	Single	Case report	/	IPAA - cancer
Groves *et al.*, 2005[34]	60	UK	Single	Retrospective analysis of St. Mark's Hospital Polyposis Registry	/	IPAA
Vroueraets *et al.*, 2004[75]	2	The Netherlands	Single	Case report	1990, 1991	IPAA – cancer
Ooi *et al.*, 2003[36]	2	Cleveland, USA	Single	Case report	/	IPAA – cancer

First author and publication date (ref.)	No. of patients	Country	Setting	Study design	Surgery performed (period)	Study population
Church *et al.*, 2003[38]	197; 62 operated in pre-pouch period and 135 in pouch period starting in 1983	Cleveland, USA	Single	Analysis of polyposis registry	1950–1999	IRA
Cherki *et al.*, 2003[76]	1	France	Single	Case report	/	IPAA - cancer
Thompson-Fawcett *et al.*, 2001[77]	33	Canada	Single	Cohort/ Prospective	/	IPAA
Church *et al.*, 2001[15]	213 (165 IRA)	Cleveland, USA	Single	Analysis of polyposis registry	/	IRA and IPAA
Brown *et al.*, 2001[78]	1	Singapore	Single	Case report	/	IPAA - cancer
Bertario *et al.*, 2000[23]	371	Italy	Multicenter	Retrospective analysis of Hereditary tumor registry	1955–1997	IRA
Vuilleumier *et al.*, 2000[79]	1	UK	Single	Case report	1990	IPAA - cancer
Jenner *et al.*, 1998[21]	55	Australia	Single	Analysis of polyposis registry	?–1994	IRA
Bassuini *et al.*, 1996[80]	1	UK	Single	Case report	1991	IPAA - cancer
Hoehner *et al.*, 1994[6]	1	Iowa, USA	Single	Case report	/	IPAA - cancer

FUP = follow up; IPAA = ileal pouch anal anastomosis; IRA = ileorectal anastomosis

and the anastomosis, one study only reported the anastomotic adenoma rate, while in the remaining seven studies the authors did not precisely define the anatomical location of the adenomas. The median age of patients at the time of surgery ranged from 15.4 to 34.6 years, with a median follow-up from surgery of 5.4 years to a median of 21.6 years. The reported rate of adenoma in the pouch body ranged from 9.4%[29] to 76.9%.[30] The proportion of HGD histology among adenomas at the polyp level ranged from 5.9% [17] to 53.2%.[31] In one study, the proportion of advanced adenomas on a per-patient basis was 11.2%.[31] The cumulative risk of adenoma development after primary IPAA was 12% and 58% at 5 and 20 years after the surgery respectively.[16] According to the analysis from Poland[32], 50% of all patients would develop LGD 15 years after the surgery, while HGD is estimated to be present in half of the patients 17.5 years after the surgery. Six studies analysed the rate of adenoma development in the neo terminal ileum, the proportion of patients with histologically confirmed adenoma varied from 4.2%[33] to 23.1%[30] with at a median follow-up from surgery of 6.5[34] to 23.1 years.[16] The cumulative risk of developing an adenoma in the neo terminal ileum was 4.4% at 20 years and increased to 36% at 30 years after the surgery as reported in the same study. The presence of pouch body adenomas was the only independent risk factor for the neo terminal ileum adenomas (OR, 2.16, P = 0.007).[35]

Cancer

Since the first case report of cancer arising in the ileal pouch of a FAP patient in 1994[6], we have identified 45 (Table 6) cancers that have developed in FAP patients after primary IPAA. Of these, 30 were located in the pouch body and 15 in the anastomosis/rectal cuff. The time from surgery to cancer diagnosis was reported for 22 patients and ranged from 2.3[36] to 33 years.[37] The information about the interval since last follow-up was reported for only 15 patients. The shortest interval between normal endoscopic surveillance and cancer diagnosis was 9 months.[16] Of the studies that reported the final outcome, 13 (28.9%) patients were alive at the last follow-up (range 8 months to 6 years) after surgical therapy and 9 patients died of disseminated cancer (1 month to 4 years after diagnosis), most despite an initial R0 resection.

Hand-sewn vs. stapled IPAA

Six studies (Supplementary Table 3) compared the rates of adenoma development at the anastomosis between hand-sewn and stapled techniques. The incidence of adenoma was lower for hand-sewn

TABLE 3. Rate of adenoma development in the neoterminal ileum in patients after ileorectal anastomosis (IRA) and ileal pouch anal anastomosis (IPAA)

First author and publication date (ref.)	Adenomas in the neoterminal ileum – after primary IPAA; n (%)	Cumulative risk for development of neoterminal adenomas	Years since surgery	Risk factor for adenomas in neoterminal ileum	Rate of adenomas in the neoterminal ileum – after primary IRA; n (%)	Years since surgery
Tajika *et al.*, 2019[16]	4/24 (16.7)	4.4% at 20 years and 36% at 30 years after primary surgery	23.1 ± 5.8		0/14 (0.0)	
Boostrom *et al.*, 2013[66]	4/33 polyps (12.0)					
Pommaretet *et al.*, 2013[35]	9/118 (6.5)			Presence of pouch adenomas (OR, 2.16, *P* = 0.007)		
Booij *et al.*, 2010[18]					5/34 (14.7) 2 patients had resection of neo-terminal ileum, one due to LGD and other due to HGD adenoma.	
Gleeson *et al.*, 2008[30]	3/13 (23.1)		Median 6.5 (0–15)		4/16 (25.0)	Median 12 (1–29)
Moussata *et al.*, 2007[17]			Mean 17.6 +-7.8(6–35) Mean from colectomy to diagnosis: 16.4+-8.5 (5–30)		10/21 (47.6) of which 2 were advanced adenomas.	
Groves *et al.*, 2005[34]	2/20 (10.0)		6 (1–14)		1/47 (2.0%)	12 (0–39)
Thompson-Fawcett *et al.*, 2001[77]	1/24 (4.2)		Median 7 (1–19)			

HGD = high grade dysplasia; LGD = low grade dysplasia

anastomosis, ranging from 0 to 33%, and for stapled anastomosis, ranging from 33.9 to 57%. The 10-year cumulative risk of adenoma development is 20–22.6% for hand-sewn anastomosis and 51.1–64% for stapled anastomosis.

Risk factors for adenoma development after primary IPAA

Nine studies analysed risk factors for adenoma development (Supplementary Table 4). None of the seven studies found a genotype-phenotype association. There was no association between colon adenoma burden at the time of surgery and subsequent development of pouch adenomas in three out of four studies. In the only positive study, none of the patients with < 200 colon polyps developed pouch adenomas, whereas almost half of the patients with > 1000 colon polyps later developed later pouch adenomas. Three studies have identified age of the pouch as a risk factor, while three others found no association between time since surgery and the rate of pouch adenomas. An association between the Spigelman score and the development of pouch adenomas was not confirmed. One study identified the presence of gastric adenomas as an independent risk factor for the development of pouch adenomas.

Discussion

Using a systematic approach, we identified a wide range of reported adenoma and cancer rates in the rectal remnant, pouch body, at IPAA and in the neoterminal ileum. The wide range in adenoma rates is probably partly due to the wide range of included studies in terms of year of publication. The equipment and quality of optical diagnosis has improved considerably in recent years, allowing better detection of adenomas and more precise examination of the pouch and rectal remnants. In addition, the risk stratification of patients at the time of surgery has also improved, allowing patients with a more aggressive phenotype to un-

TABLE 4. Patient characteristics and rate of rectal remnant cancer rate in patients after ileorectal anastomosis (IRA)

First author and publication date	Proportion of man; n / (%)	APC mutation Underwent n/ (%); Positive in; n/(%)	Follow-up (years/ months) since surgery	Years since surgery to cancer diagnosis	Age at surgery	Age at cancer diagnosis	Rectal remnant cancer rate; n/ (%)	Cumulative risk for rectal cancer	Rectal cancer mortality
Colletti *et al.*, 2022[58]	57.4%	93.6% /	/	Median of 13 years	/	/	47 / 715 (6.57)	/	14/47 (29.8%) at median follow up of 13 years.
Pasquer *et al.*, 2021[59]	95 (48.2)	/	/	/	/	/	12 / (6.1); 1 was metastatic, 2 were resected endoscopically, 10 surgically	/	/
Maehata *et al.*, 2015[20]	16 (59.3)	21 (77.8) 14 (66.7)	21.1 (3–35)	/	Median 27 years (9–66)	/	10/27 (37.0); 6/10 cancers were TisN0M0	8% at 10 years; 19% at 20 years; 57% at 30 years	3/27 (11.1)
Koskenvuo *et al.*, 2013[22]	59 (42.1)	/	Median 15 years (0–44)	/	Mean 36 years (18–71)	Cumulative risk 2% at 40 years age; 7% at 50; 13% at 60 years age and 16 % at 70 years age.	18/140 (13%)	3% at 5 years; 4% at 10 years; 11% at 20 years; 24% at 30 years after IRA	10/140 (7%); 5-year survival 55%. Cumulative risk for death due to rectal cancer after IRA: 2% at 5 years, 3% at 10 years and 9% at 30 years.
Booij *et al.*, 2010[18]	19 (44.2)	/	/	/	Median 16 (7–25)	/	3/34 (8.8)	/	2/34 (5.8)
Sinha *et al.*, 2010[26]	232 (54.3)	/ 311/427 (72.8)	Median 15 years (7–25)	/	Median 21 years (11–67)	/	48/427 (11.2%)	/	/
Yamaguchi *et al.*, 2009[25]	35 (59.3)	/	Median 8.9 years	/	Median 30 years (13–65)	/	17/59 (30%)	/	5-year survival 94%; 10-year survival 94%.
Nieuwenhuis *et al.*, 2009[27]	/	/	/	/	/	/	/	3.7% for group 1; 9.3% for group 2; 8.3% for group 3.[%]	/
Campos *et al.*, 2008[19]	/	/	91.1 (3–557)	/	Mean 45.8 years	Mean 50.6 years	6/36 (16.7)	17.2% at 5 years; 24.1% at 10 years; 43.1% after 15 years	/
Gleeson et al., 2008[30]	/	/	FUP initiated median 12 (1–29) years after surgery	/	/	40 and 59 years.	2/16 (12.5)	/	/
Bullow *et al.*, 2008[24]	401 (51.7)	/	Median 7 years (0–13). Patients were operated between 1950–2006		Median 27 (7–75)	/	60/776 (7.7%) (56/576; 10% and 4/200; 2%)	10-year cumulative risk 4.4% [95% CI 2.6–6.2] in pre-pouch era; 10-year cumulative risk 2.5% [95% CI 0–5.5] in pouch era;	/
Moussata *et al.*, 2007[17] They only watched ileal muocas above the IRA	10 (47.6)	21/21 (100.0) 14/21 (66.7)	Mean 8.4 years ± 5 since colectomy	/	/	/	0/21 (0.0)	/	/
Church *et al.*, 2003[38]	92 / (46.7)	/	Pre-pouch era: 212 months (IQR 148 months); Pouch era: 60 months (IQR 80 months)	/	Median age 23 years (IQR 15.5 years pre-pouch and 17 years pouch)	/	8 (12.9%) in the pre-pouch era and 0 in pouch era.	/	/
Bertario *et al.*, 2000[23]	206/371 (55.5)	297/371 (80.1) 200/297 (67.3)	Median 81 months	Median 102 months (1–26 years)	Mean 32 years	/	27/371 (7.3)	10 years – 7.7% 15 years – 13.1 % 20 years – 23.0%	6/371 (1.6)
Jenner *et al.*, 1998[21]	25/55 (45.0)	55/ (100.0)	Median 10 (1–31)	/	Mean age 30 (13–62)	Median 41	7/55 (12.7)	/	/

Colonic phenotype divided in 3 groups: (Group 1 - <100 polyps and mutation in codons 1–157, 312–412 and 1596–2843; Group 2 Hundred of polyps and mutation in codons 158–311, 413–1249 and 1465–1595; Group 3 Thousand of polyps and mutation in codon 125

APC = adenomatous polyposis coli; FUP = follow up

dergo primary restorative proctocolectomy while primary IRA can still be offered to patients with an attenuated phenotype or low rectal disease burden. Indeed, in the largest study of four European national polyposis registries, the cumulative risk of cancer in the rectal remnant (CRR) was 10% in patients operated in the 'pre-pouch' period and only 2% in those who were operated in the 'pouch period.[24] Similar findings have been reported from the USA[38] where 8 patients operated before

TABLE 5. Patient characteristics and rate of adenomas in patients after primary ileal pouch anal anastomosis (IPAA)

First author and publication date	Sex (man); n (%)	APC mutation Underwent; n (%); Positive in; n (%)	Distinguish between pouch body and rectal cuff	Follow-up (months/years)	Time from surgery to first adenomas (years)	Age at surgery (years)	Rate of adenomas (≥ 1 polyp)	Size of adenomas, mm	Histology of adenomas; n (%)	Number of Adenomas
Aelvoet AS *et al.*, 2023[55]	81 (56)	101 (91) 96 (86)	Yes	Median 152 (77–240)	15% at 5 years; 48% at 10 years; 85% at 20 years.	Median 24 (18–32)		Median 5 (3–15)	Tubular adenomas 31 (28%), Tubulovillous 26 (23%), Villous 5 (5%)	Prepouch ileum 4(2–13), Pouch body 20 (5–50), rectal cuff 6 (3–10)
Tajika *et al.*, 2019[26]	16 (47.1)	/	Yes	Median 21.6 (3.7–8.8)	32 (35.9) of patients showed progression of pouch adenomas during FUP	Median 34.6 (17–52)	24/34 (70.6)	2–40 mm	6 advanced adenomas (25.0)	1–300
Ganschow *et al.*, 2018[61]	100 (52.1)	133 (69.3)) ? / 133	No	Median 12.8 (9–17) for patients with pouch adenomas and (2.5–12.2) for patients without pouch adenomas;	32 (35.9) of patients showed progression of pouch adenomas during FUP	27.5 years (10.2–58.5)	90/192 (46.9) at a median of 8.5 years (0.9–25.1) after IPAA. 5 years after IPAA 84.9% patients free of adenoma; 15 years after 40.4% and 20 years after 21.9% patients were free of adenomas.	53/192 (58.9) ≤ 4 mm; 24/192 (26.7) 5 –10 mm; 13/192 (14.4) ≥ 10 mm	Tubular adenomas in 69/192 (76.7); tubulovillous adenomas in 16/192 (17.8); villous in 5/192 (5.6)	46/192 (51.1) had < 4; 14/192 (15.6) 5–10; 30/192 (33.3) > 10 adenomas
Goldstein *et al.*, 2015[63]	24 (41.0)		Yes	Mean 11.6 years +-14.6 years	Median adenoma free time interval since surgery; Cuff 10.8 years Pouch 16.9 years	Mean 30.8 years +-10.8 years	35/59 (59.0); - 20 isolated in cuff - 4 isolated in pouch body - 11 in pouch and body	/	All LGD	/
Zahid *et al.*, 2015[19]	14 (51.8)		No	Mean 9.2 years	Median; 72 months (18–249)	Median 31 years (14–65)	12/27 (44.0)	/	Only 1 polyp HGD (< 99%)	/
Kennedy *et al.*, 2014[66]	43 (45.0)		Watched only anastomosis	Mean 7.6 (0 – 24)		Mean 15.4 (4–20)	9/95 (9.4)			
Pommaretet *et al.*, 2013[36]		110 / 139 92 / 110 (Cohort included IRA, ileostomy and IPAA patients but did not distinguish between).		/	Median 15 years	25 years (9–61 years)	57/118 (48.3)	> 10 mm:12	94% LGD; 6% HGD	1–4: 22 5–20: 18 > 20: 17
Boostrom *et al.*, 2013[66]	52 (44.5)		Yes	125 months (25–423 months)	12.4 years (15–405 months)	26 years (4–60 years)	30/117 (25.6)	5.9 mm (2 mm to 20 mm)	22 LGD, 8 tubulovillous	/
Wasmuth *et al.*, 2013[67]	34 (55.7)	/	Yes (body and anastomosis)		Cumulative rate of adenomas at 28 years 17% for mucosectomy group and 75% at 15 years in a group without mucosectomy (P < 0.0001)	20 (10–49)	Anastomosis: 4/39 (10.0) vs. 14/22 (64.0) (P < 0.0001) Pouch body: 8/39 vs. 6/22 (P 0.57)			
Tonelli *et al.*, 2012[51]	/	45 (65%)	No	Median 133 months (12–288 months)	Mean 7 years (1–15 years)	33 years (17–63 years)	25 (36.0)	Mean 3 mm (1–40)	Adenomas, dysplasia not specified	Mean 8 (1–47)
Yan *et al.*, 2012[68]	30 (71.5)	/	Yes	Median 7.2 (2.2–20)		29 (16–65)	At the anastomosis 6/33 (18.2)	/	/	/
Banasiewicz *et al.*, 2011[33]	79 (47.9)	/	/	Endoscopies performed 2–19 years since surgery.	Mean 14 months to LGD; Mean 16 months to HGD. Estimated frequency LGD 15 years later 50% and for HGD 17.5 years later 50%.		21/165 (12.7)		LGD - 21/32 (65.6); HGD - 11/32 (34.4)	
Gleeson *et al.*, 2008[31]	/	/	Yes	/	FUP began median 6.5 (0–15) after surgery	/	13/13 (100): 10/13 pouch body; 2/13 anastomosis; 3/13 ileum above anastomosis	< 5 mm	/	5–30

First author and publication date	Sex (man); n (%)	APC mutation Underwent; n (%); Positive in; n (%)	Distinguish between pouch body and rectal cuff	Follow-up (months/years)	Time from surgery to first adenomas (years)	Age at surgery (years)	Rate of adenomas (≥ 1 polyp)	Size of adenomas, mm	Histology of adenomas; n (%)	Number of Adenomas
Friederich *et al.*, 2008[32]	119 (56.0)	/	/	Mean 7.9 (0.4–20.3 years)	Cumulative risk of 16% at 5-years and 42.4% at 10 years for adenoma development. Cumulative risk of 12.8% at 10 years for advanced adenoma development.	Mean 30.0 years (10–62.6 years)	47/212 (35%)	/	/	/
Campos *et al.*, 2008[17]	/	/	No	50.8 (5–228)			3/26 (11.5)			
Moussata *et al.*, 2007[25]	12 (57.1)	23/23 (100.0) 22/23 (95.7)	Yes (only polyps in the ileal mucosa of the pouch body are described)	Mean 5.4 +- 2.6 (1–11)	Mean 4.7+-3.3 years (1–14)		17/23 (74.0)	Mean size 5.2 mm +-3.4 mm; 3 polyps were > 10 mm.	LGD 16/17 (94.1); HGD 1/17 (5.9)	/
Groves *et al.*, 2005[35]	35 (58.3)	/	Between pouch and above anastomosis ileum	6 years (1–17 years)	/	32.5 years (13–66 years)	34/60 (57%) of which 5 were > 10 mm / 11 were advance adenomas	Mean size 5 mm (1–40 mm)	/	Median number 4
Thompson-Fawcett *et al.*, 2001[77]	/	20/33 (60.6) 18/20 (90.0)	Only pouch body	/	/	/	20/33 (60.0) adenomas	1–3 mm	/	Median 10 (1–100) Also lymphoid hyperplasia included

APC = adenomatous polyposis coli; FUP = follow up; HGD = high grade dysplasia; IRA = ileorectal anastomosis; LGD = low grade dysplasia

1983 (12.9%) were diagnosed with CRR compared to none of those operated after 1983 when pouch surgery was introduced at the Cleveland Clinic. Recently published data from two Japanese studies reporting an overall CRR rate of 30%[25] – 37%[20] must be interpreted with caution as carcinoma *in situ* was also included in the definition of cancer in their cohorts. The risk of metachronous cancer after IRA has been recognised early and these patients have been advised to undergo regular surveillance of the rectal remnant. Traditionally, surveillance was recommended every 3 to 12 months. This recommendation has been maintained ever since and can be also found in the recently published international guidelines (Table 1). The French national guidelines published in 2005[39] are the only ones to include the genotype information, as they recommend more frequent surveillance if the pathogenic variant is located between codons 1250–1500. However, they were published in 2005.

The main obstacle to refining recommendations for endoscopic surveillance is the lack of high-quality, prospective data. Unfortunately, we have not found a single randomised trial that has compared different surveillance strategies or aimed to identify factors that would allow risk stratification. Members of the International Society for Gastrointestinal Hereditary Tumors (InSiGHT)[40] proposed a staging system[41] and stage-specific interventions for patients with intact colon and those with IRA, but unfortunately no effort has been made to validate this staging system. Data on endoscopic treatment modalities are even more descriptive. In fact, in five international recommendations (Table 1), only Vasen *et al.*[7] recommended endoscopic removal of all polyps with dysplasia or those larger than 5 mm. Endoscopic management of these patients has therefore been influenced by expert groups. Unfortunately, preferred methods of endoscopic management were rarely described in the reviewed studies. Maehata *et al.*[20] recommend removal of all polyps larger than 8 mm. A descriptive study with a small sample size (n = 6)[42] showed that large-scale cold snare polypectomy can effectively reduce the polyp burden in the rectal remnant even in cases of very high polyp numbers. The mean number of polyps removed was 78.5 (30–155). During the follow-up (mean 10.7 months), none of the patients developed rectal cancer and there were no complications related to polypectomy. This is in contrast to another study from the USA[30], which advocates the use of ablative therapy with argon plasma coagulation. A similar

TABLE 6. Cancer rate after primary ileal pouch anal anastomosis (IPAA)

First author and publication date (ref.)	No of patients	Age at cancer diagnosis (years)	Time to cancer (years)	Interval since last surveillance endoscopy and findings	Endoscopic findings at diagnosis	Location	Staging of cancer and status
Aelvoet *et al.*, 2023[55]	3/111 (2.7%)	/	/	/	/	/	Pouch excision
Pasquer *et al.*, 2021[58]	1/92 (1.1)	30	/	1 month		Pouch body	Endoscopic resection
Ganschow *et al.*, 2018[61]	1	/	27	/	/	Pouch body	Resection and reconstruction of a new pouch - alive
Walsh *et al.*, 2016[63]	1	54	/	Regular annual surveillance	New endoscopy due to anemia and rectal blood loos	Anastomosis	T3N2Mx, resection and ileostomy, alive during last FUP.
Wasmuth *et al.*, 2013[67]	1	/	11	/	/	Rectal cuff	Resection and ileostomy - alive
Boostrom *et al.*, 2013[66]	1	/	23.7	/	/	Pouch body	Transanal resection - alive
Ozdemir *et al.*, 2013[38]	4	/	Mucosectomy group; median 11.3 years (8.3–22) Without mucosecomy; 8 years	Regular annual surveillance	/	All ATZ	? 3 underwent APR - alive 1 transanal resection – died 4 years later dissemination
Makni *et al.*, 2012[69]	1	26	10	8 months	Polyps, LGD?	Pouch body?	Pouch excision – died 12 months later dissemination
Tonelli *et al.*, 2012[51]	2	29 58	10	12 months, normal 6 months, normal	? IIa + IIc polyp	Pouch body Pouch body	Excision with ileostomy, T3N0M0, died 6 months later dissemination/ Excision with ileostomy, T2N0M0, alive after 56 month FUP
voon Roon *et al.*, 2011[70]	1	/	13	/	/	Pouch body	Excision of a pouch – died 2 years of disseminated disease
Banasiewicz *et al.*, 2011[33]	5	/	/	/	/	Pouch body	/
Ault *et al.*, 2009[71]	2	61 50	11 10	6, normal /	Pain and blood per rectum, 3 cm mass/ Sacral pain, bleeding ulcer	Pouch body / Pouch body	T2N1Mx, died of AMI prior treatment / Metastatic disease, chemotherapy
Tajika *et al.*, 2009[83]	2	55 68	8.6 20	9 months, normal No FUP	30x25 mm cancer / Polyposis and 25 x 25 mm polyp	Pouch body/ Kock's pouch body	T4N2M0 – died 1 year later T3N?M? – died (MDS)
Lee *et al.*, 2008[71]	1	/	7	/	Ulcerating tumor	Pouch body	T4N1M0, APR ileostomy. Developed metastases 2 years later.
Friederich *et al.*, 2008[32]	4	35 37 32 36	14 10.2 16.4 6.2	4.4 years, normal 2.1 years, normal No control (symptoms) 0.6 years, Tubullovilous HGD	/	All pouch body	Dukes C Dukes B Dukes B Dukes B
Linehan *et al.*, 2007[72]	1	40	10	/	Pelvic pain, discharge	Pouch body (patient had ileostomy but pouch was left in situ)	Excision. At last FUP patient was well.
Ulas *et al.*, 2006 [74]	1	/	9	/	/	Anastomosis	Dukes B, APR, metachronous cancer after 1 year
Campos *et al.*, 2005[19]	1	/	12	No FUP	Presented with rectal bleeding	Pouch body	T2N0Mx, APR and ileostomy, patient well at 6 years FUP.
Vroueraets *et al.*, 2004[75]	2	48 36	9 10	5 years normal, then 2 and 1 years (both multiple LGD adenomas refused surgery) / Regular FUP every 2 years	Presented after 1 year with rectal bleeding / Normal. Routine biopsies at subsequent FUP revealed adenoca.	Anastomosis Anastomosis	T2N0M0, APR, alive 1 year later / T4N0M0, APR, alive 8 months later
Cherki *et al.*, 2003[76]	1	35	3.5	1.5 years	/	Pouch body	T3N1M1, resection with ileostomy, died 1 month later
Ooi *et al.*, 200336	2	36 /	2 years 3 months 8 years	/ /	Symptoms of anal bleeding/ /	Anastomosis Anastomosis	T3NOMO, APR, ileostomy, died 2.5 years later dissemination / T2N0M0, transanal excision with ileostomy (refused APR), died 4 years later, dissemination
Brown *et al.*, 200178	1	44	7 years 4 months	Under FUP every 6 months	/	Anastomosis	/
Vuilleumier *et al.*, 2000[79]	1	38	7	No FUP	/	Anastomosis	Resection with ileostomy – died 12 months later dissemination
Palkar *et al.*, 1997[15]	1	39	4.7	3 months	?	Pouch body	T4NOM? - alive
Kim *et al.*, 1997[15]	1	/	/	/	/	Pouch body?	/
Bassuini *et al.*, 1996[80]	1	31	3	No FUP	/	Pouch body	/
Von Herbay *et al.*, 1996[14]	1	33	8			Anastomosis	T1N0M0
Hoehner *et al.*, 1994[7]	1	34	20	/	/	Anastomosis	/

#The data from these cases has been drawn from reviews by Tajika[14] and Smith[13] as full-text of the papers were not accessible.

FUP = follow up; HGD = high grade dysplasia; LGD = low grade dysplasia

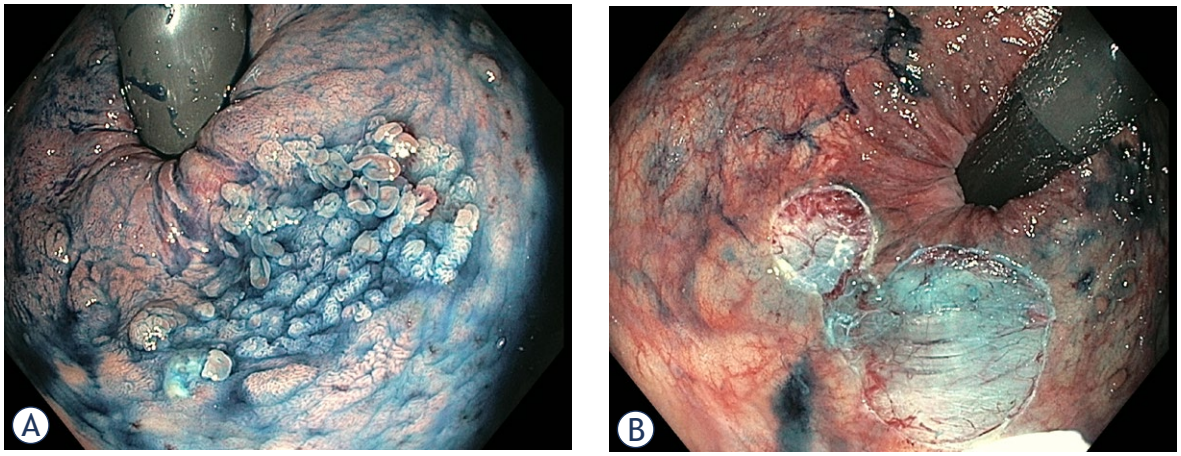


FIGURE 2. Surveillance endoscopy in a 48-year old patient with FAP after colectomy with IRA revealed 18 m LST-G **(A)**. After submucosal injection with gelofusine, indigo carmine and adrenaline, piecemeal endoscopic mucosal resection (pEMR) **(B)** was performed.

practice was supported by a study published in France in 2007.[17] National French guidelines published in 2005 recommend ablation with APC for small polyps (a few millimetres) and mucosectomy for larger polyps.[39]

Improvements in endoscopic resection techniques have also been applied to the treatment of large lesions in the rectal remnant. Recently two reports, both from Japan[43,44], have been published of successful endoscopic submucosal dissection (ESD) of 75 mm Is + IIa adenoma and residual adenoma at the IRA. In our endoscopy unit (Hospital Clinic, Barcelona) we also perform advanced endoscopic resection techniques. Figure 2 (A and B) shows a recent endoscopic mucosal resection (EMR) of an 18mm laterally spreading tumour granular type (LST-G) in the rectal remnant of a patient with FAP.

There is little data on the use of advanced imaging techniques. The study from St. Mark's hospital in London[45] showed no benefit of dye-based chromoendoscopy to detect additional adenomas in the rectal remnant. The European Society of Gastrointestinal Endoscopy (ESGE) guidelines[46] published in 2014 did not recommend the use of advanced endoscopic imaging in patients with FAP, but did not specifically differentiate between the patients with intact colon and those after surgery. On the other hand, the French Society of Endoscopy[39] recommended the use of dye-based chromoendoscopy with indigo carmine. We believe that use of dye-based chromoendoscopy in these patients does not increase the detection of clinically relevant lesions and it is not routinely performed in our unit. Considering the data on a cumulative risk of 57% for CRR 30 years after surgery[20] and the fact that adenoma development in the rectal remnant is an inevitable event[16], regular endoscopic surveillance is mandatory. Our recommendations are in line with other guidelines and our patients are recommended annual endoscopic surveillance, despite alarming data from an early study published in 2001[5] from four European registries in which 75% of patients with CRR had a negative rectoscopy within 12 months and 35% within 6 months prior to diagnosis of CRR. There was no information on the endoscopy equipment used for surveillance. We believe that the high rates of negative rectoscopies prior to cancer diagnosis may – to some extent - be influenced by the quality of endoscopy, which has been limited by the technical aspects of the equipment used in the past. This problem needs to be addressed again in the light of developments in endoscopic equipment.

When restorative proctocolectomy with IPAA was first described in 1978[47], it was believed that this operation would eliminate the risk of colorectal cancer in patients with FAP. However, a few years later, as the first pouches began to age, case reports of cancers arising in the pouch began to appear in the literature.[6] Since then, reports have become more frequent and we have identified 45 cases of cancer after primary IPAA, of which 26 arose in the ileal mucosa of the pouch body and 15 at the anastomosis. Furthermore, we now know that cancer can develop even after mucosectomy down to the dentate line[48], because even after removal of all visible rectal mucosa, some microscopic rectal columnar epithelium remains at the ATZ.[49] In the study from the Heidelberg Polyposis

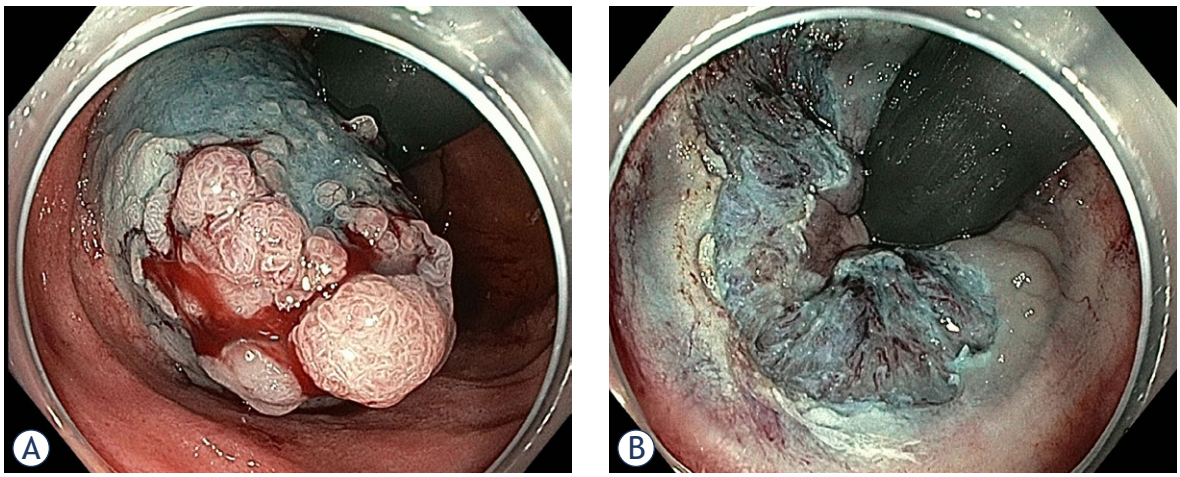


FIGURE 3. Surveillance endoscopy in a 49-year old patient with FAP after proctocolectomy with IPAA revealed 25 mm LST-G mixed type lesion in the rectal cuff. Lesion was spreading from the anastomosis to the dentate line. Patient had undergone surgery five years earlier and did not show up for endoscopy follow-up since then **(A)**. Lesion was removed with pEMR **(B)**.

Registry with 100 patients[50], rectal residual mucosa (defined as visible mucosa or detected by histology from blinded biopsies) was found in 42 (84%) cases after stapled and in 21 (42%) cases after hand-sewn anastomosis.

Researchers from Japan[16] found a 70% incidence of adenomas in the pouch body with one of the longest follow-up periods reported to date (> 20 years). Similarly, in a study from France, 74% of patients had at least one adenoma in the pouch, but with a mean follow-up of only 5.4 years. In contrast, one study found that isolated rectal cuff adenomas were more common than isolated pouch adenomas (49.1% *vs.* 6.8%), while 18.7% of patients had both pouch and rectal cuff adenomas. Cumulative 5-year, 10-year and 20-year risks for pouch adenomas were 32%, 52% and 68% in the Japanese study[16], a slightly lower 5-year cumulative risk but a similarly high 10-year risk was observed in a Dutch study[31]; 16% and 42%, but the authors of this paper did not specifically define the exact location of the adenomas. The authors also reported a 10-year cumulative risk of developing precancerous adenomas of 12.8%.

On the other hand, the adenoma rates – at least in the stapled group - seem to be higher in the studies that only looked at the anastomosis and compared hand-sewn with stapled: 0–33% *vs.* 33.9–57%. In view of these figures, it is essential that patients with primary IPAA also undergo regular endoscopic surveillance. Particular attention should be paid to the rectal cuff and anastomosis, and the pouch should be examined in both forward and retroflexed position.

International guidelines most commonly recommend annual endoscopy examination, whereas ASCO guidelines[9] advocate 'case-by-case' interval allocation. In 11 of only 12 studies that described a surveillance protocol, an interval of 12 months was recommended except in Brazil where endoscopy of the pouch was recommended every 2 years.

Interestingly, in the Netherlands pouch endoscopy was recommended every 1 to 3 years in the late 1990s but in 2001 the protocol was changed to annual endoscopic surveillance regardless of the anastomotic technique (hand-sewn or stapled).

One of the main concerns is the short interval (< 1 year) between the last normal endoscopy and the cancer diagnosis and the aggressive course of the disease despite an initial R0 resection (Supplementary Table 4). It is not entirely clear whether the adenoma-carcinoma sequence is faster in the ileal mucosa compared with the colon and rectum, or whether "negative" endoscopies prior to cancer diagnosis could be explained by the poor quality of pouch endoscopy. Chromoendoscopy improves the detection of diminutive adenomas[31] and lymphoid hyperplastic nodules[45], but its use is discouraged[33,35] for the same reasons as in the examination of rectal remnants – increased of detection of clinically irrelevant polyps. Endoscopy should be performed with a gastroscope or paediatric colonoscope, as stricture can occur at the anastomosis, especially after hand suturing.

There are no official recommendations for endoscopic management of FAP patients after IPAA. We have found considerable heterogeneity in local practice. Italian authors recommend resection of

all adenomas > 3 mm.[51] On the contrary, ablation with argon plasma coagulation is the preferred resection technique in a French study.[17] Ablative techniques were also supported by the study from the Mayo Clinic.[30] In a small descriptive cohort of only 5 patients[42], large-scale cold snare polypectomy with a mean of 110.6 (30–342) resected polyps demonstrated the efficacy of cold snare in controlling large polyp burden (> 30 polyps) with no reported polypectomy related complication. In our unit we do not use nor encourage use of argon plasma coagulation. We recommend resection of all polyps > 3 mm. Advanced resection techniques, when performed in the tertiary centres, may be a viable alternative prior to surgical resection. A case report of successful *en bloc* ESD of a 15 mm 'non-lifting' HGD adenoma in the ileal pouch has recently been published.[52] Figure 3 (A and B) shows an EMR of 25 mm LST in a patient with FAP after IPAA. The polyp was located in the rectal cuff and extended from the anastomosis to the dentate line. The procedure was performed at our Endoscopy Unit. It should be emphasised that the wall of the ileum is very thin and special care must be taken when resecting larger lesions.

Although there is no randomised trial comparing different endoscopic surveillance intervals, it is unlikely that prospective data will be available in the future. The main reason is ethical issue, as these patients are at increased risk of colorectal cancer. However, with the introduction of high quality colonoscopy and improvements in endoscopy technique, a 'negative' endoscopy before cancer diagnosis should become highly unlikely if not impossible.

Acknowledgement

Funded by Instituto de Salud Carlos III (PI19/01050) and Beca de la Marató de TV3 2020 (Beca la Marato—201932-30). Co-funded by European Regional Development Fund/European Social Fund; "A way to make Europe"/"Investing in your future". CIBERehd is funded by the Instituto de Salud Carlos III.

References

1 Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, et al. Identification of FAP locus genes from chromosome 5q21. *Science* 1991; **253:** 661-5. doi: 10.1126/science.1651562

2 Church J. Familial adenomatous polyposis. *Surg Oncol Clin N Am* 2009; **18:** 585-98. doi: 101016/jsoc200907002

3 de Campos FGCM, Perez RO, Imperiale AR, Seid VE, Nahas SC, Cecconello I. Evaluating causes of death in familial adenomatous polyposis. *J Gastrointest Surg* 2010; **14:** 1943-9. doi: 101007/s11605-010-1288-6

4 Herzig D, Hardiman K, Weiser M, You N, Paquette I, Feingold DL, et al. The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the management of inherited polyposis syndromes. *Dis Colon Rectum* 2017; **60:** 881-94. doi: 101097/DCR0000000000000912

5 Vasen HF, van Duijvendijk P, Buskens E, Bülow C, Björk J, Järvinen HJ, et al. Decision analysis in the surgical treatment of patients with familial adenomatous polyposis: A Dutch-Scandinavian collaborative study including 659 patients. *Gut* 2001; **49:** 231-5. doi: 10.1136/gut.49.2.231

6 Hoehner JC, Metcalf AM. Development of invasive adenocarcinoma following colectomy with ileoanal anastomosis for familial polyposis coli report of a case. *Dis Colon Rectum* 1994; **37:** 824-8. doi: 10.1007/BF02050149

7 Vasen HFA, Möslein G, Alonso A, Aretz S, Bernstein I, Bertario L, et al. Guidelines for the clinical management of familial adenomatous polyposis (FAP). *Gut* 2008; **57:** 704-13. doi: 101136/gut2007136127

8 Balmaña J, Balaguer F, Cervantes A, Arnold D, ESMO Guidelines Working Group. Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines. *Ann Oncol* 2013; **24(Suppl 6):** vi73-80. doi: 101093/annonc/mdt209

9 Stoffel EM, Mangu PB, Gruber SB, Hamilton SR, Kalady MF, Lau MWY, et al. Hereditary colorectal cancer syndromes: American Society of Clinical Oncology Clinical Practice Guideline Endorsement of the familial risk-colorectal cancer: European Society for Medical Oncology Clinical Practice Guidelines. *J Clin Oncol* 2015; **33:** 209-17. doi: 101200/JCO2014581322

10 Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG Clinical Guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol* 2015; **110:** 223. doi: 10.1038/ajg.2014.435

11 Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *BMJ* 2009; **339:** b2700. doi: 101136/bmjb2700

12 Zotero. About. [internet]. [cited 2029 Jul 03]. Available at: https://www-zoteroorg/about/

13 Smith JC, Schäffer MW, Ballard BR, Smoot DT, Herline AJ, Adunyah SE, et al. Adenocarcinomas after prophylactic surgery for familial adenomatous polyposis. *J Cancer Ther* 2013; **4:** 260-70. doi: 104236/jct201341033

14 Tajika M, Niwa Y, Bhatia V, Tanaka T, Ishihara M, Yamao K. Risk of ileal pouch neoplasms in patients with familial adenomatous polyposis. *World J Gastroenterol* 2013; **19:** 6774-83. doi: 103748/wjgv19i406774

15 Church J, Burke C, McGannon E, Pastean O, Clark B. Predicting polyposis severity by proctoscopy: how reliable is it? *Dis Colon Rectum* 2001; **44:** 1249-54. doi: 10.1007/BF02234779

16 Tajika M, Tanaka T, Ishihara M, Hirayama Y, Oonishi S, Mizuno N, et al. Long-term outcomes of metachronous neoplasms in the ileal pouch and rectum after surgical treatment in patients with familial adenomatous polyposis. *Endosc Int Open* 2019; **7:** E691-8. doi: 101055/a-0849-9465

17 Moussata D, Nancey S, Lapalus MG, Prost B, Chavaillon A, Bernard G, et al. Frequency and severity of ileal adenomas in familial adenomatous polyposis after colectomy. *Endoscopy* 2008; **40:** 120-5. doi: 101055/s-2007-995363

18 Booij KAC, Mathus-Vliegen EMH, Taminiau JAJM, Ten Kate FJW, Slors JFM, Tabbers MM, et al. Evaluation of 28 years of surgical treatment of children and young adults with familial adenomatous polyposis. *J Pediatr Surg* 2010; **45:** 525-32. doi: 101016/jjpedsurg200906017

19 Campos FG, Imperiale AR, Seid VE, Perez RO, da Silva e Sousa AH, Kiss DR, et al. Rectal and pouch recurrences after surgical treatment for familial adenomatous polyposis. *J Gastrointest Surg* 2009; **13:** 129-36. doi: 101007/s11605-008-0606-8

20 Maehata Y, Esaki M, Nakamura S, Hirahashi M, Ueki T, Iida M, et al. Risk of cancer in the rectal remnant after ileorectal anastomosis in patients with familial adenomatous polyposis: single center experience. *Dig Endosc* 2015; **27:** 471-8. doi: 101111/den12414

21 Jenner DC, Levitt S. Rectal cancer following colectomy and ileorectal anastomosis for familial adenomatous polyposis. *Aust N Z J Surg* 1998; **68:** 136-8. doi: 10.1111/j.1445-2197.1998.tb04724.x

22 Koskenvuo L, Renkonen-Sinisalo L, Järvinen HJ, Lepistö A. Risk of cancer and secondary proctectomy after colectomy and ileorectal anastomosis in familial adenomatous polyposis. *Int J Colorectal Dis* 2014; **29:** 225-30. doi: 101007/s00384-013-1796-4

23 Bertario L, Russo A, Radice P, Varesco L, Eboli M, Spinelli P, et al. Genotype and phenotype factors as determinants for rectal stump cancer in patients with familial adenomatous polyposis. Hereditary Colorectal Tumors Registry. *Ann Surg* 2000; **231:** 538-43. doi: 101097/00000658-200004000-00013

24 Bülow S, Bülow C, Vasen H, Järvinen H, Björk J, Christensen IJ. Colectomy and ileorectal anastomosis is still an option for selected patients with familial adenomatous polyposis. *Dis Colon Rectum* 2008; **51:** 1318-23. doi: 101007/s10350-008-9307-3

25 Yamaguchi T, Yamamoto S, Fujita S, Akasu T, Moriya Y. Long-term outcome of metachronous rectal cancer following ileorectal anastomosis for familial adenomatous polyposis. *J Gastrointest Surg* 2010; **14:** 500-5. doi: 101007/s11605-009-1105-2

26 Sinha A, Tekkis PP, Rashid S, Phillips RKS, Clark SK. Risk factors for secondary proctectomy in patients with familial adenomatous polyposis. *Br J Surg* 2010; **97:** 1710-5. doi: 101002/bjs7202

27 Nieuwenhuis MH, Bülow S, Björk J, Järvinen HJ, Bülow C, Bisgaard ML, et al. Genotype predicting phenotype in familial adenomatous polyposis: A practical application to the choice of surgery. *Dis Colon Rectum* 2009; **52:** 1259-63. doi: 101007/DCR0b013e3181a0d33b

28 Valanzano R, Ficari F, Curia MC, Aceto G, Veschi S, Cama A, et al. Balance between endoscopic and genetic information in the choice of ileorectal anastomosis for familial adenomatous polyposis. *J Surg Oncol* 2007; **95:** 28-33. doi: 101002/jso20672

29 Kennedy RD, Zarroug AE, Moir CR, Mao SA, El-Youssef M, Potter DD. Ileal pouch anal anastomosis in pediatric familial adenomatous polyposis: a 24-year review of operative technique and patient outcomes. *J Pediatr Surg* 2014; **49:** 1409-12. doi: 101016/jjpedsurg201403003

30 Gleeson FC, Papachristou GI, Riegert-Johnson DL, Boller AM, Gostout CJ. Progression to advanced neoplasia is infrequent in post colectomy familial adenomatous polyposis patients under endoscopic surveillance. *Fam Cancer* 2009; **8:** 33-8. doi: 101007/s10689-008-9203-y

31 Friederich P, de Jong AE, Mathus-Vliegen LM, Dekker E, Krieken HH, Dees J, et al. Risk of developing adenomas and carcinomas in the ileal pouch in patients with familial adenomatous polyposis. *Clin Gastroenterol Hepatol* 2008; **6:** 1237-42. doi: 101016/jcgh200806011

32 Banasiewicz T, Marciniak R, Kaczmarek E, Krokowicz P, Paszkowski J, Lozynska-Nelke A, et al. The prognosis of clinical course and the analysis of the frequency of the inflammation and dysplasia in the intestinal J-Pouch at the patients after restorative proctocolectomy due to FAP. *Int J Colorectal Dis* 2011; **26:** 1197-203. doi: 101007/s00384-011-1241-5

33 Thompson-Fawcett MW, Marcus VA, Redston M, Cohen Z, Mcleod RS. Adenomatous polyps develop commonly in the ileal pouch of patients with familial adenomatous polyposis. *Dis Colon Rectum* 2001; **44:** 347-53. doi: 10.1007/BF02234731

34 Groves CJ, Beveridge lG, Swain DJ, Saunders BP, Talbot IC, Nicholls RJ, et al. Prevalence and morphology of pouch and ileal adenomas in familial adenomatous polyposis. *Dis Colon Rectum* 2005; **48:** 816-23. doi: 101007/s10350-004-0835-1

35 Pommaret E, Vienne A, Lefevre JH, Sogni P, Florent C, Desaint B, et al. Prevalence and risk factors for adenomas in the ileal pouch and the afferent loop after restorative proctocolectomy for patients with familial adenomatous polyposis. *Surg Endosc* 2013; **27:** 3816-22. doi: 101007/s00464-013-2980-x

36 Ooi BS, Remzi FH, Gramlich T, Church JM, Preen M, Fazio VW. Anal transitional zone cancer after restorative proctocolectomy and ileoanal anastomosis in familial adenomatous polyposis: report of two cases. *Dis Colon Rectum* 2003; **46:** 1418-23. doi: 101097/01DCR0000089057202888C9

37 Ozdemir Y, Kalady MF, Aytac E, Kiran RP, Erem HH, Church JM, et al. Anal transitional zone neoplasia in patients with familial adenomatous polyposis after restorative proctocolectomy and IPAA: incidence, management, and oncologic and functional outcomes. *Dis Colon Rectum* 2013; **56:** 808-14. doi: 101097/DCR0b013e31829005db

38 Church J, Burke C, McGannon E, Pastean O, Clark B. Risk of rectal cancer in patients after colectomy and ileorectal anastomosis for familial adenomatous polyposis: a function of available surgical options. *Dis Colon Rectum* 2003; **46:** 1175-81. doi: 101097/01DCR00000843621248848

39 Saurin JC, Napoleon B, Gay G, Ponchon T, Arpurt JP, Boustiere C, et al. Endoscopic management of patients with familial adenomatous polyposis (FAP) following a colectomy. *Endoscopy* 2005; **37:** 499-501. doi: 101055/s-2005-861295

40 International Society for Gastrointestinal Hereditary Tumours, InSiGHT. [cited 2019 Mar 28]. Available at: https://wwwinsight-group.org/

41 Lynch PM, Morris JS, Wen S, Advani SM, Ross W, Chang GJ, et al. A proposed staging system and stage-specific interventions for familial adenomatous polyposis. *Gastrointest Endosc* 2016; **84:** 115-25e4. doi: 10.1016/j.gie.2015.12.029

42 Patel NJ, Ponugoti PL, Rex DK. Cold snare polypectomy effectively reduces polyp burden in familial adenomatous polyposis. *Endosc Int Open* 2016; **4:** E472-4. doi: 101055/s-0042-104114

43 Sansone S, Nakajima T, Saito Y. Endoscopic submucosal dissection of a large neoplastic lesion at the ileorectal anastomosis in a familial adenomatous polyposis patient. *Dig Endosc* 2017; **29:** 390-1. doi: 101111/den12834

44 Ishii N, Akiyama H, Suzuki K, FujitaY. Endoscopic submucosal dissection for the complete resection of the rectal remnant mucosa in a patient with familial adenomatous polyposis. *ACG Case Rep J* 2016; **3:** 172-4. doi: 1014309/crj201640

45 Groves CJ, Beveridge IG, Swain DJ, Saunders BP, Talbot IC, Nicholls RJ, et al. Prevalence and morphology of pouch and ileal adenomas in familial adenomatous polyposis. *Dis Colon Rectum* 2005; **48:** 816-23. [internet]. doi: 10.1007/s10350-004-0835-1. [cited 2019 Jan 24]. Available at: https://pubmed.ncbi.nlm.nih.gov/15747076/

46 Kamiński MF, Hassan C, Bisschops R, Pohl J, Pellisé M, Dekker E, et al. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) guideline. *Endoscopy* 2014; **46:** 435-49. doi: 101055/s-0034-1365348

47 Parks AG, Nicholls RJ. Proctocolectomy without ileostomy for ulcerative colitis. *Br Med J* 1978; **2:** 85-8. [internet]. doi: 10.1136/bmj.2.6130.85. [cited 2019 Jan 24]. Available at: https://pubmed.ncbi.nlm.nih.gov/667572/

48 Remzi FH, Church JM, Bast J, Lavery IC, Strong SA, Hull TL, et al. Mucosectomy vs stapled ileal pouch-anal anastomosis in patients with familial adenomatous polyposis: Functional outcome and neoplasia control. *Dis Colon Rectum* 2001; **44:** 1590-6. doi: 10.1007/BF02234377.

49 Kartheuser A, Stangherlin P, Brandt D, Remue C, Sempoux C. Restorative proctocolectomy and ileal pouch-anal anastomosis for familial adenomatous polyposis revisited. *Fam Cancer* 2006; **5:** 241-60. Discussion 261-2. doi: 101007/s10689-005-5672-4

50 Ganschow P, Treiber I, Hinz U, Leowardi C, Büchler MW, Kadmon M. Residual mucosa after stapled vs handsewn ileal J-pouch-anal anastomosis in patients with familial adenomatous polyposis coli (FAP) – a critical issue. *Langenbecks Arch Surg* 2015; **400:** 213-9. doi: 101007/s00423-014-1263-x

51 Tonelli F, Ficari F, Bargellini T, Valanzano R. Ileal pouch adenomas and carcinomas after restorative proctocolectomy for familial adenomatous polyposis. *Dis Colon Rectum* 2012; **55:** 322-9. doi: 101097/DCR0b013e318241e6f2

52 Sugimoto T, Yoichi T, Suzuki K, Kawai T, Yashima Y, Sato S, et al. Endoscopic submucosal dissection to treat ileal high-grade dysplasia after ileoanal anastomosis for familial adenomatous polyposis: report of a case. *Clin J Gastroenterol* 2014; **7:** 481-3. doi: 101007/s12328-014-0533-z

53 van Leerdam ME, Roos VH, van Hooft JE, Dekker E, Jover R, Kaminski MF, et al. Endoscopic management of polyposis syndromes: European Society of Gastrointestinal Endoscopy (ESGE) guideline. *Endoscopy* 2019; **51:** 877-95. doi: 101055/a-0965-0605

54 Yang J, Gurudu SR, Koptiuch C, Agrawal D, Buxbaum JL, Abbas Fehmi SM, et al. American Society for Gastrointestinal Endoscopy Guideline on the role of endoscopy in familial adenomatous polyposis syndromes. *Gastrointest Endosc* 2020; **91:** 963-82e2. doi: 101016/jgie202001028

55 Aelvoet AS, Roos VH, Bastiaansen BAJ, Hompes R, Bemelman WA, Aalfs CM, et al. Development of ileal adenomas after ileal pouch-anal anastomosis versus end ileostomy in patients with familial adenomatous polyposis. *Gastrointest Endosc* 2023; **97:** 69-77e1. doi: 101016/jgie202208031

56 Tatsuta K, Sakata M, Iwaizumi M, Sugiyama K, Kojima T, Akai T, et al. Long-term prognostic impact of metachronous rectal cancer in patients with familial adenomatous polyposis: a single-center retrospective study. *Cancer Diagn Progn* 2023; **3:** 221-9. doi: 1021873/cdp10205

57 Anele CC, Xiang J, Martin I, Hawkins M, Man R, Clark SK, et al. Regular endoscopic surveillance and polypectomy is effective in managing rectal adenoma progression following colectomy and ileorectal anastomosis in patients with familial adenomatous polyposis. *Colorectal Dis* 2022; **24:** 277-83. doi: 101111/codi15981

58 Colletti G, Ciniselli CM, Signoroni S, Cocco IMF, Magarotto A, Ricci MT, et al. Prevalence and management of cancer of the rectal stump after total colectomy and rectal sparing in patients with familial polyposis: Results from a registry-based study. *Cancers* 2022; **14:** 298. doi: 103390/cancers14020298

59 Pasquer A, Benech N, Pioche M, Breton A, Rivory J, Vinet O, et al. Prophylactic colectomy and rectal preservation in FAP: systematic endoscopic follow-up and adenoma destruction changes natural history of polyposis. *Endosc Int Open* 2021; **9:** E1014-E22. doi: 101055/a-1467-6257

60 Ardoino I, Signoroni S, Malvicini E, Ricci MT, Biganzoli EM, Bertario L, et al. Long-term survival between total colectomy versus proctocolectomy in patients with FAP: A registry-based, observational cohort study. *Tumori* 2020; **106:** 139-48. doi: 101177/0300891619868019

61 Ganschow P, Trauth S, Hinz U, Schaible A, Büchler MW, Kadmon M. Risk factors associated with pouch adenomas in patients with familial adenomatous polyposis. *Dis Colon Rectum* 2018; **61:** 1096-101. doi: 101097/DCR0000000000001157

62 Kariv R, Rosner G, Fliss-Isakov N, Gluck N, Goldstein A, Tulchinsky H, et al. Genotype-phenotype associations of APC mutations with pouch adenoma in patients with familial adenomatous polyposis. *J Clin Gastroenterol* 2019; **53:** e54-60. doi: 101097/MCG0000000000000950

63 Goldstein AL, Kariv R, Klausner JM, Tulchinsky H. Patterns of adenoma recurrence in familial adenomatous polyposis patients after ileal pouch-anal anastomosis. *Dig Surg* 2015; **32:** 421-5. doi: 101159/000439143

64 Zahid A, Kumar S, Koorey D, Young CJ. Pouch adenomas in familial adenomatous polyposis after restorative proctocolectomy. *Int J Surg* 2015; **13:** 133-6. doi: 101016/jijsu201411048

65 Kennedy RD, Potter DD, Moir CR, El-Youssef M. The natural history of familial adenomatous polyposis syndrome: a 24 year review of a single center experience in screening, diagnosis, and outcomes. *J Pediatr Surg* 2014; **49:** 82-6. doi: 10.1016/j.jpedsurg.2013.09.033

66 Boostrom SY, Mathis KL, Pendlimari R, Cima RR, Larson DW, Dozois EJ. Risk of neoplastic change in ileal pouches in familial adenomatous polyposis. *J Gastrointest Surg* 2013; **17:** 1804-8. doi: 101007/s11605-013-2319-x

67 Wasmuth HH, Tranø G, Myrvold HE, Aabakken L, Bakka A. Adenoma formation and malignancy after restorative proctocolectomy with or without mucosectomy in patients with familial adenomatous polyposis. *Dis Colon Rectum* 2013; **56:** 288-94. doi: 101097/DCR0b013e31827c970f

68 Yan Z, Liao G, Pei H. Surgical treatment of familial adenomatous polyposis: experience from a single institution in china. *Asia Pac J Clin Oncol* 2012; **8:** e23-8. doi: 101111/j1743-7563201101488x

69 Makni A, Chebbi F, Rebai W, Ayadi S, Fekih M, Jouini M, et al. Adenocarcinoma arising in the "J" pouch after total proctocolectomy for familial polyposis coli. *Tunis Med* 2012; **90:** 80-1.

70 von Roon AC, Will OCC, Man RF, Neale KF, Phillips RKS, Nicholls RJ, et al. Mucosectomy with handsewn anastomosis reduces the risk of adenoma formation in the norectal segment after restorative proctocolectomy for familial adenomatous polyposis. *Ann Surg* 2011; **253:** 314-7. doi: 101097/SLA0b013e318f3f498

71 Ault GT, Nunoo-Mensah JW, Johnson L, Vukasin P, Kaiser A, Beart RW. Adenocarcinoma arising in the middle of ileoanal pouches: report of five cases. *Dis Colon Rectum* 2009; **52:** 538-41. doi: 101007/DCR0b013e318199effe

72 Lee SH, Ahn BK, Chang HK, Baek SU. Adenocarcinoma in ileal pouch after proctocolectomy for familial adenomatous polyposis: report of a case. *J Korean Med Sci* 2009; **24:** 985-8. doi: 103346/jkms2009245985

73 Linehan G, Cahill RA, Kalimuthu SN, O'Connell F, Redmond HP, Kirwan WO. Adenocarcinoma arising in the ileoanal pouch after restorative proctocolectomy for familial adenomatous polyposis. *Int J Colorectal Dis* 2008; **23:** 329-30. doi: 101007/s00384-007-0400-1

74 Ulaş M, Neşşar G, Bostanoğlu A, Aydoğ G, Kayaalp C, Ozoğul Y, et al. Development of two cancers in the same patient after ileorectal and ileal pouch anal anastomosis for familial adenomatous polyposis. *Med Princ Pract* 2006; **15:** 83-6. doi: 101159/000089393

75 Vrouenraets BC, Van Duijvendijk P, Bemelman WA, Offerhaus GJA, Slors JFM. Adenocarcinoma in the anal canal after ileal pouch-anal anastomosis for familial adenomatous polyposis using a double-stapled technique: report of two cases. *Dis Colon Rectum* 2004; **47:** 530-4. doi: 101007/s10350-003-0073-y

76 Cherki S, Glehen O, Moutardier V, François Y, Gilly FN, Vignal J. Pouch adenocarcinoma after restorative proctocolectomy for familial adenomatous polyposis. *Colorectal Dis* 2003; **5:** 592-4. doi: 10.1046/j.1463-1318.2003.00486.x

77 Thompson-Fawcett MW, Warren BF, Mortensen NJ. A new look at the anal transitional zone with reference to restorative proctocolectomy and the columnar cuff. *Br J Surg* 1998; **85:** 1517-21. doi: 101046/j1365-2168199800875x

78 Brown SR, Donati D, Seow-Choen F. Rectal cancer after mucosectomy for ileoanal pouch in familial adenomatous polyposis: report of a case. *Dis Colon Rectum* 2001; **44:** 1714-5. doi: 10.1007/BF02234397

79 Vuilleumier H, Halkic N, Ksontini R, Gillet M. Columnar cuff cancer after restorative proctocolectomy for familial adenomatous polyposis. *Gut* 2000; **47:** 732-4. doi: 101136/gut475732

80 Bassuini MM, Billings PJ. Carcinoma in an ileoanal pouch after restorative proctocolectomy for familial adenomatous polyposis. *Br J Surg* 1996; **83:** 506. doi: 10.1002/bjs.1800830422

review

Potentially fatal complications of new systemic anticancer therapies: pearls and pitfalls in their initial management

Milena Blaz Kovac[1,2], Bostjan Seruga[2,3]

[1] Ljubljana Community Health Centre, Ljubljana, Slovenia
[2] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[3] Division of Medical Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

Radiol Oncol 2024; 58(2): 170-178.

Received 22 February 2024
Accepted 10 March 2024

Correspondence to: Assoc. Prof. Boštjan Šeruga, M.D., Ph.D., Division of Medical Oncology, Institute of Oncology Ljubljana, Zaloška cesta 2, SI-1000 Ljubljana, Slovenia. E-mail: bseruga@onko-i.si

Disclosure: No potential conflicts of interest were disclosed.

Background. Various types of immunotherapy (i.e. immune checkpoint inhibitors [ICIs], chimeric antigen receptor [CAR] T-cells and bispecific T-cell engagers [BiTEs]) and antibody drug conjugates (ADCs) have been used increasingly to treat solid cancers, lymphomas and leukaemias. Patients with serious complications of these therapies can be presented to physicians of different specialties. In this narrative review we discuss potentially fatal complications of new systemic anticancer therapies and some practical considerations for their diagnosis and initial treatment.

Results. Clinical presentation of toxicities of new anticancer therapies may be unpredictable and nonspecific. They can mimic other more common medical conditions such as infection or stroke. If not recognized and properly treated these toxicities can progress rapidly into life-threatening conditions. ICIs can cause immune-related inflammatory disorders of various organ systems (e.g. pneumonitis or colitis), and a cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) may develop after treatment with CAR T-cells or BiTEs. The cornerstones of management of these hyper-inflammatory disorders are supportive care and systemic immunosuppressive therapy. The latter should start as soon as symptoms are mild-moderate. Similarly, some severe toxicities of ADCs also require immunosuppressive therapy. A multidisciplinary team including an oncologist/haematologist and a corresponding organ-site specialist (e.g. gastroenterologist in the case of colitis) should be involved in the diagnosis and treatment of these toxicities.

Conclusions. Health professionals should be aware of potential serious complications of new systemic anticancer therapies. Early diagnosis and treatment with adequate supportive care and immunosuppressive therapy are crucial for the optimal outcome of patients with these complications.

Key words: potentially fatal toxicity; immune checkpoint inhibitor; chimeric antigen receptor T-cells; Bispecific T-cell engager; antibody dug conjugate; immunosuppressive therapy

Introduction

The outcome of patients with cancer has improved substantially over the last few decades. Most modern cancer care is delivered in the outpatient setting. Patients with cancer can develop various oncologic emergencies which can be cancer or treatment related. Patients undergoing anticancer treatment who develop acute illnesses often seek medical attention with general practitioners (GPs) and in emergency departments.[1,2] Prompt identification of oncologic emergencies, timely intervention and coordinated follow-up with oncology care teams are crucial for optimal outcome.[3] However, there may be a lack of knowledge about management of toxic complications of new anticancer

TABLE 1. Approved immune checkpoint inhibitors in the European Union

Immune checkpoint inhibitor	Target	Approved indications	
		Early cancer	Advanced cancer
Atezolizumab (Tecentriq)	PD-L1	NSCLC	Urothelial carcinoma, NSCLC, SCLC, TNBC, HCC
Avelumab (Bavencio)	PD-L1	–	Urothelial carcinoma, RCC, Merkel cell carcinoma
Cemiplimab (Libtayo)	PD-1	–	Cutaneous SCC, Basal cell carcinoma, NSCLC, Cervical carcinoma
Durvalumab (Imfinzi)	PD-L1	–	NSCLC, SCLC, HCC, Biliary tract cancer
Ipilimumab (Yervoy)	CTLA-4	–	Melanoma, RCC, NSCLC, MPM, CRC, Oesophageal carcinoma
Nivolumab (Opdivo)	PD-1	Urothelial carcinoma, melanoma, NSCLC, oesophageal and GEJ cancer	Melanoma, NSCLC, RCC, cHL, Head and neck SCC, MPM, Urothelial carcinoma, CRC, Oesophageal SCC, Gastric, GEJ or Oesophageal adenocarcinoma
Pembrolizumab (Keytruda)	PD-1	RCC, melanoma, NSCLC, TNBC	RCC, Melanoma, NSCLC, HL, Urothelial carcinoma, Head and neck SCC, Cancers with MSI-H or MMRd, Oesophageal carcinoma, Endometrial carcinoma, Cervical carcinoma, Gastric and GEJ adenocarcinoma

CRC = colorectal cancer; cHL = classical Hodgkin lymphoma; CTLA-4 = cytotoxic T-lymphocyte antigen; GEJ = gastro-oesophageal junction; HCC = hepatocellular carcinoma; HL = Hodgkin lymphoma; MMRd = mismatch repair deficiency; MPM = malignant pleural mesothelioma; NSCLC = non-small cell lung cancer; MSI-H: microsatellite instability – high; OSCC = oesophageal squamous cell carcinoma; PD-1 = program death 1; PD-L1 = program death ligand; RCC = renal cell carcinoma, SCC = squamous cell carcinoma; SCLS = small cell lung cancer; TNBC = triple-negative breast cancer

therapies such as immunotherapy among non-oncologist health providers, including emergency physicians (EPs).[4]

The most common and well-known classic oncologic emergencies are: (i) metabolic (e.g. tumour lysis syndrome [TLS], hypercalcemia, syndrome of inappropriate antidiuretic hormone), (ii) hematologic (e.g. febrile neutropenia, hyperviscosity syndrome), (iii) structural (e.g. superior vena cava syndrome, malignant epidural spinal cord compression, malignant pericardial effusion, or (iv) treatment related (e.g. chemotherapy-induced oral mucositis, radiation pneumonitis).[5] New systemic anticancer therapies can cause life-threatening complications which may be generally less-known than classical oncologic emergencies.

In this narrative review we discuss potentially fatal complications of new systemic anticancer therapies that differ from classic oncologic emergencies and provide some practical considerations for their diagnosis and initial management. For this purpose, a comprehensive search of the literature was performed through PubMed using the following key words: "immune checkpoint inhibitor", "CAR T-cells", "bispecific T-cell engager", "antibody drug conjugate", "toxicity" and "adverse event". Articles describing diagnosis and management of treatment-related toxicities, including recommendations of oncologic societies and working groups were included.

Immune checkpoint inhibitors

The immune checkpoint inhibitors (ICIs) are prescribed as monotherapy or in combination with chemotherapy and/or targeted anticancer agents in patients with both early and advanced solid cancers and Hodgkin's lymphoma (Table 1). These agents are monoclonal antibodies which enhance the immune response to cancer cells. They block negative regulators of T-cell activation, such as cytotoxic T lymphocyte associated antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and programmed cell death 1 ligand (PD-1L), and reinvigorate pre-existing T-cells (Figure 1). They target neoantigens presented by the major histocompatibility complex (MHC) molecules on the surface of cancer cells. Efficacy of ICIs may be restricted due to the lack of neoantigens presented on cancer cells, defects in expression of the MHC or in other components of the antigen-presenting machinery in cancer cells, development of resistant tumour subclones and lack of T-cells in the immunosuppressive microenvironment of the tumour.[6] ICIs are usually administered repeatedly every few weeks in the outpatient setting.

The ICIs have a unique toxicity profile distinct from that of chemotherapy and other targeted agents. As these agents enhance immune response they can cause immune-related adverse events (irAEs) (i.e. immune-related inflammatory disor-

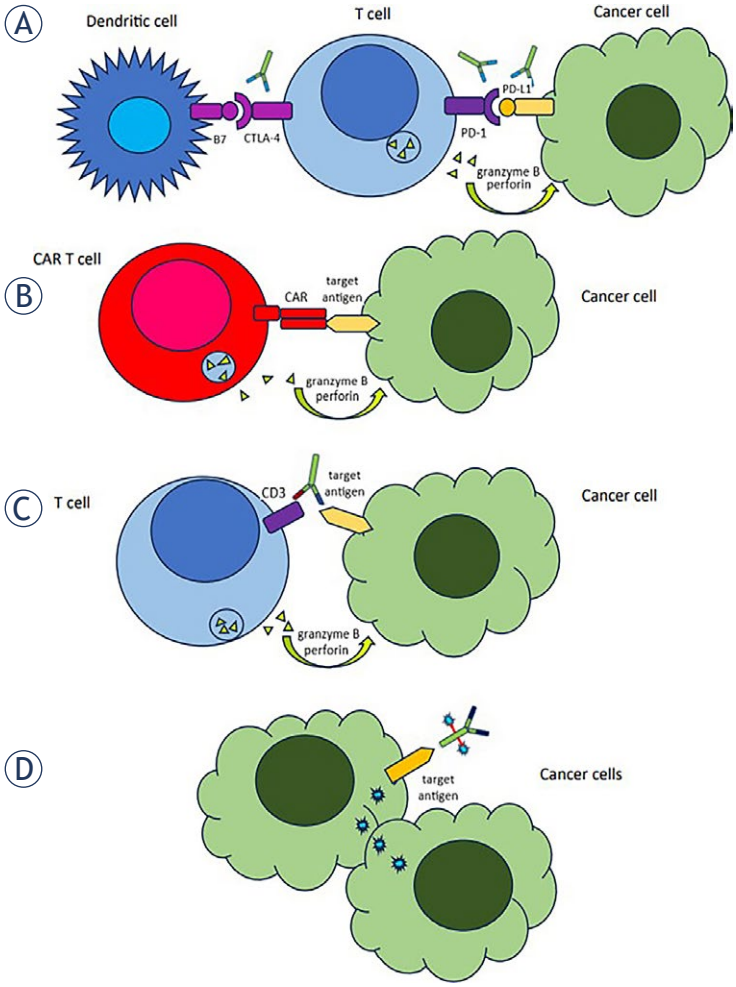


FIGURE 1. Mechanisms of action of new anticancer therapies. **(A)** Immune checkpoint inhibitor; **(B)** CAR T-cell; **(C)** Bispecific T-cell engager and **(D)** Antibody drug conjugate.

ders), which can be life-threatening. In contrast to chemotherapy and some other targeted drugs development of irAEs is more unpredictable. They can affect any organ system and can occur at any time during a patient's treatment or sometimes long after therapy with an ICI has been discontinued.[7] A majority of irAEs occur during the first four months of treatment with an ICI and they most commonly affect the skin, endocrine, gastrointestinal and pulmonary systems. Some other irAEs such as ICI-related myocarditis, hypophysitis, encephalitis and myositis are very rare but important cause of morbidity and mortality.[8,9] Early identification and treatment of irAEs is crucial to limiting their severity and duration. However, the presentation of irAEs is often non-specific and can mimic other common medical conditions such as infections, stroke, intracranial bleeding and myocardical infarction. Before treatment with an irAE is started it is very important to rule out these conditions. Importantly, mild irAEs can rapidly progress to be life-threatening conditions. Therefore, when an irAE is suspected the patient's symptoms and vital signs should be closely monitored. Detailed recommendations outlining the diagnosis, treatment and follow-up of patients with irAEs have been published by oncologic societies.[10] In general, in patients with mild or moderate symptomatic irAEs (i.e. grade ≤ 2) symptomatic treatment in the outpatient setting is recommended and early follow-up with a treating oncologist should be arranged. Exceptions are patients with symptoms suggestive of immune-related myocarditis, neurological irAEs involving the central nervous system (i.e. hypophysitis, meningitis, encephalitis and myelitis), dyspnoea or myasthenic syndromes, who should be hospitalised immediately and treated by a multidisciplinary team involving the oncologist and the corresponding organ-site specialist (Figure 2). In patients with moderate symptoms (i.e. grade 2) systemic corticosteroids (e.g. methylprednisolone 0.5–1 mg/kg/day) are recommended. All patients with severe and life-threatening irAEs (i.e. grade ≥ 3) should be immediately hospitalized and presented to the multidisciplinary team (Figure 2).[10] The cornerstones of management of severe irAEs are supportive care and immunosuppressive therapy with systemic corticosteroids (e.g. methylprednisolone 1–2 mg/kg/day), including initial high-dose pulse corticosteroids (e.g. methylprednisolone 500–1000 mg/day for three days) in some conditions. In some severe cases refractory to corticosteroids blocking of tumour necrosis factor (TNF)-α with infliximab, blocking of the interleukin-6 receptor (IL-6R) with tocilizumab, intravenous immunoglobulins (IVIGs) and mycophenolate mofetil may be beneficial.[9,10] When symptoms of the irAR are severe initiation of corticosteroids cannot be postponed and empirical antimicrobial therapy can be started concurrently with corticosteroids and discontinued when infection is excluded.[10]

Chimeric antigen receptor T-cells and bispecific T-cell engagers

CAR T-cells and BiTEs are both T-cell engaging therapies and have a similar toxicity profile. Chimeric antigen receptor (CAR) T-cells are a cell-based therapy in which patient's T-cells are extracted by leukapheresis and then genetically

TABLE 2. Approved CAR T cell therapies and bispecific T cell engagers in the European Union

Agent	Type of therapy	Target	Indications
Tisagenlecleucel (Kymriah)	CAR T	CD19	B-cell acute lymphoblastic leukaemia, Diffuse large B-cell lymphoma, Follicular lymphoma
Axicabtagene ciloleucel (Yescarta)	CAR T	CD19	Primary mediastinal large B-cell lymphoma, Diffuse large B-cell lymphoma, High grade B-cell lymphoma, Follicular lymphoma
Brexucabtagene autoleucel (Tecartus)	CAR T	CD19	Mantle-cell lymphoma, B-cell acute lymphoblastic leukaemia
Lisocabtagene maraleucel (Breyanzi)	CAR T	CD19	Follicular lymphoma grade 3B, Primary mediastinal large B-cell lymphoma, Diffuse large B-cell lymphoma
Idecabtagene vicleucel (Abecma)	CAR T	BCMA	Multiple myeloma
Ciltacabtagene autoleucel (Carvykti)	CAR T	BCMA	Multiple myeloma
Talquetamab (Talvey)	BiTE	GPRC5D/CD3	Multiple myeloma
Teclistamab (Tecvayli)	BiTE	BCMA/CD3	Multiple myeloma
Glofitamab (Columvi)	BiTE	CD20/CD3	Diffuse large B-cell lymphoma
Mosunetuzumab (Lunsumio)	BiTE	CD20/CD3	Follicular lymphoma
Tebentafusp (Kimmtrak)	BiTE	Gp100/CD3	Uveal melanoma
Teclistamab (Tecvayli)	BiTE	BCMA/CD3	Multiple myeloma

BCMA = B-cell maturation antigen; BiTE = bispecific T cell engager; CAR T = chimeric antigen receptor T-cells; CD3 = cluster of differentiation 3; CD19 = cluster of differentiation 19; CD20 = cluster of differentiation 20; GP100 = G protein 100; GPRC5D = G protein-coupled receptor, class C, group 5, member D

modified through the insertion of DNA encoding recombinant protein CAR on their surface, expanded and then administered back to the patient. Whereas the extracellular domain of the CAR recognizes a cancer-specific antigen, its intracellular domain activates the T-cell immunogenic antineoplastic response (Figure 1).[11,12] For example, tisagenlecleucel binds to the Cluster of Differentiation (CD)19 on B-cell leukaemia and lymphoma cells and activates the immune system to destroy malignant cells. CAR T-cells are now an established treatment for patients with relapsed and/or refractory B-cell lymphomas, B-cell acute lymphoblastic leukaemia and multiple myeloma (MM) (Table 2). CAR T-cells can engraft long-term and provide long-term ongoing responses against cancer cells. For most patients, CAR T-cell therapy is a one-time treatment. Before infusion of CAR T-cells patients receive lymphodepleting chemotherapy. Research into CAR T-cells is also extending to other diseases, including solid tumors, infections and autoimmune disorders. On average, patients are hospitalized for 12 days after infusion of the CAR T-cells.[13] While natural antibodies have two targeting arms that bind to the same target antigen, bispecific antibodies are engineered hybrid molecules with two distinct binding domains that target two distinct antigens. Bispecific T-cell engagers (BiTEs) bind simultaneously to a selected antigen on cancer cells and to the invariant component of the T-cell receptor complex, a CD3 chain with signalling capacity (Figure 1).[14] For example, one arm of glofitamab binds to the CD3 on T-cells and another arm to the CD20 on B-cell lymphoma cells (Table 2). BiTEs are approved for use in patients with relapsed and/or refractory B-cell lymphomas, MM and uveal melanoma (Table 2). Similar to CAR T-cells, targeting of cancer cells with BiTEs is independent of the MHC. T-cell engagement is dependent on repeated administration of the BiTEs. Premedication with systemic corticosteroids and step-up dosing reduces the risk of severe immune-related toxicities with BiTEs that are described below. It is recommended that within a step-up phase of treatment patients are hospitalized or at least remain within the proximity of a healthcare facility for a short time after the administration of a BiTE.[14,15]

The cytokine release syndrome (CRS) or cytokine storm is a result of activated T-cells, other immune cells and vascular endothelial cells,

which results in the overproduction of inflammatory cytokines. It typically manifests in the first week after therapy, rarely later.[16] While some patients experience mild, flu-like symptoms others may experience more severe and potentially life-threating complications similar to septic shock and multi-organ failure. CRS usually presents with a fever which may not respond to antipyretics, and hypotension, headache, hypoxia, rash and organ dysfunction.[17] In contrast to CRS, immune cell-associated neurologic syndrome (ICANS) is less frequent than CRS. It usually manifests around seven days after administration of therapy, rarely several weeks later, and it is often associated with preceding CRS. Patients with ICANS may present with an altered level of consciousness, aphasia, impairment of cognitive skills, motor weakness, seizures, and cerebral oedema.[17,18] The pathophysiology of ICANS is associated with the accumulation of pro-inflammatory cytokines and CAR T-cells in the central nervous system, together with the activation of resident glial cells.[19] The T-cell engaging therapies are not only associated with CRS and ICANS, but can also cause various hematologic toxicities, including haemophagocytic lymphohistiocytosis (HLH), prolonged myelosuppression, coagulopathies and tumor lysis syndrome (TLS).[20] Presentation of HLH may be similar to CRS and is characterized by a fever, cytopenia, splenomegaly, jaundice, and the pathologic finding of haemophagocytosis in bone marrow and other tissues. CRS usually precedes HLH by a few days but in rare cases HLH can develop weeks after resolution of the CRS.[21] When toxicity of T-cell engagers is suspected a treating haematologist/oncologist should be consulted and the patient should be admitted to the hospital, preferably to the cancer centre. Mild CRS and ICANS are often self-limited with proper supportive care but can rapidly progress into life-threatening conditions, which may require management in the intensive care unit (ICU). Patients with these complications require close vigilance and prompt pharmacological treatment when there is no adequate response to supportive care and/or in the case of moderate or severe symptoms (grade ≥ 2) (Table 4).[18-21] Supportive care includes antipyretics, intravenous hydration and symptomatic management of organ toxicities and constitutional symptoms in patients with CRS and intravenous hydration and aspiration precautions in patients with ICANS. When there is a combination of fever and hypotension, which does not require vasopressors (i.e. grade 2) CRS should be managed with the IL-6R antagonist tocilizumab (Figure 2). Although there is limited experience with additional therapies, alternate IL-6R antagonists such siltuximab and clazakizumab or the IL-1 receptor antagonist anakinra may be used for CRS refractory to tocilizumab.[22-23] Systemic corticosteroids should be added only in refractory, prolonged, or higher-grade CRS. Patients with moderate symptoms of ICANS and/or mild somnolence awakening to voice (i.e. grade 2) should be treated with systemic corticosteroids (e.g. dexamethasone 10 mg bid). In severe cases initial high-dose pulse corticosteroids (e.g. methylprednisolone/day 500–1000 mg of for three days) are recommended. When ICANS and CRS occur concurrently tocilizumab should be used with caution as it can lead to deterioration of ICANS (Figure 2).[22] The cornerstones of treatment of HLH are corticosteroids and an IL-6R antagonist. Both of these are contraindicated in patients with severe infection and underline the importance of arriving at a clear diagnosis prior to the initiation of treatment. In severe cases of HLH additional therapy with etoposide should

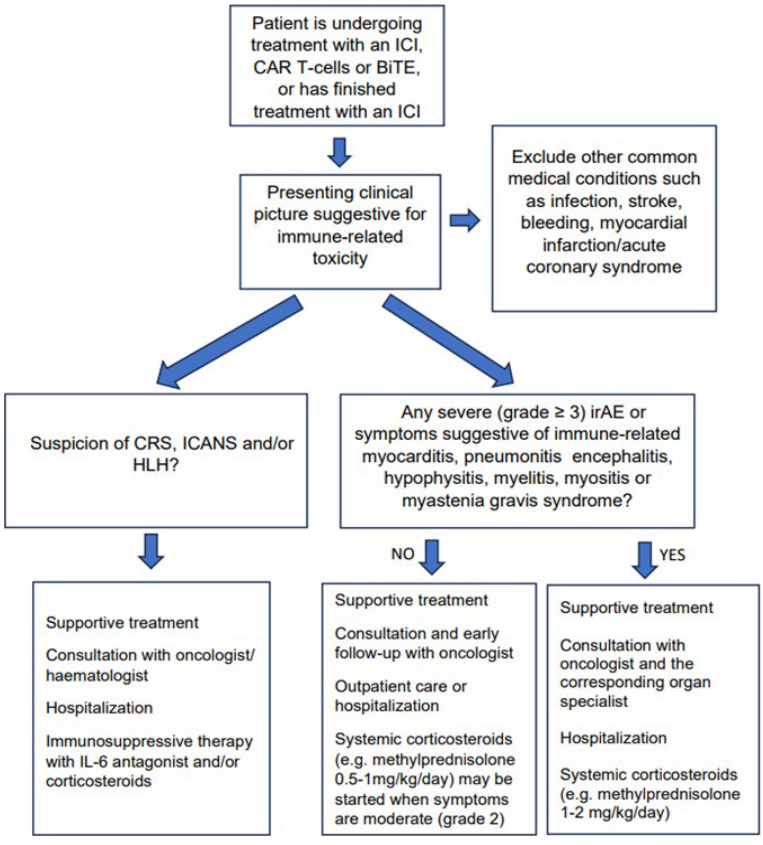


FIGURE 2. Management of toxicities of immunotherapy.

BiTE = bispecific T-cell engager; CAR = chimeric antigen receptor; CRS = cytokine release syndrome; HLH = haemophagocytic lymphohistiocytosis; ICANS = immune effector-cell associated neurotoxicity syndrome; ICI = immune checkpoint inhibitor; irAE = immune-related adverse events

TABLE 3. Grades 2 and 3 of the selected immune-related adverse events (irAEs)

irAE	Grade 2	Grade 3
Maculo-papular rash	Papules and/or pustules covering 10–30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; associated with psychosocial impact; limiting instrumental ADL; papules and/ or pustules covering > 30% BSA with or without mild symptoms	Papules and/or pustules covering > 30% BSA with moderate or severe symptoms; limiting self-care ADL; associated with local superinfection with oral antibiotics indicated
Diarrhoea/enterocolitis	Increase of 4–6 stools/day over baseline	Increase of ≥ 7 stools/day over baseline
ILD/Pneumonitis	Symptomatic (presence of new or worsening symptoms: dyspnoea, cough), medical intervention indicated, limiting instrumental ADL	Severe symptoms, oxygen indicated, limiting self-care ADL
Rheumatologic toxicity	Moderate pain, stiffness and/or weakness limiting instrumental ADL	Severe pain, stiffness and/or weakness limiting self-care ADL
Neuro-muscular toxicity	Moderate pain associated with weakness, limiting instrumental ADL	Pain associated with severe weakness, limiting self-care ADL
Hepatotoxicity	ALT or AST 3–5 x ULN	ALT > 5 x or AST < 20 x ULN
Renal toxicity	Serum creatinine >1.5–3 x above the baseline or the UNL, KDIGO stage 2: increase in serum creatinine 2–2.9 x above the baseline	Serum creatinine > 3 x above the baseline or > 3–6 x ULN, KDIGO stage 3: increase in serum creatinine > 3 x or to > 4.0 mg/dl or initiation of dialysis

ALT = alanine transaminase; ADL = activities of daily living; AST = aspartate aminotransferase; BSA = body surface area; ILD = interstitial lung disease; KDIGO = kidney disease improving global outcomes; ULN = upper limit normal

be considered.[24] Prolonged cytopenias can be treated with growth factor support and corticosteroids, and infections due to prolonged B-cell aplasia with infusion of IVIGs. Management of disseminated intravascular coagulation (DIC) is supportive, in the case of severe DIC corticosteroids and an IL-6R antagonist can be used.[22]

Antibody-drug conjugates

Antibody-drug conjugates (ADCs) have been described as 'magic bullets' of cancer treatment. The rationale behind the design of ADCs is to achieve targeted delivery of cytotoxic molecules by linking them to antibodies targeting tumour-specific antigens with the expectation of less toxicity than conventional chemotherapy (Figure 1). For example, enfortumab vedotin is a nectin-4-directed antibody and microtubule inhibitor monomethyl auristatin E conjugate. The use of antibody-drug conjugates (ADCs) is expanding rapidly, with development moving progressively from lymphomas and leukaemias to various solid cancers, and from monotherapy to combination strategies (Table 5).[25,26]

Despite their very promising design most of the currently-approved ADCs can cause severe and potentially life-threatening toxicities. Each component of the ADC, including the antibody, linker, and cytotoxic payload, may affect the extent of the ADC-induced toxicities (Table 5).[27] Apart from myelosuppression, infections and TLS other important toxicities of these agents are interstitial lung disease (ILD)/ pneumonitis, liver failure and skin toxicity (Table 5). Clinical symptoms of ILD/ pneumonitis are generally nonspecific, including dyspnoea, cough and fever and can mimic infectious pneumonia. As pneumonitis can rapidly progress to a life-threatening condition, early consultation with the oncologist and the pulmonologist is recommended. The aim of management of ADC-related pneumonitis is to suppress inflammation and prevent the build-up of irreversible lung fibrosis. Therefore, the cornerstone of treatment of symptomatic (i.e. grade ≥ 2) ILD/pneumonitis is treatment with systemic corticosteroids (e.g. methylprednisolone 1–2 mg/kg/day) (Table 3). In very severe cases initial high-dose pulse corticosteroids (e.g. methylprednisolone 500–1000 mg/ day for three days) is recommended. To prevent deterioration of ILD/ pneumonitis systemic corticosteroids may be considered even in patients with asymptomatic (i.e. grade 1) ILD/pneumonitis who have only radiologic changes in their lung. Other immunosuppressive agents are recommended in refractory cases.[28] The Steven-Johnson syndrome and toxic epidermal necrolysis are two forms of the same life-threatening skin disorder which cause rash, skin peeling, and sores of the mucous membranes. Treatment includes fluid replacement and nutrition, wound care, eye care, pain medi-

TABLE 4. Grades of the cytokine release syndrome (CRS) and the immune cell-associated neurologic syndrome ICANS

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
CRS	Fever: ≥ 38°C Hypotension: none Hypoxia: none	Fever: ≥ 38°C AND Hypotension: not requiring vasopressor AND/OR Hypoxia	Fever: ≥ 38°C AND Hypotension: requiring vasopressor AND/OR Hypoxia	Fever: ≥ 38°C AND Hypotension requiring multiple vasopressors AND/ OR Hypoxia requiring positive pressure
ICANS	ICE score: 7−9 No depressed level of consciousness	ICE score: 3−6 AND/OR Mild somnolence awaking to voice	ICE score: 0−2 AND/ OR Depressed level of consciousness awakening only to tactile stimulus AND/ OR clinical seizure focal or generalized that resolve with intervention AND/OR Focal or local oedema on neuroimaging	ICE sore: 0 AND/OR Stupor or coma AND/OR Life-threatening prolonged seizure AND/OR Diffuse cerebral oedema on neuroimaging, decerebrate or decorticate posturing or papilledema, cranial nerve VI palsy, or Cushing's triad

Immune effector cell-associated encephalopathy (ICE) assessment tool: (A) Orientation: orientation to year, month, city, and hospital: 4 points. (B) Naming: ability to name three objects: 3 points. (C) Following commands: ability to follow simple commands: 1 point. (D) Writing: ability to write a standard sentence: 1 point. (E) Attention: ability to count backward from 100 by 10: 1 point

cation, medication to reduce inflammation of the eyes and mucous membranes, antibiotics to control infection systemic high-dose corticosteroids and IVIGs. Patients with these disorders usually require admission to the ICU.[29]

Conclusions

The armamentarium of new systemic anticancer therapies is expanding rapidly. Clinical presentation of potentially fatal complications of these new

TABLE 5. Approved antibody drug conjugates in the European union and their potentially fatal toxicities

Antibody drug conjugate	Target/ cytotoxic agent	Indication	Potentially fatal complications
Belantamab mafodotin (Blenrep)	BCMA/ mcMMAF	Multiple myeloma	Pneumonitis Thrombocytopenic bleeding
Brentuximab vedotin (Adcetris)	CD30/ MMAE	Hodgkin and non-Hodgkin lymphoma	Progressive multifocal encephalopathy (reactivation of JCV) Pancreatitis ILD/Pneumonitis/ ARDS Serious infections/Opportunistic infections Severe skin reactions (SJS, TEN) Liver failure Tumor lysis syndrome
Gemtuzumab ozogamicin (Mylotarg)	CD33/ ozogamicin	AML	Liver failure (VOD/SOS) Myelosuppression Tumour lysis syndrome
Inotuzumab ozogamicin (Besponsa)	CD22/ ozogamicin	B-cell ALL	Liver failure (VOD/SOS) Myelosuppression Tumor lysis syndrome
Loncastuximab tesirine (Zynlonta)	CD19/ PBD	DLCBCL	Opportunistic infections Oedema and effusions
Polatuzumab vedotin (Polivy)	CD79b/ MMAE	DLCBCL	Neutropenic infection Opportunistic infection Progressive multifocal encephalopathy Tumor lysis syndrome
Enfortumab vedotin (Padcev)	Nectin-4/ MMAE	Advanced urothelial carcinoma	Severe skin reactions (SJS, TEN) ILD/Pneumonitis Hyperglycaemia/Diabetic ketoacidosis
Trastuzumab deruxtecan (Enhertu)	HER-2/ Dxd	Advanced breast, non-small cell lung and gastric cancer	Pneumonitis/ILD Neutropenic infection
Trastuzumab emtansine (Kadcyla)	HER-2/ Emtansine	Early and advanced breast cancer	Liver failure Haemorrhagic events ILD/Pneumonitis
Sacituzumab govitecan (Trodelvy)	Trop-2/ SN-38	Advanced breast cancer	Neutropenic infection Severe diarrhoea

ALL = acute lymphoblastic leukaemia; AML = acute myeloid leukaemia; ARDS = adult respiratory distress syndrome; BCMA = B-cell maturation antigen; CD = cluster of differentiation; DLCBCL = diffuse large cell B-cell lymphoma; Dxd = an exatecan derivative and a topoisomerase I inhibitor; HER-2 = human epidermal growth factor receptor 2; ILD = interstitial lung disease; JCV = John Cunningham virus; mcMMAF = maleimidocaproyl monomethyl auristatin F; MMAE = monomethyl auristatin E; PBD = pyrrolobenzodiazepine; SN-38 = 7-ethyl-10 hydroxycamptothecin; SJS = Steven-Johnson syndrome; TEN = toxic epidermal necrolysis; Trop-2 = trophoblast cell surface antigen 2; VOD/SOS = hepatic veno-occlusive disease/sinusoidal obstruction syndrome

therapies may be unpredictable and nonspecific and can mimic common medical conditions such as infections. Moreover, if not recognized and properly treated they can progress rapidly to life-threatening conditions. Patents with cancer who during their treatment develop acute illnesses may also present to GPs and EPs for initial workup. Therefore, it is very important that they are educated about side effects of new systemic anticancer therapies. Beside supportive care the mainstay of treatment of potentially severe toxicities of ICIs, CAR T-cells, BiTEs and sometimes of ADCs is systemic glucocorticoids and other immunosuppressive agents. In general, immunosuppressive therapy should start as soon as symptoms are mild-moderate. In patients with severe symptoms who require prompt immunosuppressive treatment concurrent empirical antimicrobial therapy may be started and continued until infectious cause of the acute illness is excluded. A multidisciplinary team involving a treating oncologist/haematologist and the corresponding organ-site specialist should be involved in the diagnosis and treatment of these treatment-related toxicities. In the changing landscape of oncology an establishment of cancer-specific urgent care centres might have an important role in the management of acutely ill patients with cancer.[30]

Acknowledgments

This work was supported by the Slovenian Research and Innovation Agency (ARIS), grant P3-0321.

References

1. Bischof JJ, Presley CJ, Caterino JM. Addressing new diagnostic and treatment challenges associated with a new age of cancer treatment. *Ann Emerg Med* 2019; **73:** 88-90. doi: 10.1016/j.annemergmed.2018.08.421
2. Mayer DK, Travers D, Wyss A, Leak A, Waller A. Why do patients with cancer visit emergency departments? Results of a 2008 population study in North Carolina. *J Clin Oncol* 2011; **29:** 2683-8. doi: 10.1200/JCO.2010.34.2816
3. Lewis MA, Hendrickson WA, Moynihan TJ. Oncologic emergencies: pathophysiology, presentation, diagnosis, and treatment. *CA Cancer J Clin* 2011; **61:** 287-314. doi: 10.3322/caac.20124
4. Alahmadi A, Altamimi H, Algarni M. Evaluation of knowledge of immunotherapy toxicities among emergency physicians in Riyadh, Saudi Arabia. *Cureus* 2022; **14:** e30325. doi: 10.7759/cureus.30325
5. Higdon ML, Higdon JA. Treatment of oncologic emergencies. *Am Fam Physician* 2006; **74:** 1873-80. PMID: 17168344
6. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12:** 252-64. doi: 10.1038/nrc3239
7. Champiat S, Lambotte O, Barreau E, Belkhir R, Berdelou A, Carbonnel F, et al. Management of immune checkpoint blockade dysimmune toxicities: a collaborative position paper. *Ann Oncol* 2016; **27:** 559-74. doi: 10.1093/annonc/mdv623
8. Palaskas N, Lopez-Mattei J, Durand JB, et al. Immune checkpoint inhibitor myocarditis: pathophysiological characteristics, diagnosis, and treatment. *J Am Heart Assoc* 2020; **9**: e013757. doi: 10.1161/JAHA.119.013757
9. Müller-Jensen L, Zierold S, Versluis JM, Boehmerle W, Huehnchen P, Matthias Endres M, et al. Characteristics of immune checkpoint inhibitor-induced encephalitis and comparison with HSV-1 and anti-LGI1 encephalitis: a retrospective multicentre cohort study. *Eur J Cancer* 2022; **175:** 224-35. doi: 10.1016/j.ejca.2022.08.009
10. Haanen J, Obeid N, Spain L, Carbonnel F, Wang Y, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2022; **33:** 1217-38. doi: 10.1016/j.annonc.2022.10.001
11. Kochenderfer JN, Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. *Nat Rev Clin Oncol* 2013; **10:** 267-76. doi: 10.1038/nrclinonc.2013.46
12. Mikkilineni L, Kochenderfer JN. CAR T cell therapies for patients with multiple myeloma. *Nat Rev Clin Oncol* 2021; **18:** 71-84. doi: 10.1038/s41571-020-0427-6
13. Kish J, Liu R, Pfeffer D, Vennam S, Lussier C, Nayak P. Real-world duration of hospitalization for CAR-T treatment: U.S. patient experience in multiple hematologic malignancies. [abstract]. Annual Meeting of the American-Society-of-Clinical-Oncology (ASCO). *J Clin Oncol* 2023; 41(Suppl S): e18896. doi: Goebeler ME, Bargou RC. T cell-engaging therapies - BiTEs and beyond. *Nat Rev Clin Oncol* 2020; **17:** 418-34. doi: 10.1038/s41571-020-0347-5
14. Ball K, Dovedi SJ, Vajjah P, Phipps A. Strategies for clinical dose optimization of T cell-engaging therapies in oncology. *mAbs* 2023; **15:** 2181016. doi: 10.1080/19420862.2023.2181016
15. Fajgenbaum DC, June CH. Cytokine storm. *N Engl J Med* 2020; **383:** 2255-73. doi: 10.1056/NEJMra2026131
16. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant* 2019; **25:** 625-38. doi: 10.1016/j.bbmt.2018.12.758
17. Hernani R, Benzaquén A, SolanC. Toxicities following CAR-T therapy for hematological malignancies. *Cancer Treat Rev* 2022: **111:** 102479. doi: 10.1016/j.ctrv.2022.102479
18. Morris EM, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. *Nat Rev Immunol* 2022; **22:** 85-96. doi: 10.1038/s41577-021-00547-6
19. Rejeski K, Subklewe M, Locke FL. Recognizing, defining, and managing CAR-T hematologic toxicities. *Hematology Am Soc Hematol Educ Program* 2023; **2023:** 198-208. doi: 10.1182/hematology.2023000472
20. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol* 2018; **15:** 47-62. doi: 10.1038/nrclinonc.2017.148.
21. Santomasso BD, Nastoupil LJ, Adkins S, Lacchetti C, Schneider BJ, Anadkat M, et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO Guideline. *J Clin Oncol* 2021; **39:** 3978-92. doi: 10.1200/JCO.21.01992
22. Strati P, Ahmed S, Kebriaei P, Nastoupil LJ, Claussen CM, Watson G, et al. Clinical efficacy of anakinra to mitigate CAR T-cell therapy-associated toxicity in large B-cell lymphoma. *Blood Adv* 2020; **4:** 3123-7. doi: 10.1182/bloodadvances.2020002328
23. Rosée PL, Horne AC, Hines M, von Bahr Greenwood T, Machowicz R, Berliner N, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood* 2019; **133:** 2465-77. doi: 10.1182/blood.2018894618
24. Drago JZ, Modi S, Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. *Nat Rev Clin Oncol* 2021; **18:** 327-44. doi: 10.1038/s41571-021-00470-8
25. Tsuchikama K, Anami Y, Ha SYY, Yamazaki CM. Exploring the next generation of antibody-drug conjugates. *Nat Rev Clin Oncol* 2024; **21:** 203-23. doi: 10.1038/s41571-023-00850-2

26. Tarantino P, Ricciuti B, Pradhan SM. Optimizing the safety of antibody-drug conjugates for patients with solid tumours. *Nat Rev Clin Oncol* 2023; **20:** 558-76. doi: 10.1038/s41571-023-00783-w

27. Rugo HS, Crossno CL, Gesthalter YB, Kelley K, Moore HB, Rimawi MF, et al. Real-world perspectives and practices for pneumonitis/interstitial lung disease associated with trastuzumab deruxtecan use in human epidermal growth factor receptor 2-expressing metastatic breast cancer. *JCO Oncol Pract* 2023; **19:** 539-46. doi: 10.1200/OP.22.00480

28. Lacouture ME, Patel AB, Rosenberg JE, Peter H O'Donnell. Management of dermatologic events associated with the nectin-4-directed antibody-drug conjugate enfortumab vedotin. *Oncologist* 2022; **27:** e223-32. doi: 10.1093/oncolo/oyac001

29. Cooksley T, Rice T. Emergency oncology: development, current position and future direction in the USA and UK. *Support Care Cancer* 2017; **25:** 3-7. doi: 10.1007/s00520-016-3470-1

review

Colitis due to cancer treatment with immune check-point inhibitors – review of literature and presentation of clinical cases

Andreja Ocepek

Department of Gastroenterology, Division of Internal Medicine, University Medical Centre Maribor, Maribor, Slovenia

Radiol Oncol 2024; 58(2): 179-185.

Received 10 Avgust 2023
Accepted 29 January 2024

Correspondence to: Andreja Ocepek, M.D., Department of Gastroenterology, Division of Internal Medicine, University Medical Centre Maribor, Ljubljanska 5, SI-2000 Maribor, Slovenia. E-mail: andreja.ocepek@ukc-mb.si

Disclosure: No potential conflicts of interest were disclosed.

Treatment with immune checkpoint inhibitors is effective in various cancers, but may be associated with immune-mediated side effects in other organs. Among the more common ones is gastrointestinal tract involvement, especially colitis. In most patients, colitis is mild or responds to corticosteroid treatment. A smaller proportion of patients, more often those treated with cytotoxic T lymphocyte antigen-4 inhibitors, may have a more severe course of colitis, even life-threatening complications. In these patients, prompt action, timely diagnosis with endoscopic evaluation and early treatment with high-dose corticosteroids and, if ineffective, rescue therapy with biologic agents such as infliximab and vedolizumab are needed. We present three cases from our clinical practice, data on incidence and clinical presentation, current recommendations regarding diagnostic approach and treatment of immune checkpoint inhibitors induced colitis.

Key words: immune checkpoint inhibitors; immune checkpoint inhibitors induced colitis; immune-mediated microscopic colitis; corticosteroid therapy; infliximab; vedolizumab

Introduction

Inhibition of immune checkpoints strengthens the body's own defences against cancer, which can be exploited to great advantage in the treatment of malignant diseases. Such drugs are antibodies against inhibitory immune regulators such as cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) and programmed death protein-1 ligand (PD-L1). Due to their involvement in the immune response, immune checkpoint inhibitors (ICPI) can trigger other immune-mediated diseases as a side effect, affecting many other organs and tissues, *e.g.* skin, lung, liver and gastrointestinal tract. The most common side effect of immunotherapy for cancer of the gastrointestinal tract is immune-mediated or ICPI-induced colitis.[1,2]

Incidence

The incidence of ICPI-induced colitis depends on three factors: the type of immunotherapy, the characteristics of the patient and the characteristics of the cancer. Colitis occurs more frequently with CTLA-4 inhibitors than with PD-1 and PD-L1 inhibitors, with combination therapy with two ICPIs, and with higher doses, although a clear dose-dependence of the occurrence and severity of colitis has not been confirmed.[3] The incidence of colitis is 3.4–22% with CTLA-4 inhibitor therapy, 0.7–12.8% with combined CTLA-4 and PD-1 inhibitor therapy, and 0.7–2.6% with PD-1/PD-L1 inhibitor monotherapy.[1,2] Another important factor is cancer itself. According to data known so far, the incidence of colitis is higher in patients with melanoma than

in patients with other cancers. For example, ipilimumab treatment causes diarrhoea in 41% of melanoma patients compared with up to 27% of lung cancer patients.[3] The stage of cancer may also influence the incidence of side-effects, although the underlying mechanism is unknown. Thus, the incidence of diarrhoea and colitis is lower in stage IV (35.3%) than in stage III (72%) of the same cancer.[1] Third factor is the patient himself. The risk of ICPI-induced colitis is higher in Caucasians and in patients with known inflammatory bowel disease (IBD), but the influence of sex, age, gut microbiota and possibly genetic predisposition (*e.g.* human leukocyte antigen (HLA) status) remains unclear.[1,3]

Clinical presentation

Symptoms and signs of ICPI-induced colitis most commonly include diarrhoea, abdominal pain, bloating, haemochesia, mucus discharge, fever and vomiting.[1,3] Diarrhoea occurs in most cases 4–8 weeks after the start of ICPI, but in some patients it may be as early as 1 week, and in others it may occur months or up to 2 years after the end of treatment.[1] The severity of colitis may range from mild, self-limiting diarrhoea to life-threatening colitis with the risk of rapid onset of complications such as ileus, toxic megacolon and intestinal perforation.[1,2] The severity of diarrhoea and colitis is defined by the Common Terminology Criteria for Adverse Events (CTCAE) in 5 grades shown in Table 1.[2,4,5] A more severe course of colitis can be expected in patients receiving CTLA-4 inhibitors (*e.g.* ipilimumab) and in patients with known IBD.[2,3]

Diagnosis

ICPI-induced colitis should be considered in all cancer patients receiving ICPI. Because of non-specific symptoms, diagnosis is based on exclusion of other conditions with a similar clinical presentation. In the differential diagnosis, we must consider infectious causes (*e.g.* clostridial diarrhoea, cytomegalovirus reactivation, *etc.*), drug-induced colitis (*e.g.* non-steroidal antirheumatic drugs, chemotherapeutic drugs, mycophenolate mofetil...), microscopic colitis, IBD flare, diverticulitis, ischaemic colitis, graft-versus-host disease, ICPI-induced pancreatitis, exocrine pancreatic insufficiency, ICPI-induced coeliac disease, thyroid dysfunction[1,4,6,7]. The recommended set of diagnostic tests and procedures is shown in Table 2.[1-3,8] In case of severe abdominal pain or suspected complications, imaging is required, most commonly with computed tomography (CT) and/or magnetic resonance imaging (MRI). Both CT and MRI are insufficiently sensitive and specific for the diagnosis of ICPI-induced colitis.[2-4]

Endoscopic and histopathological features

Endoscopic findings are non-specific. Up to 37% of patients with ICPI-induced colitis have normal endoscopic findings, but up to 90% of patients with grade 1 colitis have microscopic changes, so even macroscopically normal mucosa needs to be biopsied for histological analysis. Endoscopically, mucosal oedema, erythema, erosions, loss of vascular permeation, superficial or deep ulcers may be found.[2] Lesions may be present diffusely, segmentally or irregularly along the bowel. Single atypical cases of colonic pseudolipomatosis and collagenous colitis after atezolizumab treatment have also been described.[9,10] The Mayo endoscopic scoring system, which is routinely used to describe ulcerative colitis, can be used to describe endoscopic changes (Table 3).[4,11]

Clinical studies have shown the importance of endoscopic diagnosis in patients with CTCAE grade > 1 in predicting the need for biologic therapy, as patients with a more severe endoscopic picture according to Mayo endoscopic score of grade 3 were statistically significantly more likely to require treatment with infliximab.[12,13]

Histo-pathological features vary from acute colitis with intraepithelial neutrophilic infiltration, cryptitis and crypt abscesses, to chronic colitis with basal lymphocytic infiltration, Paneth cell metaplasia and disrupted crypt architecture, or both.[3,10] Histo-pathological assessment of the severity of inflammation using the Nancy score has also been shown to be a good predictor of the need for biologic therapy, with 50% of patients with Nancy grade 3 and 4 requiring salvage therapy with infliximab compared with 20% of patients with Nancy grade 1 and 2.[1]

Immune-mediated microscopic colitis

Immune-mediated microscopic colitis, recognised as a separate disease, presents with chronic wa-

TABLE 1. CTCAE gastrointestinal toxicity levels of ICPI[2,4]

Grade	Diarrhoea	Colitis
1	Increase in frequency of bowel movements < 4x above baseline; mild increase in ejection via stoma	Asymptomatic; clinical or diagnostic observation only; no action required
2	Increase in frequency of bowel movements 4−6x above baseline; moderate increase in ejection via stoma	Abdominal pain; mucus or blood in stool
3	Increase in frequency of bowel movements ≥ 7x above baseline, incontinence, need for hospitalisation, impaired daily self-care; marked increase in ejection via stoma	Severe or persistent abdominal pain; change in bowel movements; fever, ileus, peritoneal irritation; medical intervention required
4	Life-threatening consequences; need for urgent action	Life-threatening consequences; need for urgent action
5	Death	Death

CTCAE = Common Terminology Criteria for Adverse Events; ICPI = immune checkpoint inhibitors

tery diarrhoea. It usually occurs after treatment with anti-PD-1 or anti-CTLA-4. On colonoscopy, the intestinal mucosa is normal or mildly altered with oedema and/or erythema. Histopathological examination is the key to the diagnosis, and therefore random biopsies of apparently normal mucosa should always be performed. There are two forms of microscopic colitis. Lymphocytic colitis is characterised by intraepithelial lymphocytosis and infiltration of the lamina propria. In collagenous colitis, however, thickening of the collagenous subepithelial layer is visible on histopathology.[16]

Treatment

Treatment depends on the stage of CTCAE. In grade 1, treatment is supportive and symptomatic. Oral hydration, a soft, non-irritating diet without lactose and caffeine, and antidiarrhoic drugs (*e.g.* loperamide) are advised after exclusion of infection.[15,16] Treatment with mesalazine may be attempted.[3,17] Discontinuation of ICPI is not necessary. If symptoms do not resolve within 7–10 days or the clinical picture worsens to grade ≥ 2, a consultation with a gastroenterologist and an endoscopic examination is recommended as a starting point for further treatment.[2,4,8] In the absence of blood in the stool and/or normal endoscopic findings (Mayo grade 0), especially in confirmed microscopic colitis treatment with the topically acting corticosteroid, budesonide (extended-release tablets) at a dose of 9 mg daily may be attempted.[4,8,16] Duration of treatment depends on whether ICPI is discontinued or not. If ICPI is discontinued, budesonide treatment lasts for 8 weeks, but if ICPI is continued, budesonide treatment can be maintained continuously. In the event of a discontinuation of budesonide, a gradual dose reduction from 9 mg to 6 mg for 14 days and then 3 mg for 14 days is advised, with careful monitoring of symptoms and, if necessary, extending time intervals.[4,16] Mayo grade 1–2 colitis is treated primarily with a systemic corticosteroid. Patients without systemic signs of food-borne inflammation are prescribed

TABLE 2. Set of diagnostic tests[1-3]

Stool tests
Stool culture for pathogenic bacteria (*e.g.* Yersinia) and viruses
Clostridium difficile toxin
Parasites and parasite eggs
Pancreatic elastase
Inflammatory markers (calprotectin, lactoferrin)
PCR for CMV
Blood tests
Complete blood count
Biochemical tests (liver tests, renal function tests, amylase, lipase, albumin,...)
TSH
Inflammatory markers (CRP, ESR)
Serology for celiac disease (total IgA, tTG)
HIV serology
Imaging
CT scan of the abdomen (for severe abdominal pain)
Endoscopy of the lower GI tract (in all or at least patients with CTCAE grade ≥ 2)
Investigations before immunosuppressive/biological treatment
Serology for HAV, HBV and HCV, HIV
Quantiferon test
Chest X-ray

CMV = cytomegalovirus; CRP = C-reactive protein; CT = computed tomography; CTCAE = Common Terminology Criteria for Adverse Events; ESR = erythrocyte sedimentation rate; GI = gastrointestinal; HAV = hepatitis A virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IgA = immunoglobulin A; PCR = polymerase chain reaction; TSH = thyroid-stimulating hormone; tTG = tissue transglutaminase

TABLE 3. Mayo endoscopic score[14]

	Mayo score 0	Mayo score 1	Mayo score 2	Mayo score 3
Disease activity	Inactive disease	Mild activity	Medium activity	High activity
Features	normal mucosa	erythema, decreased vascular pattern, mild friability	marked erythema, absent vascular pattern, friability, erosions	spontaneous bleeding, ulceration

the equivalent of prednisone 0.5–1 mg/kg/day, the dose of which is tapered (by 10 mg every 5–7 days) until discontinuation after symptoms subside. In case of systemic involvement, hospitalisation is usually necessary and intravenous methylprednisolone 0,5–1 mg/kg/day is recommended in a divided dose every 12 hours. With long-term corticosteroid treatment, prophylaxis of pneumocystis infection, vitamin D and calcium replacement and blood sugar control are recommended.[4,16] The most severe endoscopic Mayo grade 3 requires treatment with higher doses of methylprednisolone 1–2 mg/kg/day intravenously and early consideration of salvage biologic therapy. In approximately 2/3 of patients, corticosteroid therapy is sufficient and is gradually weaned over 4–8 weeks.[1,8,16] However, if the colitis does not resolve with corticosteroid treatment within 2–5 days, treatment with one of the biologic agents, TNF-a inhibitor infliximab or integrin a4b7 inhibitor vedolizumab, is indicated.[2,6] The recommended dose of infliximab is 5–10 mg/kg infused at weeks 0, 2 and 6, and vedolizumab dose is 300 mg infused following the same regime.[1,3,4] Treatment can be continued with a maintenance dose every 8 weeks if needed, depending on endoscopic reassessment after 8–10 weeks and decision to continue treatment with ICPI.[2] In all patients with colitis grade ≥ 2, at least temporary discontinuation of ICPI and consideration of permanent discontinuation of CTLA-4 inhibitor or substitution with PD-1/PD-L1 inhibitor is necessary.[3] In rare cases, all treatments described so far are ineffective and refractory colitis is present. In such cases, infectious causes should be excluded again, and alternative treatments such as other immunosuppressive drugs (*e.g.* mycophenolate mofetil, tacrolimus, cyclosporine), faecal transplantation or extracorporeal photopheresis are possible.[1,3,4] Cases of treatment of refractory colitis with ustekinumab, tofacitinib and abatacept have also been described, but caution is advised due to the involvement of these drugs in anti-tumour response.[2]

Prognosis

Overall mortality from ICPI-induced colitis is 5%. A more severe disease course should be considered in patients treated with a CTLA-4 inhibitor or with dual ICPI therapy, as the disease can be progressive and rapidly leads to life-threatening complications within 14 days.[1]

In most cases, recurrence of ICPI-induced colitis after treatment with corticosteroids or biologics does not occur if ICPI treatment is discontinued. The decision to restart ICPI treatment depends on the nature of cancer, severity and likelihood of recurrence of colitis, and availability of alternative treatments. After mild grade 1 colitis has subsided, continuation with the same ICPI is advised. Likelihood of recurrence of colitis is higher with CTLA-4 inhibitor therapy or dual (CTLA-4 inhibitor + PD-1/PD-L1 inhibitor) therapy, and therefore CTLA-4 inhibitor therapy is advised against after severe colitis has subsided. In this case, a switch to a PD-1/PD-L1 inhibitor in monotherapy is possible, and concomitant maintenance therapy with a biologic may also be considered. At present, evidence for long-term safety of such combination therapy is limited.[2,4]

Clinical cases

Case 1

A 71-year-old man with metastatic non-small cell K-ras positive lung cancer was treated with the PD-1 inhibitor pembrolizumab as a second-line oncologic therapy. After the 3rd dose of immunotherapy, diarrhoea occurred, grade 2. CRP was 5 mg/L or less, faecal calprotectin was not determined. After excluding an infectious cause, he was treated with loperamide, followed by oral mesalazine and methylprednisolone at a dose of 0.5 mg/kg/day orally. Colonoscopy showed mild hyperaemia and oedema of the sigmoid mucosa, endoscopic Mayo

score 1 (Figure 1), with normal mucosa in the rest of the colon.

Random biopsies of endoscopically normal mucosa and biopsies of sigmoid mucosa were histopathologically defined as moderate-to-intense aetiologically undefined chronic colitis with predominant plasma-cell mixed-cell inflammatory infiltrate. Following the decision of the multidisciplinary team, the patient received infliximab 5 mg/kg at standard time intervals on weeks 0 and 2, which resulted in normalisation of bowel movements. Due to concomitant progression of malignant disease, treatment was not continued. The patient was considered for third line systemic oncological therapy, but his general health deteriorated and he died 6 months later due to septic pneumonia.

Case 2

A 75-year-old man with primary metastatic lung adenocarcinoma received the PD-1 inhibitor pembrolizumab as first-line systemic oncologic treatment. After 2 applications of pembrolizumab, he developed frequent discharges of small amounts of mucus. Immunotherapy was stopped, stool cultures were performed and were negative. Despite supportive symptomatic treatment and loperamide, the condition worsened and due to diarrhoea grade 3, systemic corticosteroid methylprednisolone was started at a dose of 1 mg/kg/day orally. After normalisation of bowel movements, the dose of methylprednisolone was tapered and discontinued after 6 weeks. 5 days after discontinuation, mucous discharge recurred up to 5 times daily and abdominal pain appeared, with a CRP of 157 mg/L, mild leucocytosis ($10.27x10^9$), and normal procalcitonin levels (0.11 mg/L). Emergency imaging (plain abdominal X-ray, chest X-ray, and abdominal CT) excluded life-threatening complications, and signs of pancolitis were described. The patient was admitted to hospital where, after serological investigations (viral hepatitis, HIV and Quantiferon test), he received methylprednisolone at a dose of 2 mg/kg/day intravenously. Endoscopic examination showed diffuse mucosal inflammation with ulcerations, endoscopic Mayo score 3 (Figure 2).

Histology confirmed marked active colitis with ulceration, architectural changes of the crypts, with extensive areas of acute cryptitis and crypt abscesses. Decision of multidisciplinary team was to treat the patient with infliximab at a dose of 5 mg/kg at standard time intervals (weeks 0, 2 and 6). After only one dose of infliximab, CRP dropped

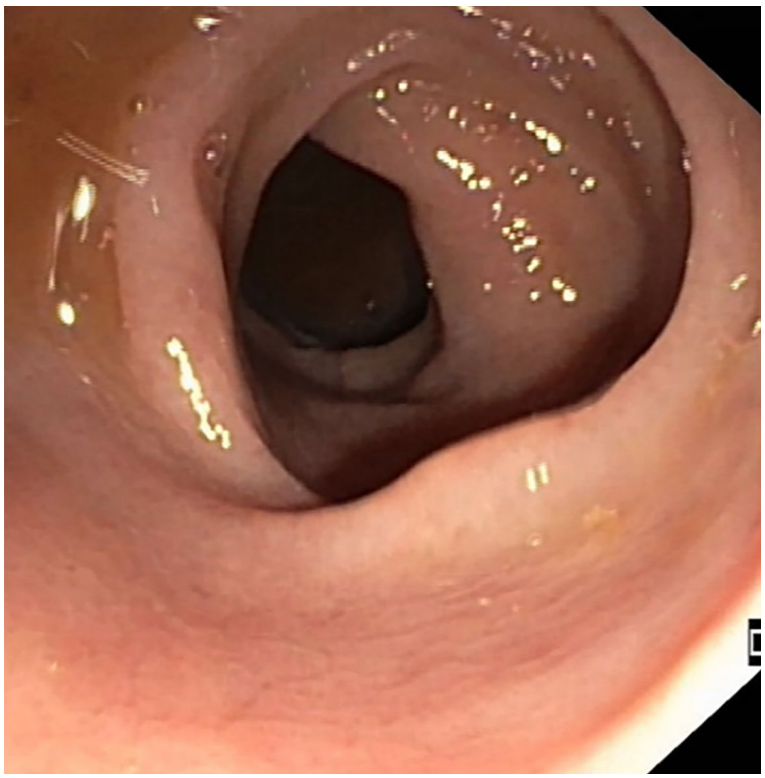


FIGURE 1. Endoscopic image of sigmoid colon in case 1.

to 16 mg/L and after the 2nd dose to normal levels, the bowel movements normalised. After the 3rd dose of infliximab, the treatment was stopped and the patient is being further managed by the treating oncologist.

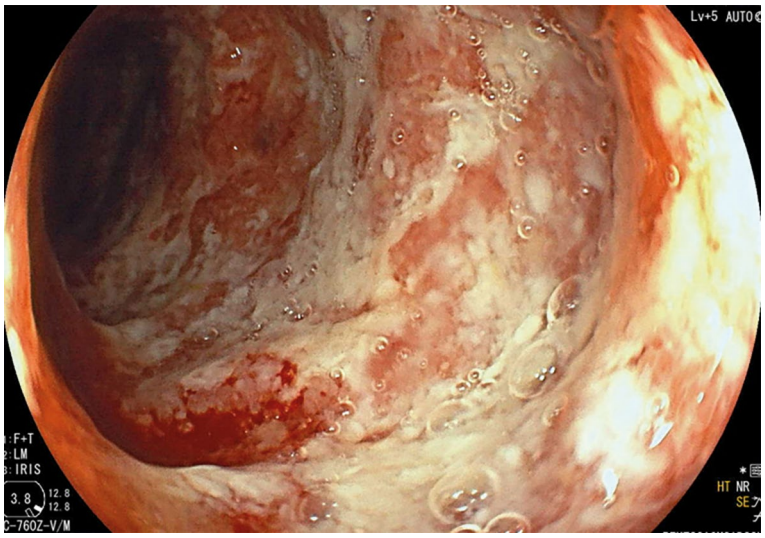


FIGURE 2. Endoscopic finding in case 2.

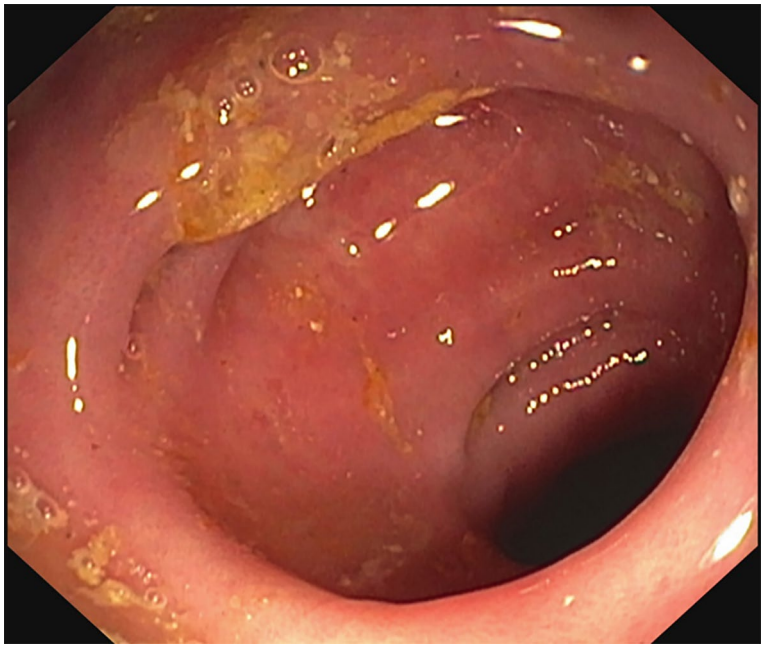


FIGURE 3. Endoscopic finding in case 3.

Case 3

A 72-year-old man received the PD-1 inhibitor nivolumab at 14-day intervals as second-line systemic oncologic treatment for metastatic renal cell carcinoma. After the first month of treatment, diarrhoea, initially grade 2, started. Due to the escalation of diarrhoea to grade 3 despite immunotherapy withdrawal and supportive treatment (dietary measures, oral rehydration, loperamide), methylprednisolone was introduced at a dose of 1 mg/kg/day, to which he responded well. The dose of methylprednisolone was gradually tapered and discontinued. 10 days after discontinuation, diarrhoea recurred and he was restarted on corticosteroid therapy and referred for colonoscopy. Endoscopy showed erythema of the sigmoid mucosa and hyperaemia of the rectal mucosa, endoscopic Mayo score 1 (Figure 3).

Histology showed infiltrates of plasma cells in the lamina propria, few lymphocytes, eosinophilic and neutrophilic granulocytes with minimal signs of cryptitis, without crypt micro-abscesses. Although histological picture was non-specific, the pathologist considered it to be consistent with ICPI-induced colitis. Following the decision of the multidisciplinary team, the patient was treated with vedolizumab at a standard dose of 300 mg intravenously at time intervals week 0, 2, 6 and 14. CT of the chest and abdomen showed stagnation of malignant disease and no signs of colitis complications. The patient continues follow-up by the treating oncologist.

Conclusions

Immunotherapy represents a breakthrough in treatment of various cancers, but can trigger immune-mediated side effects, of which ICPI-induced colitis is most common. In most cases, the course of colitis is mild or may be effectively treated with corticosteroid therapy. Small proportion of patients require treatment with biologic agents, and rarely, more severe form of colitis may trigger life-threatening complications. In these cases, cooperation between oncologist and gastroenterologist is vital to establish a rapid diagnosis with endoscopic evaluation and timely escalation of treatment.

References

1. Li H, Fu ZY, Arslan ME, Lee H, Cho D. Differential diagnosis and management of immune checkpoint inhibitor-induced colitis: a comprehensive review. *World J Exp Med* 2021; **11:** 79-92. doi: 10.5493/wjem.v11.i6.79
2. Hashash JG, Francis FF, Farraye FA. Diagnosis and management of immune checkpoint inhibitor colitis. *Gastroenterol Hepatol* 2021; **17:** 358-66. PMID: 34602898
3. Gong Z, Wang Y. Immune checkpoint inhibitor-mediated diarrhea and colitis: a clinical review. *JCO Oncol Pract* 2020; **16:** 453-61. doi: 10.1200/OP.20.00002
4. Kelly-Goss MR, Badran YR, Dougan M. Update on immune checkpoint inhibitor enterocolitis. *Curr Gastroenterol Rep* 2022; **24:** 171-81. doi: 10.1007/s11894-022-00852-7
5. SERVICES USDOHAH, Health NIO, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). Version 5.0. *J Chem Soc Dalt Trans* 2017. (cited 2024 Jul 15). Available at: https://www.meddra.org/
6. Ohwada S, Ishigami K, Yokoyama, Yoshihiro Kazama T, Masaki Y, Takahashi M, et al. Immune-related colitis and pancreatitis treated with infliximab. *Case Reports Clin J Gastroenterol* 2023; **16:** 73-80. doi: 10.1007/s12328-022-01731-4
7. Ofuji K, Hiramatsu K, Nosaka T, Naito T, Takahashi K, Matsuda H, et al. Pembrolizumab-induced autoimmune side effects of colon and pancreas in a patient with lung cancer. *Clin J Gastroenterol* 2021; **14:** 1692-9. doi: 10.1007/s12328-021-01499-z
8. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2018; **36:** 1714-68. doi: 10.1200/JCO.2017.77.6385
9. Naito T, Nosaka T, Takahashi K, Ofuji K, Matsuda H, Ohtani M, et al. A case of immune checkpoint inhibitor-related colitis with a distinctive endoscopic finding of colonic pseudolipomatosis. *Clin J Gastroenterol* 2021; **14:** 1431-6. doi: 10.1007/s12328-021-01459-7
10. Gallo A, Talerico R, Novello L, Giustiniani MC, D'Argento E, Bria E, et al. Collagenous colitis and atezolizumab therapy: an atypical case. *Clin J Gastroenterol* 2021; **14:** 165-9. doi: 10.1007/s12328-020-01276-4
11. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353:** 2462-76. doi: 10.1056/NEJMoa050516

12. Mooradian MJ, Wang DY, Coromilas A, Lumish M, Chen T, Giobbie-Hurder A, et al. Mucosal inflammation predicts response to systemic steroids in immune checkpoint inhibitor colitis. *J Immunother Cancer* 2020; **8:** 1-10. doi: 10.1136/jitc-2019-000451

13. Cheung VTF, Gupta T, Olsson-Brown A, Subramanian S, Sasson SC, Heseltine J, et al. Immune checkpoint inhibitor-related colitis assessment and prognosis: can IBD scoring point the way? *Br J Cancer* 2020; **123:** 207-15. doi: 10.1038/s41416-020-0882-y

14. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987; **317:** 1625-9. doi: 10.1056/NEJM198712243172603

15. Puzanov I, Diab A, Abdallah K, Bingham CO, Brogdon C, Dadu R, et al. Managing toxicities associated with immune checkpoint inhibitors: Consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* 2017; **5:** 1-28. doi: 10.1186/s40425-017-0300-z

16. Haanen J, Obeid M, Spain L, Carbonnel F, Wang Y, Robert C, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2022; **33:** 1217-38. doi: 10.1016/j.annonc.2022.10.001

17. Yamauchi R, Araki T, Mitsuyama K, Tokito T, Ishii H, Yoshioka S, et al. The characteristics of nivolumab-induced colitis: An evaluation of three cases and a literature review. *BMC Gastroenterol* 2018; **18:** 1-5. doi: 10.1186/s12876-018-0864-1

review

Pathogenesis and potential reversibility of intestinal metaplasia – a milestone in gastric carcinogenesis

Jan Drnovsek[1,2], Matjaz Homan[2,4], Nina Zidar[2,3], Lojze M Smid[1,2]

[1] Department of Gastroenterology, University Medical Centre Ljubljana, Ljubljana, Slovenia
[2] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[3] Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[4] Department of Gastroenterology, Hepatology and Nutrition, University Children's Hospital, Ljubljana, Slovenia

Radiol Oncol 2024; 58(2): 186-195.

Received 03 November 2023
Accepted 19 March 2024

Correspondence to: Lojze M Šmid, M.D., Ph.D., Department of Gastroenterology, University Medical Centre Ljubljana, SI-1000 Ljubljana, Slovenia. E-mail: alojz.smid@kclj.si

Disclosure: No potential conflicts of interest were disclosed.

Background. Non-cardia gastric cancer remains a major cause of cancer-related mortality worldwide, despite declining incidence rates in many industrialized countries. The development of intestinal-type gastric cancer occurs through a multistep process in which normal mucosa is sequentially transformed into hyperproliferative epithelium, followed by metaplastic processes leading to carcinogenesis. Chronic infection with *Helicobacter pylori* is the primary etiological agent that causes chronic inflammation of the gastric mucosa, induces atrophic gastritis, and can lead to intestinal metaplasia and dysplasia. Both intestinal metaplasia and dysplasia are precancerous lesions, in which gastric cancer is more likely to occur. Atrophic gastritis often improves after eradication of *Helicobacter pylori*; however, the occurrence of intestinal metaplasia has been traditionally regarded as "the point of no return" in the carcinogenesis sequence. *Helicobacter pylori* eradication heals non-atrophic chronic gastritis, may lead to regression of atrophic gastritis, and reduces the risk of gastric cancer in patients with these conditions. In this article, we discuss the pathogenesis, epigenomics, and reversibility of intestinal metaplasia and briefly touch upon potential treatment strategy.

Conclusions. Gastric intestinal metaplasia no longer appears to be an irreversible precancerous lesion. However, there are still many controversies regarding the improvement of intestinal metaplasia after *Helicobacter pylori* eradication.

Key words: *Helicobacter pylori*; intestinal metaplasia; gastric cancer

Introduction

The global burden of gastric cancer remains high, ranking fifth for incidence and third for cancer-related mortality worldwide. Early recognition of the disease can lead to potentially successful treatment; however, most patients are diagnosed at a late stage.[1](1) *H. pylori* is the main risk factor for non-cardia gastric cancer development. Although most *H. pylori*-positive individuals remain asymptomatic, the infection predisposes them to the development of chronic gastritis[2], which can be followed by the inflammation–atrophy–metaplasia–dysplasia– carcinoma sequence, known as the Correa cascade.[3] Both chronic atrophic gastritis and intestinal metaplasia are considered precancerous conditions, as they independently confer risk for the development of dysplasia and gastric cancer.[4]

H. pylori infection is associated with a 3-fold increase in the lifetime risk for developing non-cardia gastric cancer, and *H. pylori* infection is believed to cause at least 75% of all gastric cancer.[5] The eradication reduces the risk of gastric cancer

in patients with non-atrophic and atrophic gastritis and effectively heals non-atrophic chronic gastritis. It may also lead to the regression of atrophic gastritis.[6] On the other hand, short-term cancer risk in patients with established intestinal metaplasia does not seem to change significantly with *H. pylori* eradication[7,8], and intestinal metaplasia has thus been considered irreversible. This concept has been challenged in recent years by studies with longer follow up, in which regression of intestinal metaplasia has been observed after *H. pylori* eradication.[9,10] This short review summarizes the role of *H. pylori* in intestinal metaplasia and non-cardia gastric cancer, reviews gastric intestinal metaplasia pathogenesis, and briefly discusses evidence regarding its reversibility.

The following keywords and MeSH terms were used for online searches: [(gastric) AND (metaplasia) OR (intestinal) AND ((regression) OR (reversibility) OR (reversible))]. Reference lists of suitable studies and related previous review articles were reviewed manually to increase search yield and identify other related studies. All searches were restricted to original studies published in the English language.

Helicobacter pylori infection and intestinal metaplasia

H. pylori, a microaerophilic, spiral-shaped, Gram-negative bacterium, colonizes the gastric epithelium in over half of the adult population worldwide. Its prevalence varies widely, ranging from 30% in industrialized regions to 90% in developing countries and Eastern Asia.[11,12] *H. pylori* stands as the most potent single risk factor for non-cardia gastric cancers, including adenocarcinoma and lymphoma[13] and was classified as a class I carcinogen by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) in 1994. Gastric adenocarcinoma is generally divided into two main histological subtypes: diffuse and intestinal, and *H. pylori* contributes to the risk of both.[14]

Diffuse-type gastric adenocarcinomas, characterized by poorly differentiated infiltrating neoplastic cells without a clear glandular structure, predominantly occur in younger patients. Their development does not require long-standing chronic inflammation, and *H. pylori*'s exact role in this subtype remains unclear. Diffuse-type cancer is associated with interference in cell adhesion, polarity, and proliferation, all caused by *H. pylori* infection, leading to the cleavage of E-cadherin, abnormal intracellular accumulation of β-catenin, TP53 mutations, and reduced p27 protein expression.[15] On the other hand, intestinal-type gastric adenocarcinoma emerges later in life and consists of irregular glandular structures formed by well-differentiated cancer cells. This type represents the terminal phase of the chronic inflammation-atrophy-metaplasia-dysplasia-carcinoma sequence, initiated by *H. pylori*-induced gastritis.[16] Atrophic gastritis and gastric intestinal metaplasia, which evolve over decades of chronic infection, are thus established pre-neoplastic lesions for intestinal-type gastric adenocarcinoma.[17] This sequence allows for the possibility of primary prevention strategies involving either population-based or targeted screening to identify patients with precancerous lesions who may need subsequent surveillance.[18]

H. pylori utilizes urease activity to neutralize the acidic conditions in the host stomach at the infection's onset. The bacterium's flagella-mediated motility enables movement toward host gastric epithelium cells. This movement, followed by interactions between bacterial adhesins and host cell receptors, facilitates successful colonization and persistent infection. Some strains of *H. pylori* release effector proteins and toxins, such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), which can damage host tissue.[19] A direct correlation exists between the number of virulence factors in an *H. pylori* strain and the frequency of associated advanced gastric mucosa pathology.[20] However, the characterization of *H. pylori* virulence genes' individual roles is complex due to the interaction of methodological[21], bacterial, and host factors[19], often leading to conflicting results and interpretations.

Intrabacterial urease activity is required for *H. pylori* acid resistance, and this activity is regulated by the proton-gated urea channel UreI, which permits urea entry only under acidic conditions and thus prevents lethal alkalization during times of relative neutrality. The urease gene cluster is composed of seven genes, including catalytic subunits (*ureA*/*B*), an acid-gated urea channel (*ureI*), and accessory assembly proteins (*ureE-H*).[22] Urease can also protect against host innate immune response by modulation of phagosome pH following phagocytosis and promotion of *H. pylori* survival inside megasomes.[23]

Flagella-mediated motility is essential for colonization of the gastric mucosa by *H. pylori*. Loss of any component of the motility and chemotaxis systems abolishes the ability of *H. pylori* to

infect the stomach and establish colonization.[24-26] Infection with *H. pylori* that exhibits higher motility may show enhanced bacterial density, triggering a more pronounced inflammatory response in the upper stomach, and can thus be associated with severe pathological outcomes.[27] The flagellar filament consists of two flagellins (FlaA and FlaB) encoded by *flaA* and *flaB*.[28] FIaA elicits host antibody response and can be used as a marker of *H. pylori* infection; host anti-FlaA titer correlates with *H. pylori* colonization density[29] and the presence of gastric intestinal metaplasia.[30]

The interaction of bacterial adhesins with host cellular receptors protects *H. pylori* from displacement by the forces generated by peristalsis. This bacterial adherence plays an important role in both the initial colonization and long-term persistence of *H. pylori* in the human gastric mucosa[31] and is necessary for the tight adherence of the bacteria to gastric epithelial cells, which facilitates subsequent delivery of bacterial toxins.[32] The *H. pylori* genome encodes a variety of outer membrane proteins (OMPs); several OMPs have been described in detail to date, with most studies focusing on *babA2*, *oipA*, *homB*, and *sabA* genes.[19] BabA is one of the most studied *H. pylori* adhesins. BabA is capable of binding to Lewis b and related ABO antigens on gastric epithelial cells[33], which may play a crucial role in the development of *H. pylori* related gastric pathology such as severe gastritis, peptic ulcers, and gastric adenocarcinoma.[21,34] *BabA* positive strains appear to be associated with worse clinical outcomes in several studies[35-37], while another study found no correlation between the presence of *babA2* positive strains and atrophy or intestinal metaplasia.[21] *HomB* may be strongly associated with gastric cancer in certain populations[38] and display little measurable virulence in others.[39]

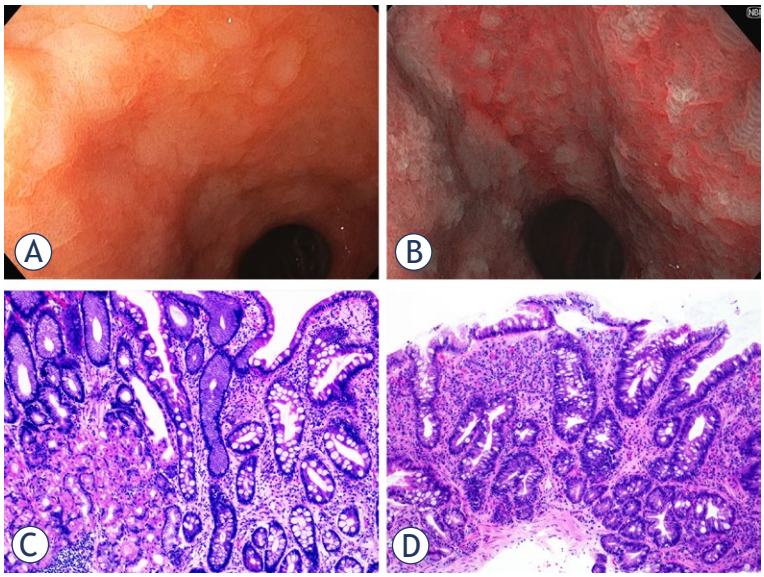


FIGURE 1. Gastric intestinal metaplasia, endoscopic **(A, B)** and histological **(C, D)** appearance. Gastric intestinal metaplasia is endoscopically characterized by the presence of grey-white velvety or slightly nodular elevated patches, which are clearly demarcated against the surrounding pink gastric mucosa, as illustrated in image A of antral gastric mucosa under white light. Narrow band imaging (NBI, depicted in image **B**) further enhances the visualization of mucosal and vascular patterns by employing optical filters to narrow the bandwidth of light. This technique offers superior contrast compared to white light endoscopy, thereby improving the detection of metaplastic transformation. Histologically, gastric intestinal metaplasia can be classified into either complete (as seen in image **C**) or incomplete types (as shown in image **D**). Image **C** demonstrates preserved oxyntic mucosa (on the left) adjacent to intestinal metaplasia of the complete type, which features enterocytes with a well-defined brush border, alongside well-formed goblet cells and Paneth cells. In contrast, image **D** illustrates the intestinal metaplasia of the gastric mucosa of the incomplete type, characterized by goblet cells of variable size and intervening mucin-secreting columnar cells that lack a brush border (both images are hematoxylin and eosin-stained, original magnification 10x).

Attachment of *cagA*-positive *H. pylori* to host gastric epithelial cells initiates and facilitates the formation of the bacterial type IV secretion system, involved in the delivery of CagA into host epithelial cells.[32] The translocated CagA protein localizes to the inner surface of the plasma membrane via interactions with phosphatidylserine and subsequently undergoes tyrosine phosphorylation by the Src family protein tyrosine kinase. However, once injected into the cytoplasm, CagA can alter host cell signaling in both a phosphorylation-dependent and phosphorylation-independent manner. The phosphorylated CagA binds to the phosphatase SHP-2, forming CagA-SPH-2 complex, and affects the adhesion, spreading, and migration of the cell.[40,41] CagA can also affect the host cell in a phosphorylation-independent manner by stimulating the gastric epithelium cells to secrete IL-8, which strongly affects the level of mucosal inflammation.[42,43]

The CagA-SHP-2 complex is predominantly located in atrophic gastric mucosa and is associated with the transition to atrophic gastritis and possibly intestinal metaplasia.[41] Deregulation of the SHP-2 role by CagA is functionally similar to the effect of the gain-of-function mutation of the SHP-2 gene observed in other human malignancies.[44] CagA interference with intracellular signaling may thus lead to deregulation of cellular growth, apoptosis, and elevated cell motility. This can result in increased cell turnover, which in turn leads to the accumulation of further genetic changes favoring neoplastic cell transformation.[45] Unsurprisingly, infection with *cagA*-positive strains markedly in-

TABLE 1. Patients' related predictive risk factors for gastric intestinal metaplasia

Risk Factor	Odds ratio (OD)	Key findings	References
Race			
White	1	Hispanic and Asian patients have an increased risk for GIM	Tan MC *et al.* (2022)[94] Akpoigbe K *et al.* (2022)[95]
Asian	2.83–3		
Hispanic	2.10–5.6		
Age (> 50 years)	1.5–2.03	Risk increases with age, possibly due to accumulated exposure to risk factors.	Aumpan N *et al.* (2021)[96] Tan MC *et al.* (2020)[97]
Male gender	1.55–2.09	Probably due to genetics and exposure to other risk factors	Aumpan N *et al.* (2020)[98] Leung WK *et al.* (2005)[99]
Chronic gastritis	3.68–5.76	Chronic inflammation is leads to IM.	Yoo YE *et al.* (2013)[100] Tatsuta M *et al.* (1993)[101]
***H. pylori* infection**	2.47–3.65	Strong correlation with IM, especially with *CagA* positive strains.	Aumpan N *et al.* (2021)[96] Nguyen T *et al.* (2021)[102]
Family history of gastric cancer	1.5–3.8	Patients with a first-degree relative with gastric cancer have an increased risk of neoplastic progression	Nieuwenburg SAV *et al.* (2021)[103] Reddy KM *et al.* (2006)[104]
Alcohol consumption	1.27–1.54	Alcohol intake was independently associated with increased risk of developing AG and IM	Holmes HM *et al.* (2021)[105] Kim K *et al.* (2020)[106]
Tobacco smoking	1.54–2.75	Tobacco smoking is a risk factor for gastric IM.	Morais S *et al.* (2014)[107] Thrift AP *et al.* (2022)[108]
Blood group A	1.39–1.42	Blood group A is associated with higher risk of GIM	Mao Y *et al.* (2019)[109] Rizatto C *et al.* (2013)[110]
Bile reflux	unknown	Bile acids not only interefere with gastric mucosa but also regulate multiple carcinogenic pathways	Wang M *et al.* (2023)[111] Yu J *et al.* (2019)[112]
Salt consumption	0.37–1.53	Salt intake may increase progression to advanced gastric precancerous lesions	Dias-Neto M *et al.* (2010)[113] Song JH *et al.* (2017)[114]
Industrially processed food	unknown	Dietary exposure to *N*-nitroso–containing compounds has been shown to increase the promotion of gastric carcinogenesis	Wiseman M (2008)[115] Jencks DS *et al.* (2018)[116]

creases the risk of gastric cancer.[46] *CagA*-positive strains are responsible for 60% of *H. pylori* infections in individuals worldwide.[47-49] Strains isolated in East Asian countries such as Japan, China, and Korea are almost all *CagA*-positive.[50] Furthermore, CagA protein can be divided into the Western-type CagA and East Asian-type CagA. The affinity of the East Asian-type CagA to SHP-2 is significantly higher than that of the Western-type CagA and is more likely to be associated with gastric cancer.[40,51]

VacA, another key toxin involved in *H. pylori* pathogenesis, binds to host epithelial cells after secretion from the bacteria. It is then internalized and causes the accumulation of large intracellular vesicles (vacuolation), interferes with mitochondria, and causes apoptosis of host cells.[52] VacA also appears to disrupt the balance of cell proliferation and death by affecting genes that regulate the cell cycle.[53] *H. pylori* strains producing VacA differ in the potency of cytotoxin, in both its activity (allele s1 is more active than s1) and binding (allele m1 is more effective than m2).[54] A meta-analysis of 33 studies (1,446 cases and 2,697 controls in total) confirmed the correlation between the *vacA s1* genotype and the risk of atrophic gastritis, intestinal metaplasia, and gastric cancer. The *vacA m1* genotype was associated with intestinal metaplasia and gastric cancer but did not significantly correlate with atrophic gastritis.[55]

Pathogenesis of gastric intestinal metaplasia

Gastric intestinal metaplasia is defined as the replacement of normal gastric epithelium in the antral or oxyntic mucosa with intestinal epithelium, consisting of intestinal cell types including Paneth, goblet, and absorptive cells.[56] These metaplastic glands are characterized by modification of the surrounding stroma and by reorganization of the crypts, with displacement of the proliferative zone from the neck region to the base of the crypts.[57] Intestinal metaplasia can be classified as either limited (when confined to one anatomical region) or extensive, if two gastric regions are involved (Figure 1).

Complete intestinal metaplasia is characterized by small intestinal-type mucosa with mature absorptive cells, and a brush border, with a notable loss of gastric mucin markers (MUC1, MUC5AC, MUC6) and an acquisition of the intestinal mucin

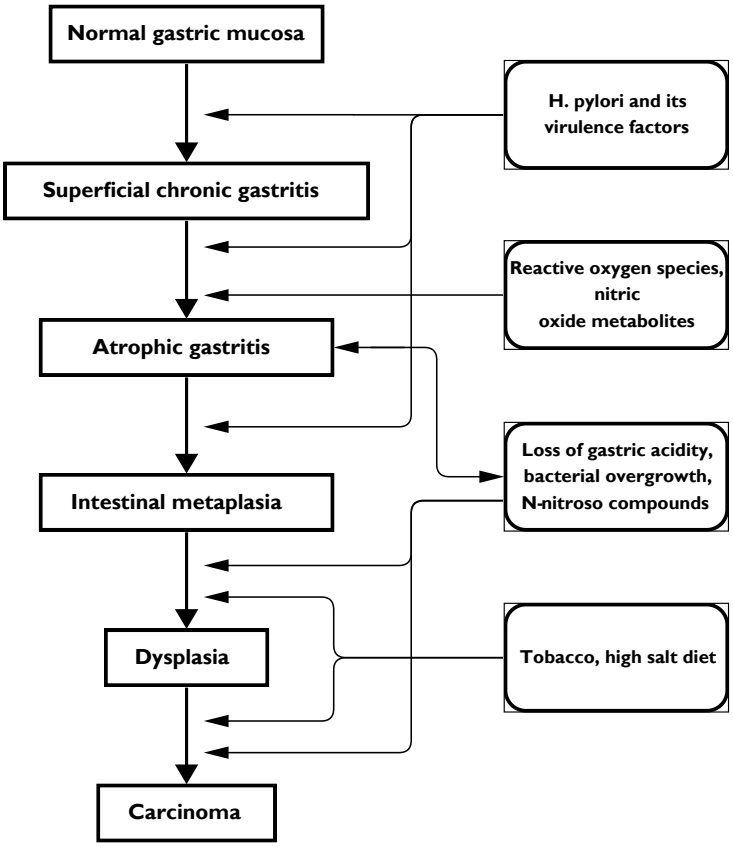


FIGURE 2. Pathogenesis of intestinal metaplasia and gastric adenocarcinoma – the Corea cascade. This stepwise process starts with chronic gastritis triggered by *H. pylori* infection. The likelihood of developing gastric cancer is higher in individuals infected with virulent strains of *H. pylori*, unhealthy diets (rich in salt and smoked foods), low iron levels, and harmful lifestyle choices, including smoking. Persistent inflammation results in the damage and eventual loss of acid-producing parietal cells, causing reduced stomach acidity (hypochlorhydria) and eventually no stomach acid production (achlorhydria). This reduction in acidity allows for the colonization of the stomach by detrimental, pro-inflammatory microbiota. These bacteria can produce genotoxic and pro-inflammatory metabolites and carcinogens, directly contributing to the transformation of stomach epithelial cells into malignant cells.

MUC2. On the other hand, incomplete intestinal metaplasia is characterized by columnar "intermediate" cells at various differentiation stages, irregular mucin droplets, and a lack of a brush border, while still maintaining gastric mucin markers alongside the presence of intestinal mucin MUC2.[58,59] Earlier gastric metaplasia classifications relied on traditional mucin staining methods (such as periodic acid-Schiff, Alcian blue, and high iron diamine) and cell morphology. This methodology defined three intestinal metaplasia grades: Type I, which encompasses absorptive cells, Paneth cells, and goblet cells that secrete sialomucins; Type II, consisting of goblet and columnar cells secreting sialomucins; and Type III, involving goblet and columnar cells secreting sulfomucins. Presently, Type I aligns with the complete type, while Types II and III correspond to the incomplete type in the contemporary classification.[58]

The Correa cascade is a widely accepted model of the pathogenesis of gastric cancer (Figure 2).[3] This cascade commences with the emergence of chronic mucosal inflammation, mediated by polymorphonuclear and mononuclear cells. It evolves through a multifactorial process, steered by various factors including *H. pylori*, host genetics, environmental elements, and diet, propelling further alterations in the gastric mucosa towards atrophy, metaplasia, and ultimately, cancer.[60-62]

Annually, an estimated 0.1%, 0.25%, 0.6%, and 6% of Western patients with atrophic gastritis, intestinal metaplasia, and mild-to-moderate or severe dysplasia, respectively, progress to gastric cancer.[62] In contrast, East Asian populations demonstrate a higher risk, with about 1.8%, 10%, and 73% of patients with atrophic gastritis, intestinal metaplasia, and dysplasia, respectively, progressing to gastric cancer each year.[63] Patients with incomplete intestinal metaplasia encounter a 3.3-fold higher relative risk of developing gastric cancer compared to those with complete intestinal metaplasia. Furthermore, extensive intestinal metaplasia is linked with a 2.1-fold higher relative risk of progression compared to limited gastric metaplasia.[64,65]

Host factors that are associated with higher risk for non-cardia gastric cancer are similar to risk factors for development of intestinal metaplasia (Table 1) and include advanced age, male sex, family history, and smoking. More than two thirds of all gastric cancers are diagnosed after the age of 55, and roughly two thirds of non-cardia cancers are found in male patients.[66] The reason for the latter observation is most likely multifactorial. The difference can be partly attributed to smoking (which is more prevalent in men) and partly to the protective role of estrogen, since increased fertility and late menopause both reduce the risk of gastric cancer in women.[67] Individuals with blood type A have a 20% higher chance of developing gastric cancer when compared to other blood types, according to a prospective blood donor cohort study.[68]

Ethnicity also plays an important role in gastric cancer risk. The incidence of non-cardia gastric cancer in individuals of African-American, East Asian, or Pacific Islander descent is almost twice that observed in Caucasians.[69] A similar pattern was seen in the analysis of intestinal metaplasia

prevalence. A study that reviewed 800,000 gastric biopsies taken in the United States showed 20% prevalence of gastric metaplasia in people of East Asian descent, 12% prevalence in Hispanics, and 8% in all other ethnic backgrounds.[62]

Tobacco smoking is the second most important environmental factor in gastric cancer pathogenesis, accounting for 11% of all cases.[70] Tobacco use increases the risk of intestinal metaplasia and doubles the risk of its progression to dysplasia, according to a large Chinese population-based study.[71]

Bile acid reflux into the gastric lumen produces repetitive gastric mucosal injury, which predisposes patients to intestinal metaplasia and gastric cancer in *H. pylori*-positive patients.[72] Bile acids increase the expression of CDX2, an intestinal-specific transcription factor that directs and maintains intestinal differentiation in gastric mucosa[73], and indirectly damage cellular DNA by induction of oxidative stress and production of reactive oxygen species[74], which promote intestinal metaplasia and the further accumulation of mutations, leading to increased cancer risk.

The role of diet (being an obvious potential factor in gastric disorders) has been extensively studied in gastric cancer pathogenesis. High salt consumption is associated with increased risk of *H. pylori* infection and upregulation of *cagA* expression.[75,76] Dietary use of processed or preserved meat using smoke or salt is positively and dose-dependently associated with non-cardia gastric cancer.[77] Nitrite and nitrate additives form N-nitroso carcinogenic compounds when they combine with amino acids.

Similar carcinogens are formed by ingestion of haem (and meat) in the human gastrointestinal tract.[78] Vegetables and fruits in the diet have a protective role[79], and folic acid supplementation has been shown to reduce *H. pylori* related gastric inflammation and dysplasia in murine models.[80]

Reversibility of intestinal metaplasia

Large prospective trials of *H. pylori* eradication for non-cardia gastric cancer prevention failed to show a reduction in gastric cancer incidence after eradication in a subpopulation of patients with pre-existing gastric intestinal metaplasia or extensive atrophic gastritis.[7,81] Intestinal metaplasia has thus been considered irreversible, and its occurrence is considered to be the histological point of no return in the carcinogenic cascade.

These assumptions appeared to be confirmed by prospective studies designed to evaluate the effect of *H. pylori* eradication on intestinal metaplasia and atrophic gastritis in eradicated subjects. A marked regression of histologic changes associated with acute and chronic gastritis was observed after eradication in one of these studies; however, the level of mucosal atrophy and intestinal metaplasia remained unchanged one year after *H. pylori* eradication.[82] Similar results with no regression in intestinal metaplasia were reported in a more recent detailed histological analysis of 88 antral biopsies taken in patients with intestinal metaplasia prior to and several months after *H. pylori* eradication.[83] Several other smaller studies, all with short intervals of observation, reported similar results.[84,85] On the other hand, a number of prospective studies with longer observation intervals report the partial regression of intestinal metaplasia.[10,86-87] Hwang *et al*. postulated that the reason for this apparent discrepancy might stem simply from the slow pace of the process under observation.[10] The partial reversibility of intestinal metaplasia after *H. pylori* eradication is also indirectly supported by a meta-analysis that confirmed reduced gastric cancer incidence in all levels of baseline risk, including patients with gastric metaplasia.[88] Another recent meta-analysis directly addressed the natural course of intestinal metaplasia. Its regression was observed in 32%, and its persistence in 43%, of 20 relevant studies.[89]

A recent study of genomic and epigenomic profiling of intestinal metaplasia by Huang *et al*.[90] also addressed the regression of intestinal metaplasia. Eighty-two eradicated patients with intestinal metaplasia were included in an assessment of correlates between molecular features and clinical outcome. At the end of surveillance period, 6 patients had developed dysplasia or cancer, 61 showed no change, and regression of intestinal metaplasia was observed in 15 patients. The level of DNA methylation changes correlated with the tendency to progress and was highest among progressors, intermediate in the stable group, and low in patients with intestinal metaplasia regression. Furthermore, *H. pylori* burden correlated with DNA methylation levels only in the intermediate group, but not in the methylation-high group, which could explain the failure of *H. pylori* eradication to stabilize or reverse intestinal metaplasia in these patients. Levels of aberrant DNA methylation could thus indicate the point of no return within the scope of intestinal metaplasia.

Folate is water soluble vitamin that acts a as a methyl group donor in DNA methylation and plays an important role in epigenetic regulation.[91] Folic acid (FA) supplementation has been shown to reduce the risk of gastric cancer in 7-year prospective randomized trial of 216 patients with chronic atrophic gastritis.[92] All 5 observed gastric cancer cases occurred outside the group of FA-treated patients. Furthermore, the use of FA for 12 months was associated with more frequent reversal of both, atrophy and intestinal metaplasia in comparison to patients receiving placebo. These observations were confirmed by recent meta-analysis of the role of FA supplementation in reversal of gastric precancerous conditions.[93] Daily doses of 20–30 mg of FA in the duration of 3-6 months were associated with significant reversal of both, atrophic gastritis and intestinal metaplasia (RR: 1.77, 95% CI: 1.32–2.37).[93]

Conclusions

The long-held belief that intestinal metaplasia of the gastric mucosa represents an irreversible precursor to cancer has increasingly been questioned in recent years. The concept of a 'point of no return' in the progression toward gastric cancer is now understood to be more complex than histomorphological changes alone. Consequently, the histological subtypes of gastric intestinal metaplasia must be considered during the planning of patient surveillance due to their varying potential for neoplastic transformation. Additionally, epigenomic alterations and molecular profiling could prove valuable in identifying the pro-carcinogenic transformation of intestinal metaplasia in patients without established risk factors. The eradication of *H. pylori* remains a critical step towards the potential reversibility of intestinal metaplasia; however, identifying patients at high risk of progression to cancer continues to be essential. The question of intestinal metaplasia progression despite *H. pylori* eradication could be addressed by examining changes in DNA methylation levels. Furthermore, non-*H. pylori* related host risk factors in the pathogenesis of gastric cancer are under thorough investigation. Significant challenges remain, such as accurately quantifying these factors and determining their exposure duration to assess their actual impact on intestinal metaplasia progression accurately. Recent studies highlighting the role of bile acids, N-nitroso–containing compounds, and deficiencies in vitamin C and folate have shown promise, yet their clinical relevance remains to be fully elucidated. An enduring unresolved issue is the long-term monitoring of these individuals, where the patchy nature of intestinal metaplasia could lead to sampling errors and potentially incorrect assessments of intestinal metaplasia reversibility.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68:** 394-424. doi: 10.3322/caac.21492
2. de Brito BB, da Silva FAF, Soares AS, Pereira VA, Santos MLC, Sampaio MM, et al. Pathogenesis and clinical management of Helicobacter pylori gastric infection. *World J Gastroenterol* 2019; **25:** 5578-89. doi: 10.3748/wjg.v25.i37.5578
3. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process − First American Cancer Society award lecture on cancer epidemiology and prevention. *Cancer Res* 1992; **52:** 6735-40. PMID: 1458460
4. Kapadia CR. Gastric atrophy, metaplasia, and dysplasia: a clinical perspective. *J Clin Gastroenterol* 2003; **36:** S29-36; discussion S61-62. doi: 10.1097/00004836-200305001-00006
5. Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of Helicobacter pylori infection with gastric carcinoma: a meta-analysis. *Am J Gastroenterol* 1999; **94:** 2373-9. doi: 10.1111/j.1572-0241.1999.01360.x
6. Rokkas T, Rokka A, Portincasa P. A systematic review and meta-analysis of the role of Helicobacter pylori eradication in preventing gastric cancer. *Ann Gastroenterol* 2017; **30:** 414-23. doi: 10.20524/aog.2017.0144
7. Wong BC-Y, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. China Gastric Cancer Study Group. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291:** 187-94. doi: 10.1001/jama.291.2.187
8. Chen H-N, Wang Z, Li X, Zhou Z-G. Helicobacter pylori eradication cannot reduce the risk of gastric cancer in patients with intestinal metaplasia and dysplasia: evidence from a meta-analysis. *Gastric Cancer* 2016; **19:** 166-75. doi: 10.1007/s10120-015-0462-7
9. Hwang Y-J, Kim N, Lee HS, Lee JB, Choi YJ, Yoon H, et al. Reversibility of atrophic gastritis and intestinal metaplasia after Helicobacter pylori eradication – a prospective study for up to 10 years. *Aliment Pharmacol Ther* 2018; **47:** 380-90. doi: 10.1111/apt.14424
10. Kodama M, Murakami K, Okimoto T, Sato R, Uchida M, Abe T, et al. Ten-year prospective follow-up of histological changes at five points on the gastric mucosa as recommended by the updated Sydney system after Helicobacter pylori eradication. *J Gastroenterol* 2012; **47:** 394-403. doi: 10.1007/s00535-011-0504-9
11. Mišak Z, Hojsak I, Homan M. Review: Helicobacter pylori in pediatrics. *Helicobacter* 2019; **24 (Suppl 1):** e12639. doi: 10.1111/hel.12639
12. Goh K-L, Chan W-K, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implications. *Helicobacter* 2011; **16 (Suppl 1):** 1-9. doi: 10.1111/j.1523-5378.2011.00874.x
13. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325:** 1127-31. doi: 10.1056/NEJM199110173251603
14. Hansson LR, Engstrand L, Nyrén O, Lindgren A. Prevalence of Helicobacter pylori infection in subtypes of gastric cancer. *Gastroenterology* 1995; **109:** 885-8. doi: 10.1016/0016-5085(95)90398-4
15. Ansari S, Gantuya B, Tuan VP, Yamaoka Y. Diffuse gastric cancer: a summary of analogous contributing factors for its molecular pathogenicity. *Int J Mol Sci* 2018; **19:** 2424. doi: 10.3390/ijms19082424
16. Correa P, Haenszel W, Cuello C, Zavala D, Fontham E, Zarama G, et al. Gastric precancerous process in a high risk population: cohort follow-up. *Cancer Res* 1990; **50:** 4737-40.

17. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. *J Cancer Prev* 2015; **20:** 25-40. doi: 10.15430/JCP.2015.20.1.25

18. Pimentel-Nunes P, Libânio D, Marcos-Pinto R, Areia M, Leja M, Esposito G, et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. *Endoscopy* 2019; **51:** 365-88. doi: 10.1055/a-0859-1883

19. Šterbenc A, Jarc E, Poljak M, Homan M. Helicobacter pylori virulence genes. *World J Gastroenterol* 2019; **25:** 4870-884. doi: 10.3748/wjg.v25.i33.4870

20. Höcker M, Hohenberger P. Helicobacter pylori virulence factors – one part of a big picture. *Lancet* 2003; **362:** 1231-3. doi: 10.1016/S0140-6736(03)14547-3

21. Šterbenc A, Lunar MM, Homan M, Luzar B, Zidar N, Poljak M. Prevalence of the Helicobacter pylori babA2 gene in children mainly depends on the PCR primer set used. *Can J Infect Dis Med Microbiol* 2020; **2020:** 4080248. doi: 10.1155/2020/4080248

22. Mobley HL, Island MD, Hausinger RP. Molecular biology of microbial ureases. *Microbiol Rev* 1995; **59:** 451-80. doi: 10.1128/mr.59.3.451-480.1995

23. Schwartz JT, Allen L-AH. Role of urease in megasome formation and Helicobacter pylori survival in macrophages. *J Leukoc Biol* 2006; **79:** 1214-25. doi: 10.1189/jlb.0106030

24. Eaton KA, Suerbaum S, Josenhans C, Krakowka S. Colonization of gnotobiotic piglets by Helicobacter pylori deficient in two flagellin genes. *Infect Immun* 1996; **64:** 2445-8. doi: 10.1128/iai.64.7.2445-2448.1996

25. Kim JS, Chang JH, Chung SI, Yum JS. Molecular cloning and characterization of the Helicobacter pylori fliD gene, an essential factor in flagellar structure and motility. *J Bacteriol* 1999; **181:** 6969-76. doi: 10.1128/JB.181.22.6969-6976.1999

26. Howitt MR, Lee JY, Lertsethtakarn P, Vogelmann R, Joubert L-M, Ottemann KM, et al. ChePep controls Helicobacter pylori infection of the gastric glands and chemotaxis in the Epsilonproteobacteria. *mBio* 2011; **2:** e00098-11. doi: 10.1128/mBio.00098-11

27. Kao CY, Sheu BS, Sheu SM, Yang HB, Chang WL, Cheng HC, et al. Higher motility enhances bacterial density and inflammatory response in dyspeptic patients infected with Helicobacter pylori. *Helicobacter* 2012; **17:** 411-6. doi: 10.1111/j.1523-5378.2012.00974.x

28. Lertsethtakarn P, Ottemann KM, Hendrixson DR. Motility and chemotaxis in Campylobacter and Helicobacter. *Annu Rev Microbiol* 2011; **65:** 389-410. doi: 10.1146/annurev-micro-090110-102908

29. Tian W, Jia Y, Yuan K, Huang L, Nadolny C, Dong X, et al. Serum antibody against Helicobacter pylori FlaA and risk of gastric cancer. *Helicobacter* 2014; **19:** 9-16. doi: 10.1111/hel.12095

30. Toyoshima O, Nishizawa T, Sakitani K, Yamakawa T, Takahashi Y, Yamamichi N, et al. Serum anti-Helicobacter pylori antibody titer and its association with gastric nodularity, atrophy, and age: a cross-sectional study. *World J Gastroenterol* 2018; **24:** 4061-8. doi: 10.3748/wjg.v24.i35.4061

31. Yamaoka Y. Roles of Helicobacter pylori BabA in gastroduodenal pathogenesis. *World J Gastroenterol* 2008; **14:** 4265-72. doi: 10.3748/wjg.14.4265

32. Backert S, Clyne M, Tegtmeyer N. Molecular mechanisms of gastric epithelial cell adhesion and injection of CagA by Helicobacter pylori. *Cell Commun Signal* 2011; **9:** 28. doi: 10.1186/1478-811X-9-28

33. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 1998; **279:** 373-7. doi: 10.1126/science.279.5349.373

34. Gerhard M, Lehn N, Neumayer N, Borén T, Rad R, Schepp W, et al. Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin. *PNAS* 1999; **96:** 12778-83. doi: 10.1073/pnas.96.22.12778

35. Oleastro M, Gerhard M, Lopes AI, Ramalho P, Cabral J, Sousa Guerreiro A, et al. Helicobacter pylori virulence genotypes in Portuguese children and adults with gastroduodenal pathology. *Eur J Clin Microbiol Infect Dis* 2003; **22:** 85-91. doi: 10.1007/s10096-002-0865-3

36. Olfat FO, Zheng Q, Oleastro M, Voland P, Borén T, Karttunen R, et al. Correlation of the Helicobacter pylori adherence factor BabA with duodenal ulcer disease in four European countries. *FEMS Immunol Med Microbiol* 2005; **44:** 151-6. doi: 10.1016/j.femsim.2004.10.010

37. Oliveira AG, Santos A, Guerra JB, Rocha GA, Rocha AMC, Oliveira CA, et al. babA2- and cagA-positive Helicobacter pylori strains are associated with duodenal ulcer and gastric carcinoma in Brazil. *J Clin Microbiol* 2003; **41:** 3964-6. doi: 10.1128/jcm.41.8.3964-3966.2003

38. Abadi ATB, Rafiei A, Ajami A, Hosseini V, Taghvaei T, Jones KR, et al. Helicobacter pylori homB, but not cagA, is associated with gastric cancer in Iran. *J Clin Microbiol* 2011; **49:** 3191-7. doi: 10.1128/JCM.00947-11

39. Šterbenc A, Poljak M, Zidar N, Luzar B, Homan M. Prevalence of the Helicobacter pylori homA and homB genes and their correlation with histological parameters in children. *Microb Pathog* 2018; **125:** 26-32. doi: 10.1016/j.micpath.2018.09.005

40. Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, et al. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci U S A* 2002; **99:** 14428-33. doi: 10.1073/pnas.222375399

41. Yamazaki S, Yamakawa A, Ito Y, Ohtani M, Higashi H, Hatakeyama M, et al. The CagA protein of Helicobacter pylori is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa. *J Infect Dis* 2003; **187:** 334-7. doi: 10.1086/367807

42. Kikuchi K, Murata-Kamiya N, Kondo S, Hatakeyama M. Helicobacter pylori stimulates epithelial cell migration via CagA-mediated perturbation of host cell signaling. *Microbes Infect* 2012; **14:** 470-6. doi: 10.1016/j.micinf.2011.12.003

43. Boonyanugomol W, Chomvarin C, Hahnvajanawong C, Sripa B, Kaparakis-Liaskos M, Ferrero RL. Helicobacter pylori cag pathogenicity island (cag-PAI) Involved in bacterial internalization and IL-8 induced responses via NOD1- and MyD88-dependent mechanisms in human biliary epithelial cells. *PLoS One* 2013; **8:** e77358. doi: 10.1371/journal.pone.0077358

44. Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, et al. Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. *Cancer Res* 2004; **64:** 8816-20. doi: 10.1158/0008-5472.CAN-04-1923

45. Hatakeyama M. Oncogenic mechanisms of the Helicobacter pylori CagA protein. *Nat Rev Cancer* 2004; **4:** 688-94. doi: 10.1038/nrc1433

46. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A* 1996; **93:** 14648-53. doi: 10.1073/pnas.93.25.14648

47. Homan M, Luzar B, Kocjan BJ, Orel R, Mocilnik T, Shrestha M, et al. Prevalence and clinical relevance of cagA, vacA, and iceA genotypes of Helicobacter pylori isolated from Slovenian children. *J Pediatr Gastroenterol Nutr* 2009; **49:** 289-96. doi: 10.1097/MPG.0b013e31818f09f2

48. Chiurillo MA, Moran Y, Cañas M, Valderrama E, Granda N, Sayegh M, et al. Genotyping of Helicobacter pylori virulence-associated genes shows high diversity of strains infecting patients in western Venezuela. *Int J Infect Dis* 2013; **17:** e750-6.doi: 10.1016/j.ijid.2013.03.004

49. Homan M, Hojsak I, Kolaček S. Helicobacter pylori in pediatrics. *Helicobacter* 2012; **17 (Suppl 1):** 43-8. doi: 10.1111/j.1523-5378.2012.00982.x

50. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999; **37:** 2274-9. doi: 10.1128/JCM.37.7.2274-2279.1999

51. Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of Helicobacter pylori. *Gastroenterology* 2004; **127:** 514-23. doi: 10.1053/j.gastro.2004.06.006

52. Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (VacA), a key toxin for Helicobacter pylori pathogenesis. *Front Cell Infect Microbiol* 2012; **2:** 92. doi: 10.3389/fcimb.2012.00092

53. Hisatsune J, Nakayama M, Isomoto H, Kurazono H, Mukaida N, Mukhopadhyay AK, et al. Molecular characterization of Helicobacter pylori VacA induction of IL-8 in U937 cells reveals a prominent role for p38MAPK in activating transcription factor-2, cAMP response element binding protein, and NF-kappaB activation. *J Immunol* 2008; **180:** 5017-27. doi: 10.4049/jimmunol.180.7.5017

54. Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270:** 17771-7. doi: 10.1074/jbc.270.30.17771

55. Abdi E, Latifi-Navid S, Latifi-Navid H, Safarnejad B. Helicobacter pylori vacuolating cytotoxin genotypes and preneoplastic lesions or gastric cancer risk: a meta-analysis. *J Gastroenterol Hepatol* 2016; **31:** 734-44. doi: 10.1111/jgh.13256

56. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20:** 1161-81. doi: 10.1097/00000478-199610000-00001

57. Mutoh H, Sakurai S, Satoh K, Osawa H, Tomiyama T, Kita H et al. Pericryptal fibroblast sheath in intestinal metaplasia and gastric carcinoma. *Gut* 2005; **54:** 33-9. doi: 10.1136/gut.2004.042770

58. Reis CA, David L, Correa P, Carneiro F, de Bolós C, Garcia E, et al. Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. *Cancer Res* 1999; **59:** 1003-7. PMID: 10070955

59. Capelle LG, de Vries AC, Haringsma J, Ter Borg F, de Vries RA, Bruno MJ, et al. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. *Gastrointest Endosc* 2010; **71:** 1150-8. doi: 10.1016/j.gie.2009.12.029

60. Lim JH, Kim N, Lee HS, Choe G, Jo SY, Chon I, et al. Correlation between endoscopic and histological diagnoses of gastric intestinal metaplasia. *Gut Liver* 2013; **7:** 41-50. doi: 10.5009/gnl.2013.7.1.41

61. Marques-Silva L, Areia M, Elvas L, Dinis-Ribeiro M. Prevalence of gastric precancerous conditions: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2014; **26:** 378-87. doi: 10.1097/MEG.0000000000000065

62. Song H, Ekheden IG, Zheng Z, Ericsson J, Nyrén O, Ye W. Incidence of gastric cancer among patients with gastric precancerous lesions: observational cohort study in a low risk Western population. *BMJ* 2015; **351:** h3867. doi: 10.1136/bmj.h3867

63. Huang RJ, Choi AY, Truong CD, Yeh MM, Hwang JH. Diagnosis and management of gastric intestinal metaplasia: current status and future directions. *Gut Liver* 2019; **13:** 596-603. doi: 10.5009/gnl19181

64. González CA, Sanz-Anquela JM, Gisbert JP, Correa P. Utility of subtyping intestinal metaplasia as marker of gastric cancer risk. A review of the evidence. *Int J Cancer* 2013; **133:** 1023-32. doi: 10.1002/ijc.28003

65. Gupta S, Tao L, Murphy JD, Camargo MC, Oren E, Valasek MA, et al. Race/ethnicity-, socioeconomic status-, and anatomic subsite-specific risks for gastric cancer. *Gastroenterology* 2019; **156:** 59-62.e4. doi: 10.1053/j.gastro.2018.09.045

66. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am* 2002; **11:** 235-56. doi: 10.1016/s1055-3207(02)00002-9

67. Derakhshan MH, Liptrot S, Paul J, Brown IL, Morrison D, McColl KEL. Oesophageal and gastric intestinal-type adenocarcinomas show the same male predominance due to a 17 year delayed development in females. *Gut* 2009; **58:** 16-23. doi: 10.1136/gut.2008.161331

68. Edgren G, Hjalgrim H, Rostgaard K, Norda R, Wikman A, Melbye M, et al. Risk of gastric cancer and peptic ulcers in relation to ABO blood type: a cohort study. *Am J Epidemiol* 2010; **172:** 1280-5. doi: 10.1093/aje/kwq299

69. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M et al. SEER cancer statistics review, 1975-2016, National Cancer Institute. Bethesda, MD. Available at: https://seer.cancer.gov/csr/1975_2016/, based on November 2018 SEER data submission, posted to the SEER web site, April 2019. Updated April 9, 2020. (cited 2021 Feb 22).

70. Trédaniel J, Boffetta P, Buiatti E, Saracci R, Hirsch A. Tobacco smoking and gastric cancer: review and meta-analysis. *Int J Cancer* 1997; **72:** 565-73. doi: 10.1002/(sici)1097-0215(19970807)72:4<565::aid-ijc3>3.0.co;2-o

71. Kneller RW, You WC, Chang YS, Liu WD, Zhang L, Zhao L, et al. Cigarette smoking and other risk factors for progression of precancerous stomach lesions. *J Natl Cancer Inst* 1992; **84:** 1261-6. doi: 10.1093/jnci/84.16.1261

72. Tatsugami M, Ito M, Tanaka S, Yoshihara M, Matsui H, Haruma K, et al. Bile acid promotes intestinal metaplasia and gastric carcinogenesis. *Cancer Epidemiol Biomarkers Prev* 2012; **21:** 2101-7. doi: 10.1158/1055-9965.EPI-12-0730

73. Li T, Guo H, Li H, Jiang Y, Zhuang K, Lei C, et al. MicroRNA-92a-1–5p increases CDX2 by targeting FOXD1 in bile acids-induced gastric intestinal metaplasia. *Gut* 2019; **68:** 1751-63. doi: 10.1136/gutjnl-2017-315318

74. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat Res* 2005; **589:** 47-65. doi: 10.1016/j.mrrev.2004.08.001

75. Lee S-A, Kang D, Shim KN, Choe JW, Hong WS, Choi H. Effect of diet and Helicobacter pylori infection to the risk of early gastric cancer. *J Epidemiol* 2003; **13:** 162-8. doi: 10.2188/jea.13.162

76. Loh JT, Torres VJ, Cover TL. Regulation of Helicobacter pylori cagA expression in response to salt. *Cancer Res* 2007; **67:** 4709-15. doi: 10.1158/0008-5472.CAN-06-4746

77. Takahashi M, Nishikawa A, Furukawa F, Enami T, Hasegawa T, Hayashi Y. Dose-dependent promoting effects of sodium chloride (NaCl) on rat glandular stomach carcinogenesis initiated with N-methyl-N'-nitro-N-nitrosoguanidine. *Carcinogenesis* 1994; **15:** 1429-32. doi: 10.1093/carcin/15.7.1429

78. Cross AJ, Pollock JRA, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res* 2003; **63:** 2358-60.

79. Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc* 2008; **67:** 253-6. doi: 10.1017/S002966510800712X

80. Ta G, Yi K, Mc S, Mv G, W S, S M, et al. Folic acid increases global DNA methylation and reduces inflammation to prevent Helicobacter-associated gastric cancer in mice. *Gastroenterology* 2012; **142:** 824-33.e7. doi: 10.1053/j.gastro.2011.12.058

81. Yanaoka K, Oka M, Ohata H, Yoshimura N, Deguchi H, Mukoubayashi C et al. Eradication of Helicobacter pylori prevents cancer development in subjects with mild gastric atrophy identified by serum pepsinogen levels. *Int J Cancer* 2009; **125:** 2697-703. doi: 10.1002/ijc.24591

82. Sung JJ, Lin SR, Ching JY, Zhou LY, To KF, Wang RT, et al. Atrophy and intestinal metaplasia one year after cure of H. pylori infection: a prospective, randomized study. *Gastroenterology* 2000; **119:** 7-14. doi: 10.1053/gast.2000.8550

83. Kiriyama Y, Tahara T, Shibata T, Okubo M, Nakagawa M, Okabe A, et al. Gastric-and-intestinal mixed intestinal metaplasia is irreversible point with eradication of Helicobacter pylori. *Open Journal of Pathology* 2016; **6:** 93-104. doi: 10.4236/ojpathology.2016.62012

84. Satoh K, Kimura K, Takimoto T, Kihira K. A follow-up study of atrophic gastritis and intestinal metaplasia after eradication of Helicobacter pylori. *Helicobacter* 1998; **3:** 236-40. PMID: 9844064

85. Kodama M, Okimoto T, Ogawa R, Mizukami K, Murakami K. Endoscopic atrophic classification before and after H. pylori eradication is closely associated with histological atrophy and intestinal metaplasia. *Endosc Int Open* 2015; **3:** E311-7. doi: 10.1055/s-0034-1392090

86. Lu B, Chen M-T, Fan Y-H, Liu Y, Meng L-N. Effects of Helicobacter pylori eradication on atrophic gastritis and intestinal metaplasia: a 3-year follow-up study. *World J Gastroenterol* 2005; **11:** 6518-20. doi: 10.3748/wjg.v11.i41.6518

87. Zhou L, Sung JJY, Lin S, Jin Z, Ding S, Huang X, et al. A five-year follow-up study on the pathological changes of gastric mucosa after H. pylori eradication. *Chin Med J (Engl)* 2003; **116:** 11-4. PMID: 12667379

88. Lee Y-C, Chiang T-H, Chou C-K, Tu Y-K, Liao W-C, Wu M-S, et al. Association between helicobacter pylori eradication and gastric cancer incidence: a systematic review and meta-analysis. *Gastroenterology* 2016; **150:** 1113-24.e5. doi: 10.1053/j.gastro.2016.01.028

89. Akbari M, Tabrizi R, Kardeh S, Lankarani KB. Gastric cancer in patients with gastric atrophy and intestinal metaplasia: a systematic review and meta-analysis. *PLoS One* 2019; **14:** e0219865. doi: 10.1371/journal.pone.0219865

90. Huang KK, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan ALK, et al. Genomic and Epigenomic profiling of high-risk intestinal metaplasia reveals molecular determinants of progression to gastric cancer. *Cancer Cell* 2018; **33:** 137-50.e5. doi: 10.1016/j.ccell.2017.11.018

91. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. *Adv Nutr* 2012; **3:** 21-38. doi: 10.3945/an.111.000992

92. Zhu S, Mason J, Shi Y, Hu Y, Li R, Wahg M, et al. The effect of folic acid on the development of stomach and other gastrointestinal cancers. *Chin Med J (Engl)* 2003; **116:** 15-9. PMID: 12667380

93. Lei J, Ren F, Li W, Guo X, Liu Q, Gao H, et al. Use of folic acid supplementation to halt and even reverse the progression of gastric precancerous conditions: a meta-analysis. *BMC Gastroenterol* 2022; **22:** 370. doi: 10.1186/s12876-022-02390-y

94. Tan M, Jamali T, Nguyen TH, Galvan A, Sealock RJ, Khan A, et al. Race/ethnicity and birthplace as risk factors for gastric intestinal metaplasia in a multiethnic united states population. *Am J Gastroenterol* 2022; **117:** 280-7. doi: 10.14309/ajg.0000000000001576

95. Akpoigbe K, Culpepper-Morgan J, Nwankwo O, Genao A. Predicting gastric intestinal metaplasia in a high-risk population. *Cureus* 2022; **14:** e31502. doi: 10.7759/cureus.31502

96. Aumpan N, Vilaichone Rk, Pornthisarn B, Chonprasertsuk S, Siramolpiwat S, et al. Predictors for regression and progression of intestinal metaplasia (IM): a large population-based study from low prevalence area of gastric cancer (IM-predictor trial). *PLoS One* 2021 **16:** e0255601. doi: 10.1371/journal.pone.0255601

97. Tan MC, Mallepally N, Liu Y, El-Serag HB, Thrift AP. Demographic and lifestyle risk factors for gastric intestinal metaplasia among US veterans. *Am J Gastroenterol* 2020; **115:** 381-7. doi: 10.14309/ajg.0000000000000498

98. Aumpan N, Vilaichone RK, Nunanan P, Chonprasertsuk S, Siramolpiwat S, et al. Predictors for development of complete and incomplete intestinal metaplasia (IM) associated with H. pylori infection: a large-scale study from low prevalence area of gastric cancer (IM-HP trial). *PLoS One* 2020; **15:** e0239434. doi: 10.1371/journal.pone.0239434

99. Leung WK, Ng EK, Chan WY, Auyeung AC, Chan KF, Lam CC, et al. Risk factors associated with the development of intestinal metaplasia in first-degree relatives of gastric cancer patients. *Cancer Epidemiol Biomarkers Prev* 2005; **14:** 2982-86. doi: 10.1158/1055-9965.EPI-05-0181

100. Joo YE, Park HK, Myung DS, Baik GH, Shin JE, Seo GS, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia: a nationwide multicenter prospective study in Korea. *Gut Liver* 2013; **7:** 303-10. doi: 10.5009/gnl.2013.7.3.303

101. Tatsuta M, Iishi H, Nakaizumi A, Okuda S, Taniguchi H, Hiyama T, et al. Fundal atrophic gastritis as a risk factor for gastric cancer. *Int J Cancer* 1993; **53:** 70-4. doi: 10.1002/ijc.2910530114

102. Nguyen TH, Tan MC, Liu Y, Rugge M, Thrift AP, El-Serag HB. Prevalence of gastric intestinal metaplasia in a multiethnic US veterans' population. *Clin Gastroenterol Hepatol* 2021; **19:** 269-76. doi: 10.1016/j.cgh.2020.03.015

103. Nieuwenburg SAV, Mommersteeg MC, Eikenboom EL, Yu B, den Hollander WJ, Holster IL, et al. Factors associated with the progression of gastric intestinal metaplasia: a multicenter, prospective cohort study. *Endosc Int Open* 2021; **9:** 297-305. doi: 10.1055/a-1314-6626

104. Reddy KM, Chang JI, Shi JM, Wu BU. Risk of gastric cancer among patients with intestinal metaplasia of the stomach in a US integrated health care system. *Clin Gastroenterol Hepatol* 2016; **14:** 1420-5. doi: 10.1016/j.cgh.2016.05.045

105. Holmes HM, Jove AG, Tan MC, El-Serag HB, Thrift AP. Alcohol consumption and the risk of gastric intestinal metaplasia in a U.S. Veterans population *PLoS One* 2021; **16:** e0260019. doi: 10.1371/journal.pone.0260019. PMID: 34780551

106. Kim K, Chang Y, Ahn J, Yang HJ, Ryu S. Low levels of alcohol consumption and risk of intestinal metaplasia: a cohort study. *Cancer Epidemiol Biomarkers Prev* 2020; **29:** 2633-41. doi: 10.1158/1055-9965.EPI-20-0858

107. Morais S, Rodrigues S, Amorim L, Peleteiro B, Lunet N. Tobacco smoking and intestinal metaplasia: systematic review and meta-analysis. *Dig Liver Dis* 2014; **46:** 1031-7. doi: 10.1016/j.dld.2014.08.034

108. Thrift AP, Jove AG, Liu Y, Tan MC, El-Serag HB. Associations of duration, intensity, and quantity of smoking with risk of gastric intestinal metaplasia. *J Clin Gastroenterol* 2022; **56:** e71-e6. doi: 10.1097/MCG.0000000000001479

109. Mao Y, Yang W, Qi Q, Yu F, Wang T, Zhang H, et al. Blood groups A and AB are associated with increased gastric cancer risk: evidence from a large genetic study and systematic review. *BMC Cancer* 2019; **19:** 164. doi: 10.1186/s12885-019-5355-4

110. Rizzato C, Kato I, Plummer M, Muñoz N, Stein A, Jan van Doorn L, et al. Risk of advanced gastric precancerous lesions in Helicobacter pylori infected subjects is influenced by ABO blood group and cagA status. *Int J Cancer* 2013; **133:** 315-22. doi: 10.1002/ijc.28019

111. Wang M, Lou E, Xue Z. The role of bile acid in intestinal metaplasia. *Front Physiol* 2023; **14:** 1115250. doi: 10.3389/fphys.2023.1115250

112. Yu J, Zheng J, Qi J, Yang K, Wu Y, Wang K, et al. Bile acids promote gastric intestinal metaplasia by upregulating CDX2 and MUC2 expression via the FXR/NF-κB signalling pathway. *Int J Oncol* 2019; **54:** 879-92. doi: 10.3892/ijo.2019.4692

113. Dias-Neto M, Pintalhao M, Ferreira M, Lunet N. Salt intake and risk of gastric intestinal metaplasia: systematic review and meta-analysis. *Nutr Cancer* 2010; **62:** 133-47. doi: 10.1080/01635580903305391

114. Song JH, Kim YS, Heo NJ, Lim JH, Yang SY, Chung GE, et al. high salt intake is associated with atrophic gastritis with intestinal metaplasia. *Cancer Epidemiol Biomarkers Prev* 2017; **26:** 1133-8. doi: 10.1158/1055-9965.EPI-16-1024

115. Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc* 2008; **67:** 253-6. doi: 10.1017/S002966510800712X

116. Jencks DS, Adam JD, Borum ML, Koh JM, Stephen S, Doman DB. Overview of current concepts in gastric intestinal metaplasia and gastric cancer. *Gastroenterol Hepatol (N Y)* 2018; **14:** 92-101. PMID: 29606921

research article

Utility of clinical and MR imaging parameters for prediction and monitoring of response to capecitabine and temozolomide (CAPTEM) therapy in patients with liver metastases of neuroendocrine tumors

Maria Ingenerf[1], Christoph Auernhammer[2,3], Roberto Lorbeer[1], Michael Winkelmann[1], Shiwa Mansournia[1], Nabeel Mansour[1], Nina Hesse[1], Kathrin Heinrich[4], Jens Ricke[1,2], Frank Berger[1], Christine Schmid-Tannwald[1,2]

[1] Department of Radiology, University Hospital, LMU Munich, Germany;
[2] ENETS Centre of Excellence, Interdisciplinary Center of Neuroendocrine Tumours of the GastroEnteroPancreatic System at the University Hospital of Munich (GEPNET-KUM), University Hospital of Munich, Munich, Germany
[3] Department of Internal Medicine 4, University Hospital, LMU Munich, Munich, Germany
[4] Department of Medicine III, University Hospital, University of Munich, Munich, Germany

Radiol Oncol 2024; 58(2): 196-205.

Received 8 December 2023
Accepted 20 February 2024

Correspondence to: Christine Schmid-Tannwald, Ph.D., M.D., Department of Radiology, University Hospital, LMU Munich, Germany; Email: Christine.schmid-tannwald@med.uni-muenchen.de

Disclosure: No potential conflicts of interest were disclosed.

Background. This study explores the predictive and monitoring capabilities of clinical and multiparametric MR parameters in assessing capecitabine and temozolomide (CAPTEM) therapy response in patients with neuroendocrine tumors (NET).

Patients and methods. This retrospective study (n = 44) assessed CAPTEM therapy response in neuroendocrine liver metastases (NELM) patients. Among 33 monitored patients, as a subgroup of the overall study cohort, pretherapeutic and follow-up MRI data (size, apparent diffusion coefficient [ADC] values, and signal intensities), along with clinical parameters (chromogranin A [CgA] and Ki-67%), were analyzed. Progression-free survival (PFS) served as the reference. Responders were defined as those with PFS ≥ 6 months.

Results. Most patients were male (75%) and had G2 tumors (76%) with a pancreatic origin (84%). Median PFS was 5.7 months; Overall Survival (OS) was 25 months. Non-responders (NR) had higher Ki-67 in primary tumors (16.5 *vs.* 10%, p = 0.01) and increased hepatic burden (20% *vs.* 5%, p = 0.007). NR showed elevated CgA post-treatment, while responders (R) exhibited a mild decrease. ADC changes differed significantly between groups, with NR having decreased ADCmin (-23%) and liver-adjusted ADCmean/ADCmean liver (-16%), compared to R's increases of ADCmin (50%) and ADCmean/ADCmean liver (30%). Receiver operating characteristic (ROC) analysis identified the highest area under the curve (AUC) (0.76) for a single parameter for Δ ADC mean/ liver ADCmean, with a cut-off of < 6.9 (76% sensitivity, 75% specificity). Combining Δ Size NELM and Δ ADCmin achieved the best balance (88% sensitivity, 60% specificity) outperforming Δ Size NELM alone (69% sensitivity, 65% specificity). Kaplan-Meier analysis indicated significantly longer PFS for Δ ADCmean/ADCmean liver < 6.9 (p = 0.024) and Δ Size NELM > 0% + Δ ADCmin < -2.9% (p = 0.021).

Conclusions. Survival analysis emphasizes the need for adapted response criteria, involving combined evaluation of CgA, ADC values, and tumor size for monitoring CAPTEM response in hepatic metastasized NETs.

Key words: neuroendocrine tumors; liver metastases; CAPTEM therapy; clinical parameters; MR imaging; treatment response

Introduction

Neuroendocrine tumors (NETs) encompass a diverse group of neoplasms originating from neuroendocrine cells, with a predilection for the gastrointestinal (GI) tract, pancreas, and pulmonary system.[1] Their indolent progression often leads to delayed diagnosis, rendering curative surgical resection unfeasible.

Among the therapeutic options for metastatic or progressive cases, Capecitabine and Temozolomide (CAPTEM) chemotherapy has emerged as an effective and safe systemic regimen, particularly benefiting patients with well-differentiated pancreatic NETs.[2,3] Response rates range widely from 17% to 70%, and progression-free survival (PFS) spans 4 to 38.5 months.[1,4-6] Previous investigations into clinical biomarkers like O6-methylguanine DNA methyltransferase (MGMT) expression, alternative lengthening of telomeres (ALT) activation, and Ki-67 index have yielded conflicting results.[1,7] Thus, the imperative arises for predictive biomarkers to mitigate treatment failures and needless exposure to toxicity.[1] As such, there is a growing interest in evaluating imaging parameters for prognostic and monitoring purposes in oncologic therapies.

In addition to morphological changes like tumor size, MRI has the capability to display structural and functional data such as diffusion-weighted imaging (DWI). Incorporating both morphological and functional data, multiparametric MRI could offer a more comprehensive insight into subtle shifts in tumor behavior, especially in small growing tumors such as NET. Parameters such as signal intensity (SI) on T1-weighted or T2-weighted images, tumor vascularization, and apparent diffusion coefficient (ADC) derived from DWI are increasingly scrutinized for their predictive and monitoring potential across various therapy regimens.[8-11] Notably, no prior study has assessed the utility of these MRI parameters for monitoring therapy or predicting CAPTEM response in patients with hepatic metastasized NETs. Therefore, this study aims to evaluate clinical, morphological, and functional imaging factors for their ability to predict and monitor therapy response in metastatic NET patients undergoing CAPTEM treatment.

Patients and methods

Patients

This retrospective study received approval from the local research ethics committee with decision Number 23-0183 and the requirement for written informed patient consent was waived. We consecutively enrolled patients with histologically confirmed, resected or advanced NETs with liver metastases, all of whom received CAPTEM therapy and underwent pretherapeutic MRI at our department. Furthermore, in the sub-analysis focused on therapy monitoring, we incorporated all individuals from this cohort who underwent subsequent MRI examinations (Figure 1). The timeframe for therapy initiation ranged from April 2013 to June 2022. The decision to commence CAPTEM therapy was reached through consensus in an interdisciplinary tumor conference certified for NETs (ENETS Center of Excellence) for each patient.

MR imaging

All patients were positioned supine in a 1.5 T MR system (Siemens Healthcare, Erlangen, Germany). For signal reception a phased-array coil was utilized. Images were acquired in accordance with our standard liver imaging protocol. The following sequences were employed for evaluation:

1. A single shot T2-weighted sequence (HASTE).
2. T1-weighted 3D GRE sequences with fat suppression (VIBE) prior to and at 20, 50, and 120 seconds (dependent on circulation time) post intravenous contrast injection (EOB- Bayer Pharma, Germany; 25 µmol/kg body weight).
3. Diffusion-weighted sequences with b-values of 50 and 800 s/mm².

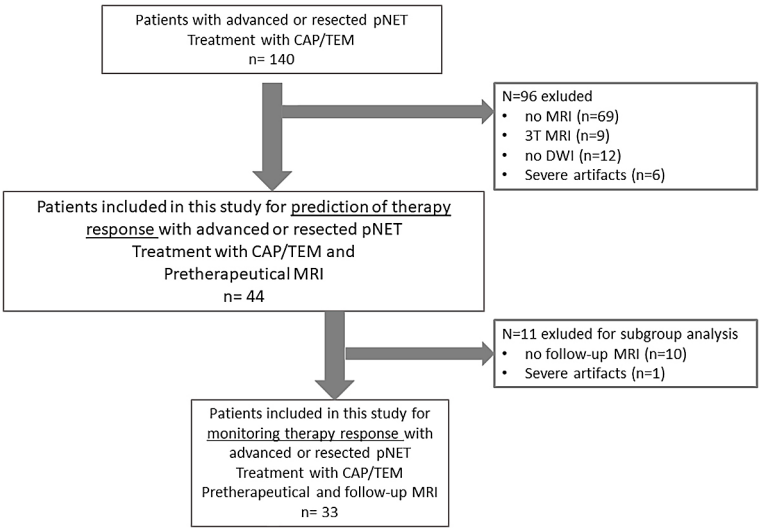


FIGURE 1. Flow-chart of including process of patients.

CAP/TEM = capecitabine and temozolomide; DWI = diffusion-weighted imaging

TABLE 1. Patients characteristics

	Baseline N = 44	Follow-Up N = 33
Age (years)	60.4 (50.5; 70.2)	
Males	33 (75.0%)	
Time initial diagnosis – therapy start	685 (199; 1230)	
Clinical parameter		
Hepatic tumor burden (%)	10 (5 ;40)	
CgA (ng/ml)	610 (119; 2093)	647 (261; 2357)
Bilirubin (mg/dl)	0.6 (0.4; 0.8)	0.7 (0.6; 0.9)
Grading		
1	1 (2.4%)	
2	32 (76.2%)	
3	6 (14.3%)	
NEC = 4	3 (7.1%)	
Ki-67 primary tumor (%)	15 (8;20)	
Localization primary tumor		
Pancreas	37 (84.1%)	
Lung	7 (15.9%)	
MRI parameter		
NELM		
Size (mm)	28 (19;36)	24.5 (18;38.5)
T1 non-contrast/T1 liver	0.62 (0.53;0.68)	0.68 (0.56;0.75)
T2/T2 liver	1.63 (1.16;2.07)	1.66 (1.21;2.17)
ADCmin	448.5 (242.5;628.5)	549 (341;848)
ADCmean	903 (708.5;1069.5)	969 (764;1250)
ADCmin/ADCmin liver	0.80 (0.60;0.93)	0.85 (0.51;1.32)
ADCmean/ADCmean liver	0.82 (0.74;0.96)	0.99 (0.65;1.32)
% arterial vascularization	42.5 (15;80)	22.5 (5;74.5)**
PNET		
Size (mm)	43 (32;70)	43 (29.5;52)
T1 non-contrast /T1 pancreas	0.63 (0.59;0.76)	0.68 (0.61;0.84)
T2/T2 pancreas	1.38 (0.85;1.67)	1.08 (0.83;1.34)
ADCmin	604.5 (237;648)	628 (499.5;758.5)
ADCmean	985 (810;1150)	1042.5 (939;1167)
ADCmin/ADCmin pancreas	0.69 (0.41;1.11)	0.73 (0.58;0.85)
ADCmean/ADCmean pancreas	1.01 (0.78;1.19)	0.89 (0.72;0.97)
% arterial vascularization	15 (10;80)	7 (5;45)

Data are given as median (25th and 75th percentile) or number (percentage); *p < 0.05; **p < 0.01; ***p < 0.001 from Wilcoxon signed-rank test; ADC = apparent diffusion coefficient; CgA = chromogranin A; d = days; NEC = neuroendocrine cancer; NELM = neuroendocrine liver metastasis; PNET = pancreatic neuroendocrine tumor

4. After a 15-minute delay, a fat-suppressed T1-weighted VIBE 3D GRE sequence identical to the earlier one.

All sequences utilized parallel imaging with an acceleration factor of 2. ADC maps were computed from the acquired DWI-MR images, incorporating all b-values.

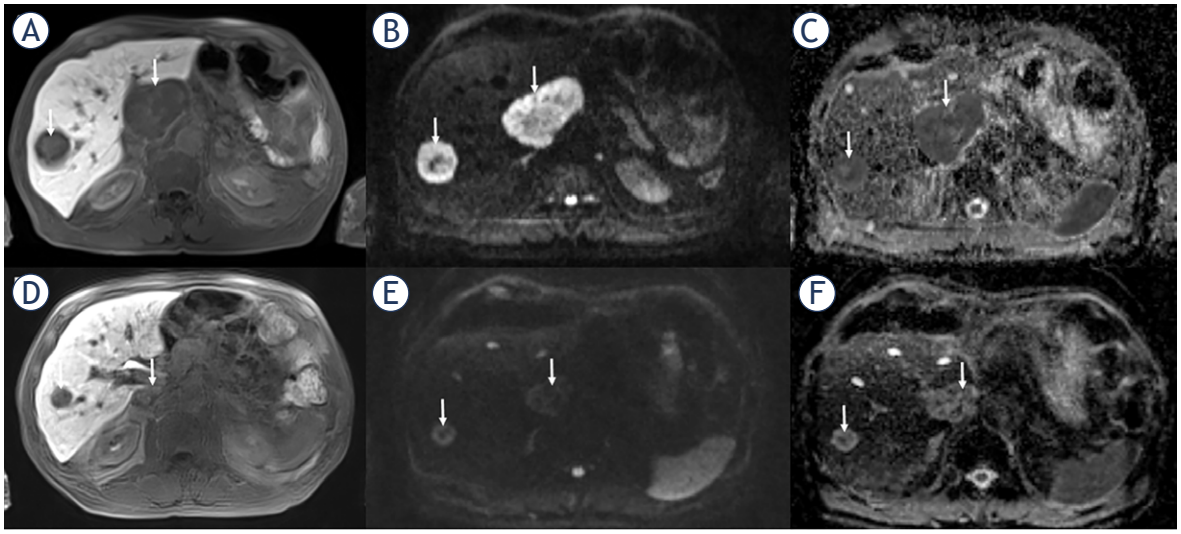


FIGURE 2. A 72-year-old man with liver metastasis of pancreatic NET classified as responder with a PFS of 38 months. The baseline axial contrast-enhanced T1- weighted image (hepatobiliary phase) **(A)** shows hypointense lesions (arrows) in segment 8 and exophytic in segment 1. The metastases show **(B)** restricted diffusion (arrows) with high signal on axial DW-MR image b = 800 s/mm^2 and dark signal (arrows) on ADC map **(C)**. After initiation of CAPTEM, the metastases (arrows) exhibited a decrease in size **(D)** On the axial DW-MR image b = 800 s/mm^2, the metastasis (arrow) **(E)** demonstrated less hyperintense signal to liver and predominantly hyperintense signal (circle) on the ADC map **(F)** indicating less restricted diffusion compared to the pre-interventional image.

ADC = apparent diffusion coefficient; CAPTEM = capecitabine and temozolomide; DW-MR = diffusion-weighted magnetic resonance; NET = neuroendocrine tumor; PFS = progression-free survival; PR =partial remission; TARE = transarterial radioembolization

Image analysis

Two board-certified radiologists, blinded to the patients' clinical and follow-up data, reviewed all MRI data in consensus. They randomly identified, on the pretherapeutic MRI, two hepatic metastases per patient that were larger than 1 cm in size, along with the primary tumor if it hadn't been previously resected. Inclusion criteria for metastases encompassed a homogeneous appearance and absence of artifacts within the lesion across all sequences. The image review took place in two separate sessions, both achieving consensus: 1) pretherapeutic MRI, and for the sub-analysis 2) post-therapeutic MRI, with a three-week interval between each session.

For quantitative analysis, the size of liver metastases and NETs were measured on the hepatobiliary and arterial phases, respectively. ADCmean and ADCmin values of the tumorous lesions were calculated by manually placing circular regions-of-interest (ROIs) on the slice with the largest tumor extent on DWI, excluding structures near the rim to avoid partial volume effects. Signal intensity (SI) values on non-contrast T1-weighted and T2-weighted images were recorded by outlining ROIs of the lesions as large as possible. Percentage of arterial enhancement was visually assessed by the two radiologists in consensus. Additionally, ADC mean and ADC min values, as well as T2-weighted and T1-weighted SI values of the normal liver, pancreas, and spleen, were measured by placing circular ROIs in tumor-free tissue areas. Additionally, SI of the normal liver was measured on the hepatobiliary phase. Tumor-to-organ ratios, including tumor-to-spleen (T/S) ratio and tumor-to-liver (T/L) ratio of SI and ADC, were calculated.

Standard of reference and response to treatment

Clinical and surgical records were compiled by a third radiologist. Histopathological confirmed diagnoses of NET, along with their respective Ki-67 indices, were obtained for each patient. Tumor grading adhered to the 2017 WHO Tumor Classification Guideline (G1: Ki-67 Index < 3%, G2: Ki-67 Index 3–20%, and G3 neuroendocrine tumor/neuroendocrine cancer [NET/NEC]: Ki-67 Index > 20%). Given that the primary tumor was resected in 31 out of 44 patients, rendering RECIST 1.1. assessment of treatment response heterogeneous, evaluation of

TABLE 2. Differences in baseline clinical and imaging tumor parameters between responder and non-responder

	Non-responder (< 6 months PFS) N = 23	Responder (≥ 6 months PFS) N = 21	p-value
Age	57.8 (44.1;71.1)	61.7 (55.8;68.8)	0.953
Males	16 (69.6%)	17 (81.0%)	0.494
Time ID – Therapy start (d)	851 (426;1552)	396 (153;1004)	0.115
Clinical parameter			
Hepatic tumor burden (%)	5 (5;20)	20 (10;40)	**0.007**
CgA	592 (116;2031)	616 (156.5;2745)	0.706
Bilirubin	0.6 (0.4;0.8)	0.6 (0.3;0.9)	0.859
Grading			0.234
1	0 (0%)	1 (5%)	
2	15 (68.2%)	17 (85%)	
3	4 (18.2%)	2 (10%)	
NEC = 4	3 (13.6%)	0 (0%)	
Ki-67 primary tumor (%)	16.5 (10;30)	10.0 (5;15)	**0.013**
Localization primary tumor			0.232
Pancreas	21 (91.3%)	16 (76.2%)	
Lung	2 (8.7%)	5 (23.8%)	
MRI parameter			
NELM			
Size (mm)	25.5 (17;33.5)	29.8 (21.8;37.5)	0.348
T1 non-contrast/T1 liver	0.60 (0.53;0.68)	0.64 (0.54;0.74)	0.263
T2/T2 liver	1.62 (1.2;2.07)	1.69 (1.12;2.06)	0.903
ADCmin	506 (228;639)	424 (243;606)	0.827
ADCmean	852.5 (674;1059)	911 (790.5;1082.5)	0.495
ADCmin/ADCmin liver	0.80 (0.63;0.93)	0.74 (0.51;1.03)	0.846
ADCmean/ADCmean liver	0.82 (0.68;0.93)	0.86 (0.78;1.02)	0.342
% arterial vascularization	45 (15;85)	36.3 (15;72.5)	0.494
PNET			
Size (mm)	38 (30;44)	75.5 (65;85.5)	**0.024**
T1 non-contrast /T1 pancreas	0.60 (0.58;0.71)	0.71 (0.63;0.8)	0.258
T2/T2 pancreas	1.38 (0.84;1.67)	1.38 (1.11;1.5)	0.777
ADCmin	604.5 (237;648)	527 (316.5;698)	1.000
ADCmean	893 (789;1055)	1084 (996.5;1256)	0.157
ADCmin/ADCmin pancreas	0.79 (0.41;1.18)	0.63 (0.44;0.8)	0.480
ADCmean/ADCmean pancreas	1.09 (0.66;1.31)	0.97 (0.96;1.1)	0.888
% arterial vascularization	10 (5;70)	65 (30;85)	0.130

Data are given as median (25th and 75th percentile); p-values are from Wilcoxon rank-sum (Mann-Whitney) test or Fisher's exact test; ADC = apparent diffusion coefficient; CgA = chromogranin A; NELM = neuroendocrine liver metastasis; PFS = progression-free survival; PNET = pancreatic neuroendocrine tumor

treatment response was conducted through PFS. This was measured in months from the initiation of CAPTEM until progression, as determined by the local interdisciplinary tumor board's comprehensive assessment of all performed imaging studies (CT, PET/CT, MRI). Responders were defined by

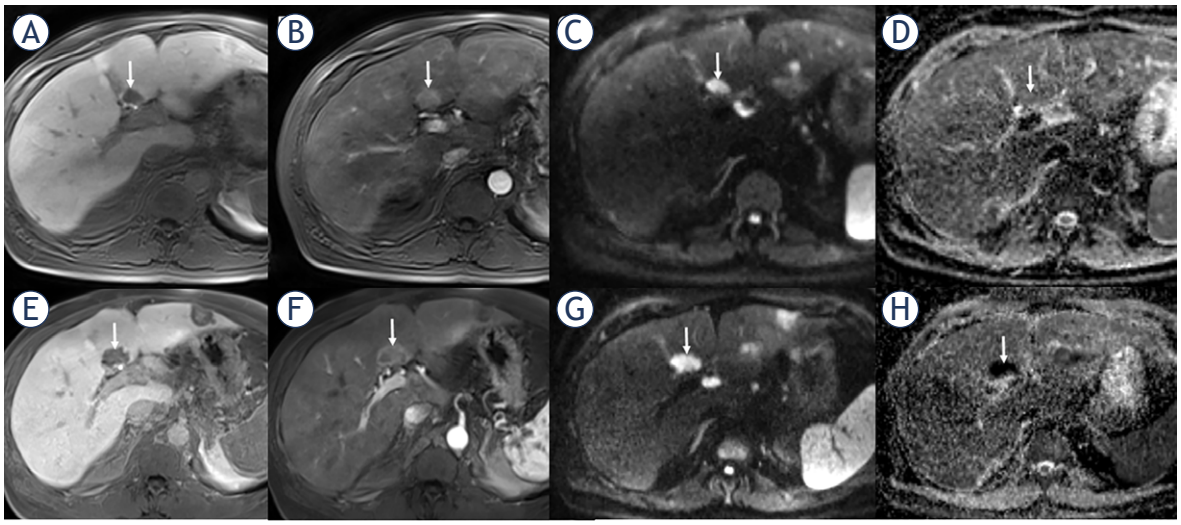


FIGURE 3. A 56-year-old man with liver metastasis of pancreatic NET classified as nonresponder with a PFS of 3 months. The baseline axial contrast-enhanced T1- weighted image (hepatobiliary phase) **(A)** shows a hypointense lesion (arrow) in segment 4A. The metastasis shows a strong artrerial enhancement **(B)** and restricted diffusion (arrow) with high signal on axial DW-MR image b = 800 s/mm^2 **(C)** and dark signal (arrow) on ADC map **(D)**. After 3 months under CAPTEM, the metastasis (arrow) **(E)** exhibited an increase in size; however, it shows less arterial enhancement **(F)**. On the axial DW-MR image b = 800 s/mm^2, the metastasis (arrow) demonstrated hyperintense signal to liver and increasing hypointense signal on the ADC map indicating increasing restricted diffusion compared to the baseline image ADC, apparent diffusion coefficient.

ADC = apparent diffusion coefficient; CAPTEM = capecitabine and temozolomide; DW-MR = diffusion-weighted magnetic resonance; NET = neuroendocrine tumor; PFS = progression-free survival

PFS ≥ 6 months, while non-responders (NR) were defined by PFS < 6 months, respectively.

Statistical analysis

Continuous data were summarized by median with interquartile range (IQR) and categorical data by numbers and percentages. Differences between baseline and follow-up parameters were assessed by Wilcoxon signed-rank test for paired samples. Differences of baseline characteristics and parameter changes until follow-up between non-responder and responder were investigated by Wilcoxon rank-sum test for unpaired samples or Fisher's exact test. The area under the receiver operating characteristic (ROC) curve (AUC) was estimated according to logistic regression models predicting non-responder by selected imaging and clinical parameters. Two AUC values were compared by chi^2-test. Sensitivity, specificity, and the Youden-Index were calculated for median-dichotomized parameters. Overall survival (OS) and PFS curves with median survival times were calculated by Kaplan-Meier analysis and compared by log rank-test between individuals separated by the median for selected parameters. Individuals were censored in case of death, progression or end of study. A p-value < 0.05 was considered to indicate statistical significance. All analyses were conducted with Stata 16.1 (Stata Corporation, College Station, TX, U.S.A.).

Results

Patients' characteristics

A total of 44 patients, comprising 86 neuroendocrine liver metastases (NELM) and 14 primary pancreatic NETs were included for the evaluation of prognostic factors for PFS. A subset of 33 patients, with corresponding 66 NELM and 12 pNETs, was identified for the sub-analysis of therapy monitoring. Baseline MRI scans were obtained 19d (IQR 1; 61) prior to CAPTEM initiation, and the time interval between baseline MRI and follow-up MRI was 130 days (IQR 113; 161). Most patients were male (75%), had G2 tumors (76%), and the primary tumor originated in the pancreas (84%). Detailed patient characteristics are presented in Table 1.

In the baseline cohort, the overall median PFS was 5.7 months (IQR 3.6; 15.0), and median OS was 25.0 months (interquartile range [IQR] 16.3; 45.3). Responder in the baseline group tended to have a slightly longer median OS 35.0 m (IQR 19.4; 53.4)

TABLE 3. Differences in change of clinical and imaging tumor parameters between responder and non-responder

Change between baseline and follow-up (%)	Non-responder (< 6 months PFS) N = 17	Responder (≥ 6 months PFS) N = 16	p-value
Clinical parameter			
CgA	61.2 (-8.3;251.9)	-1.5 (-69.3;19)	**0.036**
Bilirubin	0 (-20;40)	8.3 (-15.3;133.3)	0.312
MRI parameter			
NELM			
Size (mm)	20 (-4.7;50)	-8.0 (-20.1;2.2)	**0.038**
T1 non-contrast/T1 liver	5.4 (-3.8;32.6)	-6.8 (-13.6;11.2)	0.078
T2/T2 liver	1.6 (-9.2;24.1)	-5.7 (-26.2;32.8)	0.589
ADCmin	-22.8 (-41.1;40.2)	49.7 (-6.7;146.4)	**0.037**
ADCmean	-3.5 (-18.4;14.1)	11.7 (-3.4;75.4)	**0.056**
ADCmin/ADCmin liver	-32.3 (-46.2;70.8)	47.5 (12.7;251.7)	0.113
ADCmean/ADCmean liver	-16.3 (-30.6;6.9)	30.0 (6.9;90.4)	**0.011**
% arterial vascularization	-16.7 (-75;-5.9)	-16.7 (-50.0;11.8)	0.298
PNET			
Size (mm)	2.3 (-5.4;20)	-55 (-60;-17.8)	**0.013**
T1 non-contrast /T1 pancreas	7.4 (-3.8;36.7)	-5 (-19.7;1.9)	0.116
T2/T2 pancreas	-16.6 (-22;1.2)	-36.1 (-40.3;-10.1)	0.229
ADCmin	14.4 (-13.7;260.8)	18.7 (-33.2;48.9)	0.782
ADCmean	8.3 (-4.5;29.3)	4.0 (-26.3;4.6)	0.405
ADCmin/ADCmin pancreas	-3.6 (-29;76.6)	53 (-18.4;80.9)	0.518
ADCmean/ADCmean pancreas	-23.2 (-35.5;4.5)	-5.7 (-14.3;0.2)	0.518
% arterial vascularization	-50 (-80;0)	-50 (-80;0)	0.851

Data are given as median (25th and 75th percentile); p-values are from Wilcoxon rank-sum (Mann-Whitney) test; ADC = apparent diffusion coefficient; CgA = chromogranin A; NELM = neuroendocrine liver metastasis; PFS = progression-free survival; PNET = pancreatic neuroendocrine tumor

compared to non-responders, with a median OS 21.4 month (IQR 15.0; 38.3). According to RECIST 1.1,21 patients were rated as stable disease (SD), 3 patients were rated as partial response, and 9 patients were graded as progressive disease.

When comparing baseline and follow-up parameters, no differences were observed, except for arterial vascularization of NELM, which was significantly lower at follow-up time.

Differences between non-responders (NR) and responders (R) at baseline

The comparison of baseline clinical and imaging parameters between the two response groups revealed that NR had a significantly higher Ki-67 of the primary tumor (16.5% *vs.* 10.0%, p = 0.01) with three patients graded as neuroendocrine cancer (NEC) in the NR group (none in the R group). Responders showed a significantly higher hepatic tumor burden (20% *vs.* 5%, p = 0.007). There were no differences in imaging parameters of the NELM, while for the pNETs size varied significantly between response groups with greater diameters of the baseline pNET in R compared to NR (76 mm *vs.* 38 mm, p = 0.02). However, the statistical evaluation of pNET was limited by the small number of patients with non-resected pNET in our cohort (14 and 12 respectively).

Differences of parameter change between non-responders (NR) and responders (R)

After treatment initiation there was a significant difference in the change of chromogranin A (CgA) between response groups, with an increase in NR compared to a mild decrease in R (61% *vs.* -2%, p < 0.04). Regarding imaging parameters, there were significant differences in the changes of the size of both NELM (20% *vs.* -8%, p = 0.038) and pNET (2% *vs.* -55% p < 0.013) between the two response groups.

Additionally, changes of ADC in NELM differed significantly between response groups, with a decrease in both ADCmin (-23%) and the liver adjusted ADCmean / ADCmean liver ratio (-16%) in NR, compared to an increase in R of both ADCmin (50%) and ADCmean / ADCmean liver (30%). Notably there were no differences in changes in arterial vascularization and signal intensity (SI) on T1w and T2w images between response groups.

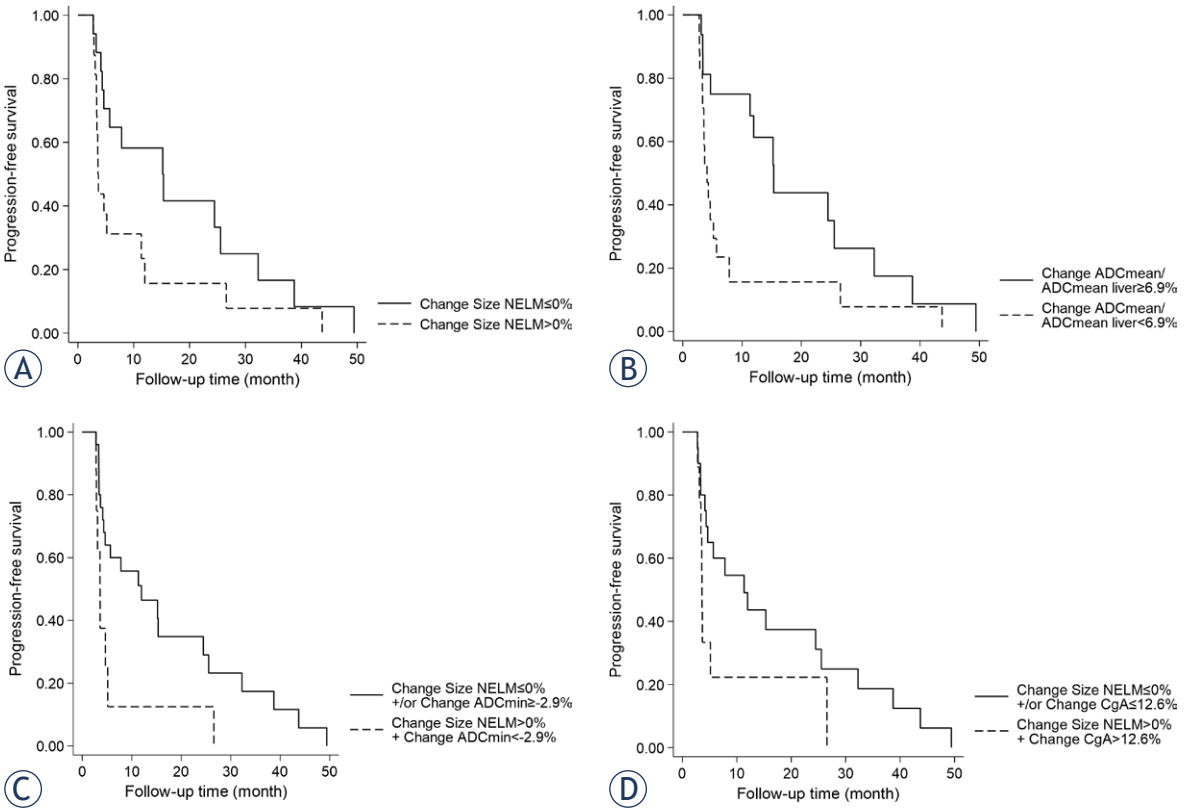


FIGURE 4. (A) Survival analysis for Δ size of NELM with a cut-off of ≤ 0% for responder. This cut-off revealed a slightly longer median PFS time of 12.2 *vs*. 3.6 month (p = 0.062). **(B)** The median cut-off for Δ ADCmean/ADCmean liver showed a significantly longer median PFS time of 15.3 compared to 4.1 month (p = 0.024). Both the combination of Δ size of NELM > 0% and Δ ADCmin < - 2.9% and the combination of Δ size of NELM > 0% and Δ CgA > 12.6% could differentiate patients with a longer median PFS time. Median PFS of the group with Δ size of NELM > 0% and Δ ADCmin < -2.9% was 3.6 m compared to 12 months (p = 0.021) in the group not fulfilling these criteria or a maximum of one criterion. Median PFS of the group with Δ size of NELM > 0% and Δ CgA > 12.6% was 3.6 m compared to 11.3 months (p = 0.072) in the group not fulfilling these criteria or a maximum of one criterium.

ADC = apparent diffusion coefficient; CgA = chromogranin A; NELM = neuroendocrine liver metastasis; PFS = progression-free survival

ROC and survival analysis of selected clinical and imaging parameters

ROC analysis of the previously selected imaging and clinical parameters revealed AUC values differing from 0.71 (Δ Size NELM and Δ ADCmin) to 0.76 (Δ ADC mean/ Liver ADCmean) for classifying non-responders *vs*. responders. The highest AUC for a single parameter was found for Δ ADC mean/ Liver ADCmean, with a median cut-off of < 6.9 which yielded a sensitivity of 76% and a specificity of 75%. The combination of Δ Size NELM and Δ CgA or Δ ADC mean/ Liver ADCmean could each slightly, though not significantly, improve AUC (0.79 and 0.77 respectively), while the combination of Δ Size NELM and Δ ADCmin yielded the best balance for sensitivity and specificity with 88% and 60% compared to 69% and 65% respectively for Δ Size NELM alone. Subsequent Kaplan-Meier survival analysis, utilizing the respective median cut-off values (Table 4 and Figure 4) for the parameters, revealed significantly longer PFS times for Δ ADCmean/ADCmean liver < 6.9 (p = 0.024) and the combination of Δ Size NELM > 0% + Δ ADCmin < -2.9% (p = 0.021).

Discussion

In this study, we explored the utility of clinical, morphological, and functional imaging parameters in assessing the response and predicting outcomes in metastatic NETs treated with CAPTEM. Our results underscore the significance of multiparametric MRI, in conjunction with established clinical factors, for evaluating therapy response.

The median PFS in our baseline cohort was 5.7 months, which is on the lower end of the range of

TABLE 4. ROC analysis of the previously selected imaging and clinical parameters

	AUC	Cut-off (Median)	Sensitivity (%)	Specificity (%)	Youden-Index
Ki-67%	0.72	> 15	69	59	0.28
Hepatic tumor burden	0.73	< 10	84	72	0.56
Δ CgA	0.73	> 12.6	67	64	0.31
ΔSize NELM	0.71	> 0	**69**	**65**	**0.34**
Δ Size PNET	-	> -2.7	100	50	0.50
Δ ADCmin	0.71	< -2.9	65	63	0.28
Δ ADCmean/ADCmean liver	0.76	< 6.9	76	75	0.51
Δ Size NELM+ Δ CgA	**0.79**	> 0/> 12.6	78	60	0.38
Δ Size NELM+ Δ ADCmin	**0.70**	> 0/< -2.9	**88**	**60**	**0.48**
Δ Size NELM+ Δ ADCmean/ ADCmean liver	**0.77**	> 0/< 6.9	78	58	0.36

All p > 0.05; ADC = apparent diffusion coefficient; AUC = area under the curve; CgA = chromogranin A; NELM = neuroendocrine liver metastasis; PNET = pancreatic neuroendocrine tumor

the review by Arrivi *et al*., which reported a median PFS between 4 to 38.5 months.[6] Discrepancies may be attributed to the predominance of GEP-NENs (GEP-NENs) in their study. Our median OS aligned well with Arrivi *et al*. report, at 25 months, compared to their range of 8 to 108 months. Disease control rate in our cohort was consistent with the literature, at 73% versus 77%.[6]

Comparison of baseline parameters between non-responders (NR) and responders (R) revealed higher Ki-67 levels (> 15%) in NR, contrasting with some studies suggesting improved response to CAPTEM in tumors with higher Ki-67.[6,12] The applicability of Ki-67 as a predictive/prognostic biomarker for CAPTEM therapy in NETs remains controversial. Other authors suggested that there was no correlation between tumor grade, mitotic rate, or Ki-67 and tumor response to CAPTEM as the cytotoxic activity of temozolomide is not limited to mitosis but encompasses the entire cell cycle.[7,13]

Responders in our cohort exhibited a higher hepatic tumor burden at baseline, potentially indicating a better response in advanced disease stages. Follow-up analysis revealed marked CgA increases in non-responders versus mild decreases in responders. CgA is considered the most sensitive general marker for the diagnosis of NET[14], and has been shown to be associated with survival and treatment response[15-18] in follow-up, however optimal cut-offs remain controversial.[19]

Changes in size of metastases and primary tumors differed significantly between response groups, and ROC analysis showed an AUC for Δsize NELM of 0.71 with an optimal cut-off of > 0% to define non-response. Generally, we found that cut-offs for tumor progression (≥20%) or response (≥30%) according to RECIST 1.1 were barely reached in our cohort (median Δsize NELM for NR = 20%, and for R = -8%). Therefore, it is critical to adapt treatment response criteria to the rather slow evolution of most NETs to ameliorate management of NET patients and design of clinical trials with better study end points.[19]

An effort to enhance therapy response assessment included the development of mRECIST criteria, initially proposed for hepatocellular carcinoma[20] and now also proposed an alternative to RECIST for GEP-NETs.[21] Despite well-developed capillary networks in NETs, and previous indications of DCE-CT perfusion parameters predicting outcomes in NETs undergoing targeted therapies[19,22], our study revealed a significant decrease in arterial vascularization in both NELM and pNETs after initiating CAPTEM treatment. However, notably, there was no discernible difference between responder and non-responder groups, challenging the utility of mRECIST in this context.

Notably, our investigation revealed significant differences in ADCmin changes and the ratio of ADCmean divided by ADCmean of the liver between response groups. ROC analysis demonstrated the highest AUC for ΔADCmean/Liver ADCmean, with corresponding cut-offs effectively stratifying patients with longer PFS. Combining changes in tumor size (Δsize NELM) with CgA or ADCmin showed slight improvements in sensitivities compared to size-based evaluation alone. Although no study has specifically analyzed the

value of ADC for NETs undergoing CAPTEM treatment, existing reports underscore the potential prognostic value of ADC for other treatment strategies.[23-25]

Acknowledging study limitations, including its retrospective design and small sample size, future prospective studies with larger cohorts are warranted for validation.

Conclusions

Our study, among the first to assess multiparametric MRI for monitoring CAPTEM response in hepatic metastasized NETs, suggests the importance of combined evaluation of CgA, ADC values, and tumor size. Our study underscores the complexity of monitoring CAPTEM response in hepatic metastasized NETs, calling for adapted response criteria for slow-growing tumors like NETs, where conventional size-based criteria may not be reached.

References

5. Wang W, Zhang Y, Peng Y, Jin KZ, Li YL, Liang Y, et al. A Ki-67 index to predict treatment response to the capecitabine/temozolomide regimen in neuroendocrine neoplasms: a retrospective multicenter study. *Neuroendocrinology* 2021; **111:** 752-63. doi: 10.1159/000510159

6. Dogan I, Tastekin D, Karabulut S, Sakar B. Capecitabine and temozolomide (CAPTEM) is effective in metastatic well-differentiated gastrointestinal neuroendocrine tumors. *J Dig Dis* 2022; **23:** 493-9. doi: 10.1111/1751-2980.13123

7. Al-Toubah T, Pelle E, Valone T, Haider M, Strosberg JR. Efficacy and toxicity analysis of capecitabine and temozolomide in neuroendocrine neoplasms. *J Natl Compr Canc Netw* 2021; **20:** 29-36. doi: 10.6004/jnccn.2021.7017

8. Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT, et al. First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer* 2011; **117:** 268-75. doi: 10.1002/cncr.25425

9. Ramirez RA, Beyer DT, Chauhan A, Boudreaux JP, Wang YZ, Woltering EA. The role of capecitabine/temozolomide in metastatic neuroendocrine tumors. *Oncologist* 2016; **21:** 671-5. doi: 10.1634/theoncologist.2015-0470

10. Arrivi G, Verrico M, Roberto M, Barchiesi G, Faggiano A, Marchetti P, et al. Capecitabine and temozolomide (CAPTEM) in advanced neuroendocrine neoplasms (NENs): a systematic review and pooled analysis. *Cancer Manag Res* 2022; **14:** 3507-23. doi: 10.2147/cmar.S372776

11. Cives M, Ghayouri M, Morse B, Brelsford M, Black M, Rizzo A, et al. Analysis of potential response predictors to capecitabine/temozolomide in metastatic pancreatic neuroendocrine tumors. *Endocr Relat Cancer* 2016; **23:** 759-67. doi: 10.1530/erc-16-0147

12. Zhang G, Xu Z, Zheng J, Wang M, Ren J, Wei X, et al. Prognostic value of multi b-value DWI in patients with locally advanced rectal cancer. *Eur Radiol* 2023; **33:** 1928-37. doi: 10.1007/s00330-022-09159-7

13. Ingenerf M, Kiesl S, Winkelmann M, Auernhammer CJ, Rübenthaler J, Grawe F, et al. Treatment assessment of pNET and NELM after everolimus by quantitative MRI parameters. *Biomedicines* 2022: **10:** 2618**.** doi: 10.3390/biomedicines10102618

14. Yuan W, Yu Q, Wang Z, Huang J, Wang J, Long L. Efficacy of diffusion-weighted imaging in neoadjuvant chemotherapy for osteosarcoma: a systematic review and meta-analysis. *Acad Radiol* 2022; **29:** 326-34. doi: 10.1016/j.acra.2020.11.013

15. Luo Y, Pandey A, Ghasabeh MA, Pandey P, Varzaneh FN, et al. Prognostic value of baseline volumetric multiparametric MR imaging in neuroendocrine liver metastases treated with transarterial chemoembolization. *Eur Radiol* 2019; **29:** 5160-71. doi: 10.1007/s00330-019-06100-3

16. Strosberg JR, Cives M, Brelsford M, Black M, Meeker A, Ghayouri M. Identification of response predictors to capecitabine/temozolomide in metastatic pancreatic neuroendocrine tumors. *J Clin Oncol* 2015; **33:** 4099. doi: 10.1200/jco.2015.33.15_suppl.4099

17. Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* 2004; **4:** 296-307. doi: 10.1038/nrc1319

18. 14. Baudin E, Bidart JM, Bachelot A, Ducreux M, Elias D, Ruffié P, et al. Impact of chromogranin A measurement in the work-up of neuroendocrine tumors. *Annalf Oncol* 2001; **12 Suppl 2:** S79-82. doi: 10.1093/annonc/12.suppl_2.s79

19. Yao JC, Pavel M, Phan AT, Kulke MH, Hoosen S, St Peter J, et al. Chromogranin A and neuron-specific enolase as prognostic markers in patients with advanced pNET treated with everolimus. *J Clin Endocrinol Metab* 2011; **96:** 3741-9. doi: 10.1210/jc.2011-0666

20. Chou WC, Chen JS, Hung YS, Hsu JT, Chen TC, Sun CF, et al. Plasma chromogranin A levels predict survival and tumor response in patients with advanced gastroenteropancreatic neuroendocrine tumors. *Anticancer Res* 2014; **34:** 5661-9.

21. Chou WC, Hung YS, Hsu JT, Chen JS, Lu CH, Hwang TL, et al. Chromogranin A is a reliable biomarker for gastroenteropancreatic neuroendocrine tumors in an Asian population of patients. *Neuroendocrinology* 2012; **95:** 344-50. doi: 10.1159/000333853

22. Tsai H-J, Hsiao C-F, Chang JS, Chen L-T, Chao Y-J, Yen C-J, et al. The Prognostic and predictive role of chromogranin A in gastroenteropancreatic neuroendocrine tumors – a single-center experience. *Front Oncol* 2021; **11:** 741096. doi: 10.3389/fonc.2021.741096

23. de Mestier L, Dromain C, d'Assignies G, Scoazec J-Y, Lassau N, Lebtahi R, et al. Evaluating digestive neuroendocrine tumor progression and therapeutic responses in the era of targeted therapies: state of the art. *Endocr Relat Cancer* 2014; **21:** R105-R120. doi: 10.1530/erc-13-0365

24. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30:** 52-60. doi: 10.1055/s-0030-1247132

25. Merino-Casabiel X, Aller J, Arbizu J, García-Figueiras R, González C, Grande E, et al. Consensus document on the progression and treatment response criteria in gastroenteropancreatic neuroendocrine tumors. *Clin Transl Oncol* 2018; **20:** 1522-8. doi: 10.1007/s12094-018-1881-9

26. Yao JC, Phan AT, Fogleman D, Ng CS, Jacobs CB, Dagohoy CD, et al. Randomized run-in study of bevacizumab (B) and everolimus (E) in low- to intermediate-grade neuroendocrine tumors (LGNETs) using perfusion CT as functional biomarker. *J Clin Oncol* 2010; **28:** 4002. doi: 10.1200/jco.2010.28.15_suppl.4002

27. Ingenerf MK, Karim H, Fink N, Ilhan H, Ricke J, Treitl KM, et al. Apparent diffusion coefficients (ADC) in response assessment of transarterial radioembolization (TARE) for liver metastases of neuroendocrine tumors (NET): a feasibility study. *Acta Radiol* 2022; **63:** 878-88. doi: 10.1177/02841851211024004

28. Min JH, Kang TW, Kim YK, Kim SH, Shin KS, Lee JE, et al. Hepatic neuroendocrine tumour: apparent diffusion coefficient as a potential marker of prognosis associated with tumour grade and overall survival. *Eur Radiol* 2018; **28:** 2561-71. doi: 10.1007/s00330-017-5248-3

29. Vandecaveye V, Dresen RC, Pauwels E, Binnebeek SV, Vanslembrouck R, Baete K, et al. Early whole-body diffusion-weighted MRI helps predict long-term outcome following peptide receptor radionuclide therapy for metastatic neuroendocrine tumors. *Radiol Imaging Cancer* 2022; **4:** e210095. doi: 10.1148/rycan.210095

research paper

Long-term outcome of multilayer flow modulator in aortic aneurysms

Karlo Pintaric[1,2], Lucka Boltezar[3,4], Nejc Umek[5], Dimitrij Kuhelj[1,4]

[1] Clinical Institute of Radiology, University Medical Center Ljubljana, Slovenia
[2] Department of Radiology, Faculty of Medicine, University of Ljubljana, Slovenia
[3] Department of Medical Oncology, Institute of Oncology Ljubljana, Slovenia
[4] Faculty of Medicine, University of Ljubljana, Slovenia.
[5] Institute of Anatomy, Faculty of Medicine, University of Ljubljana, Slovenia

Radiol Oncol 2024; 58(2): 206-213.

Received 12 January 2024
Accepted 20 February 2024

Correspondence to: Assoc. Prof. Dimitrij Kuhelj, M.D., Ph.D., Clinical Institute of Radiology, University Medical Center Ljubljana, Zaloška 7, SI-1000 Ljubljana, E-mail: dimitrij.kuhelj@kclj.si

Disclosure: No potential conflicts of interest were disclosed.

Background. This retrospective study investigated the efficacy of endovascular treatment with multilayer flow modulators (MFMs) for treating aortic aneurysms in high-risk patients unsuitable for conventional treatments.

Patients and methods. Conducted from 2011 to 2019 at a single center, this retrospective observational study included 17 patients who underwent endovascular treatment with MFMs. These patients were selected based on their unsuitability for traditional surgical or endovascular procedures. The study involved meticulous pre-procedural planning, precise implantation of MFMs, and follow-up using CT angiography. The primary focus was on volumetric and flow volume changes in aneurysms, along with traditional diameter measurements. Moreover, the technical success and post-procedural complications were also registered.

Results. The technical success rate was 100%, and 30-day procedural complication rate was 17.6%. Post-treatment assessments revealed that 11 out of 17 patients showed a decrease in flow volume within the aneurysm sac, indicative of a favorable hemodynamic response. The median decrease in flow volume was 12 ml, with a median relative decrease of 8%. However, there was no consistent reduction in aneurysm size; most aneurysms demonstrated a median increase in volume for 46 ml and median increase in diameter for 18 mm.

Conclusions. While MFMs offer a potential alternative for high-risk aortic aneurysm patients, their effectiveness in preventing aneurysm expansion is limited. The results suggest that MFMs can provide a stable hemodynamic environment but do not reliably reduce aneurysm size. This underscores the need for ongoing vigilance and long-term monitoring in patients treated with this technology.

Key words: multilayer flow modulator; aortic aneurism; long-term follow up; volumetric measurements

Introduction

Endovascular management of aortic aneurysms with stent grafts has been possible for over 30 years. One of the challenges of the procedure is the preservation of flow in the aortic branches and the prevention of ischaemia. There have been many attempts to overcome this problem, including using fenestrated and branched stent grafts, as well as snorkel and chimney techniques.[1,2] However, all these technical solutions are relatively complex and require appropriate anatomical conditions and highly skilled operators since the learning curves for the implantation of these devices are

very prolonged.[3] As an alternative, multilayer flow modulators (MFMs) have been developed. These are 3-layer stents designed to laminate the flow through the aneurysm so that it gradually becomes thrombotic without affecting the flow in the branches, simultaneously decreasing the peak stress on the aneurysm wall.[4] This feature is particularly important in the aortic arch and thoracoabdominal region.

Most studies on MFM in the aorta have relied on measurements of aneurysm diameters, which is the most practical and time-efficient method. However, this approach does not provide comprehensive information on gradual thrombosis of the aneurysm and morphological changes in the aneurysm itself.[5–8] Volumetric measurements and measurements of the flow volume through the aneurysm could provide additional information about the behaviour of aneurysms over time and, thus, about the efficiency of MFM stents. Accordingly, our study aimed to evaluate the safety and efficacy of MFMs implanted between 2011 and 2019 in a single centre cohort, using volumetric and flow volume assessments along with diameter measurements.

Patients and methods

This retrospective observational study was approved by the Republic of Slovenia National Medical Ethics Committee (Permit No. 55/05/14) and was conducted in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki). We analyzed a cohort of consecutive patients who underwent endovascular treatment for aortic aneurysms using MFMs (Cardiatis, Isnes, Belgium) at the University Clinical Center, Ljubljana, between March 2011 and October 2019. Our institution started using MFMs in 2011, and since then, we have implanted MFMs in 17 patients with aortic aneurysms.

Only patients unsuitable for open surgical treatment or other endovascular procedures were considered for endovascular treatment with MFM by a multidisciplinary board held for each patient. The exclusion criteria were rupture of aortic aneurysm, stenosis of branch arteries (arteries of head and neck, visceral and iliac arteries), occlusion of the aortoiliac segment, prior endovascular or surgical treatment of the same aneurysm, mycotic aneurysm, myeloproliferative blood disorders, known coagulopathies, and expected survival less than six months.[9] For each patient, a consultation was held with Cardiatis (Isnes, Belgium) before the procedure to obtain their consent for MFM implantation.

Planning of the procedure was primarily performed by experienced interventional radiologists using computed tomographic (CT) angiography. Critical parameters assessed and measured prior to MFM implantation included the status of outgoing arteries (especially eventual stenoses), the largest diameter of the aneurysm, the diameter of the healthy vessel above and below the aneurysm, and the length of the aneurysm. Diagnostic images were shared with the MFM manufacturer and operator, who subsequently performed size of device determination, applying an oversizing of 10–15%. The total volume and flow volume of the aneurysms were estimated using the OsirX (Pixmeo, Geneve, Switzerland). These two parameters were also assessed in the last follow-up CT angiographies.

All procedures were performed under general anesthesia. Prophylactic antibiotics were administered prior to the procedure, and 5000 IU of Heparin was administered during it. Bilateral access in the groin was achieved, and a large-bore sheath (22–24 Fr) was implanted at the site with larger iliac arteries through the common femoral artery to accommodate the MFM delivery system. The contralateral side was utilized to provide a diagnostic catheter, essential for precise MFM implantation. The MFM was carefully implanted using a slow release and push-pull technique to ensure adequate sealing of the affected aortic segment. In all patients, complete percutaneous hemostasis was successfully achieved using the ProStar XL/ProGlide systems (Abbott Laboratories, IL, USA). Following the procedure, all patients were prescribed lifelong treatment with acetylsalicylic acid and a three-month course of clopidogrel. Figure 1 shows volume rendering of aorta with implanted MFM and fluoroscopy image of implanted MFM in thoracoabdominal aorta.

The follow-up period in this study extended from the date of MFM implantation to the most recent CT angiography available in the hospital information system. Patient survival status, as of May 2023, was determined based on the cause of death listed in the National Registry.

For each patient, the technical success of the intervention, procedure-related complications, adherence to usage instructions, and mortality were documented. Technical success was defined as the accurate deployment of MFM at the targeted sites with the absence of any leaks at the attachment sites or the junctions of different device components.[5]

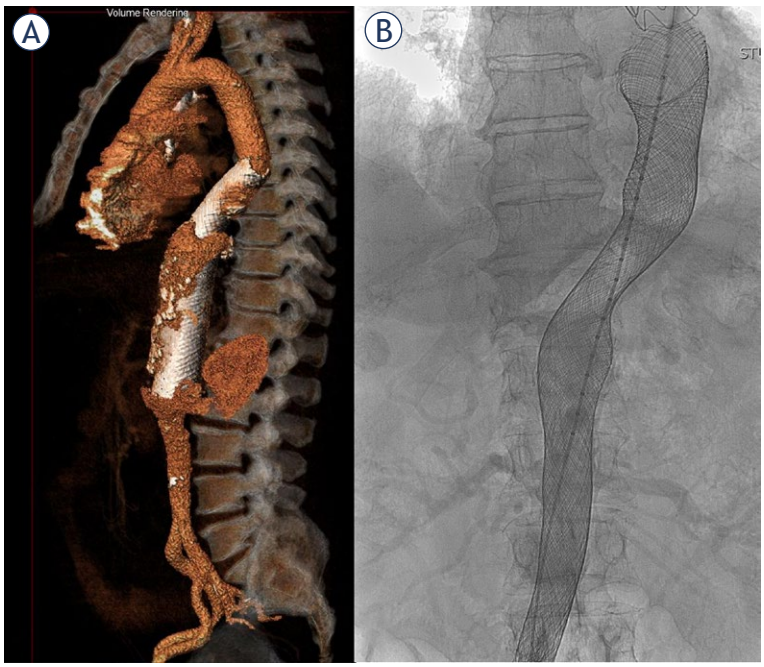


FIGURE 1. Volume rendering of implanted multilayer flow modulators (MFMs) **(A)** and fluoroscopic image of implanted MFM **(B)** in thoracoabdominal aorta.

Serious adverse events such as cerebral stroke, renal ischemia, paraplegia, and aneurysm rupture were recorded. Complications attributed directly to the procedure were monitored at one month and twelve months after intervention. Furthermore, any potential reinterventions were noted, including the timing and cause for these additional procedures.

Additionally, the aneurysm diameters and volumetric data were calculated. Volumetric assessments of the aneurysms (total volume of the aneurysm sac and volume of aneurysm with retained flow) were performed prior to the implantation of the MFM and repeated in the last available CT angiography for each patient. The volumetric measurements were performed by Cardiatis (Isnes, Belgium). Incidences of branch occlusions, if any, were also reported.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 10 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as median, interquartile range (IQR), range, frequency, and proportion. The comparison of numerical variables was performed using the Mann-Whitney test. Spearman's correlations were used to test variable correlations. A *p*-value of 0.05 was considered statistically significant.

Results

Demographic data and clinical characteristics

During the study period, 17 patients were treated with MFM for aortic aneurysms, including 16 males and one female. The median age was 68 years (IQR: 66–78, range: 48–81 years). Four MFMs were implanted in aortic arch, six in thoracic aorta, one in thoracoabdominal aorta and two in abdominal aorta. The age of the patients at MFM implantation, the year of the first endovascular treatment, and the location of the placed MFM are shown in Table 1. All procedures were performed electively. The median duration of follow-up was 25 months, with an IQR of 13–58 months and an overall range of 7–76 months.

Procedure and procedural complications

The procedure was performed according to the manufacturer's instructions in 70.6% of patients. In other patients aneurysm was larger then 6.5 cm, which is not according to the manufacturer's instruction of use.[8] However, all procedures were performed with the consent of manufacturer. The technical success rate was 100%, and a total of 23 stents were implanted to treat all 17 patients.

The 30-day procedural complication rate was 17.6% (3/17). One patient required an additional stent graft after a few days to achieve an optimal proximal seal, as no additional MFM was available during the procedure. One patient was diagnosed with a type A dissection at CTA follow-up, which was attributed to the procedure itself, while one other was diagnosed with a dissection of the celiac trunk. Note, that the aforementioned type A dissection was stable on following CTAs, and cardiovascular surgeouns did not decide for surgical treatment. Additionally, one patient suffered a cerebral infarction due to a pre-existing stenosis of the left carotid artery and brachiocephalic trunk (however, poor adherence to antiplatelet medication was noted in this patient). None of the patients required open surgery due to these complications, and none developed paraplegia, end-organ failure, or aneurysm rupture.

Mortality

The intraoperative and 30-day mortality rates were both 0%. After 12 months, the aneurysm-related mortality rate was 5.9% (1 in 17 patients). As of May 2023, 5 of the 17 (29.4%) patients were still alive, while 12 (71.6%) had died. Three of these deaths were due to aneurysm ruptures that occurred 9 months, 40 months, and 51 months after MFM implantation. The remaining deaths were attributed to other causes, including head and neck cancer, pneumonia, myocardial infarction, and suicide.

Aortic branch occlusions

Aortic branch occlusions following the MFM implantation occurred in 5 patients (29.4%) – one patient developed chronic renal failure due to stenosis of the left renal artery (which did not require haemodialysis). Vascular occlusion in the other patients included stenosis of the superior mesenteric artery, left subclavian artery, superior mesenteric artery and the celiac trunk (with robust collaterals from the inferior mesenteric artery), and the left carotid artery and the brachiocephalic trunk.

TABLE 1. Age of patients at procedure, year of procedure, and location of multilayer flow modulator (MFM)

Patient	Age	Procedure year	Location of MFM
1.	48	2012	Thoracic aorta
2.	79	2012	Thoracic aorta
3.	80	2012	Thoracic aorta
4.	73	2013	Aortic arch
5.	65	2013	Thoracic aorta
6.	67	2016	Aortic arch
7.	68	2016	Thoracic aorta
8.	63	2016	Abdominal aorta
9.	71	2016	Thoracic aorta
10.	80	2017	Abdominal aorta
11.	67	2017	Aortic arch
12.	65	2017	Aortic arch
13.	76	2018	Thoracoabdominal aorta
14.	75	2018	Thoracoabdominal aorta
15.	81	2018	Abdominal aorta
16.	66	2018	Abdominal aorta
17.	66	2019	Thoracoabdominal aorta

Reinterventions

Reintervention was required in 7 patients (41.2%) as shown in Table 2. Most reinterventions were performed in the early years of our practice due to type 1 leaks.

Changes in aneurysm size after MFM implantation

The median aneurysm volume before MFM implantation was 309 ml (IQR: 223–452 ml, range 32–856 ml) with a median diameter of 58 mm (IQR: 51–68 mm, range 26–96 mm). At the last follow–up, the median aneurysm volume was 355 ml (IQR: 237–608 ml, range: 62–881 ml), while the median diameter was 76 mm (IQR: 57–92 mm, range 27–103 mm).

Based on volume measurements, five patients (29.4%) experienced shrinkage of the aneurysm at the last follow-up, two (11.8%) experienced no volume change over time, and ten (58.5%) experienced an enlargement of the aneurysm during the observation period. In latter patients, the median enlargement of the sac was 96 ml (IQR: -15–117 ml, range -84–718 ml) in volume and 15 mm (IQR: 3–27 mm, range: -7–49 mm) in diameter. Nine of these patients had an enlargement of the aneurysm for more than 10% of the initial volume.

Per the measurements of the maximum diameter, fifteen patients (88.2%) experienced an increase in the diameter of the aneurysm, one patient experienced shrinkage, and in one patient, the diameter remained stable during the follow-up period. Patient-specific measurements are presented in Table 3.

There were no significant correlations between the aneurysm volume before MFM implantation and absolute or relative change in the aneurysm volume after MFM implantation ($p = 0.9167$ and $p = 0.4473$, respectively). We only noted a significant positive correlation between the aneurysm volume before MFM implantation and the aneurysm volume at the last follow-up after MFM implantation ($\varrho = 0.71$, $p = 0.0013$) and between aneurysm volume and maximal diameter ($\varrho = 0.86$, $R^2 = 0.74$, $p = 0.0001$).

There was a significant positive correlation between follow-up duration and both absolute and relative changes in aneurysm volume and diameter (absolute volume: $\varrho = 0.62$, $p = 0.0084$, relative volume: $\varrho = 0.70$, $p = 0.0017$, absolute diameter: $\varrho = 0.65$, $p = 0.0046$, relative diameter: $\varrho = 0.66$, $p =$

TABLE 2. Time and cause of reinterventions after multilayer flow modulator (MFM) implantation

Patient number	Time after MFM implantation (months)	Cause of reintervention; reintervention undertaken
1.	25	Endoleak type I and enlargement of the aneurysm; implantation of another MFM
2.	3	Insufficient proximal seal and collateral flow, resulting in enlargement of the aneurysm; implantation of another MFM
3.	1	Insufficient proximal seal; implantation of another MFM
4.	8	Enlargement of aneurysm and partial stenosis of subclavian artery; implantation of another MFM into previous MFM
6.	13	Stenosis of brachiocephalic truncus; implantation of a stent, which resulted in a stroke
9.	9	Migration of the MFM; implantation of another MFM
10.	74	Displacement of MFM and stent graft; implantation of another stent graft

0.0040). Therefore, we normalized absolute and relative changes in aneurysm size to follow-up duration; however, again, there were no significant correlations between initial aneurysm size and normalized absolute and relative changes in aneurysm size after MFM implantation. Moreover, there were no significant correlations between initial aneurysm size or change in aneurysm size and patient age.

Three patients (17.6%) had complete occlusion of the aneurysm sac with a thrombus around the implanted MFM, twelve patients (70.6%) still had partial flow in the aneurysm sac (four of them, however, with only minimal presence of contrast medium on CT angiography), and two patients (11.8%) had a completely non-occluded aneurysm sac with flow still present in the aneurysm sac.

Changes in aneurysm flow volume after MFM implantation

Six patients had an increase in flow volume (35.3%), and 11 patients had a decrease in flow volume (64.7%) at the last follow-up, which is considered a favourable haemodynamic outcome. The median volume before MFM implantation was 183 ml (IQR: 159–262 ml, range: 18–468 ml), and the median flow volume at the last follow-up was 168 ml (IQR: 125–268 ml, range: 6–555 ml). The median change in flow volume was -12 ml (IQR: -36–31 ml, range: -94–124 ml), and the median relative decrease in flow volume was 8% (IQR: -19%–15%, range: -64%–49%). We found no significant correlation between the patient's age, duration of follow-up, or initial size of the aneurysm and the change in absolute or relative flow volume. There was also no significant correlation between absolute or relative change in aneurysm volume or diameter and absolute or relative flow volume.

Discussion

We studied consecutive patients treated with MFM stents for aortic aneurysms between 2011 and 2019 in our centre. We found that flow volume decreased in 11 out of 17 patients after MFM implantation, which is considered a favourable hemodynamic response; however, this did not correlate with a decrease in aneurysm sac size. In 15 out of 17 patients, the aneurysm sac increased in diameter, which was also accompanied by an increase in volume in 10 out of 17 patients.

Our institution was one of the first centers to perform treatment with MFM stents for aortic aneurysms. This study, therefore, has one of the longest follow-up periods currently published in the literature. The literature review by Pinto *et al*. concluded that the implantation of MFMs is safe with few complications, although no randomized studies were available.[9] Most of the studies published to date included a few patients, usually less than 30, with a relatively short follow-up period of no more than 12 months.[5–8,10–15] A larger study with 103 patients was published by Sultan *et al*. in 2014 but with a short median follow-up of only six months.[15] There are two studies that report a median follow-up time of 22 months: one from Ireland with 14 patients included[11] and the other from Italy with only 8 patients included.[13]

The literature mostly reports measurements of the aneurysm maximal diameters.[5–8,10–15] To our knowledge, our study is the first to evaluate volumetric measurements before MFM implantation

TABLE 3. Aneurysm size before and after multilayer flow modulator (MFM) implantation

Patient number	Aneurysm volume (ml)		Aneurysm diameter (mm)		Difference between the last follow-up and before the MFM implantation		Duration of follow-up (months)
	Before MFM implantation	At last follow-up	Before MFM implantation	At last follow-up	Volume (ml [%])	Diameter (mm [%])	
1.	345	854	65	107	509 [147]	42 [64]	76
2.	888	920	122	130	32 [4]	8 [7]	9
3.	309	943	70	110	634 [205]	40 [57]	50
4.	255	355	57	93	100 [39]	36 [63]	69
5.	75	62	26	27	-13 [-17]	1 [4]	14
6.	197	330	44	76	133 [68]	32 [73]	49
7.	343	549	65	85	206 [60]	20 [31]	28
8.	311	266	48	68	-45 [-14]	20 [42]	3
9.	575	667	76	81	92 [16]	5 [7]	14
10.	539	530	97	90	-9 [-2]	-7 [-7]	40
11.	32	62	34	50	30 [94]	16 [47]	48
12.	411	411	54	55	0 [0]	1 [2]	33
13.	493	409	53	57	-84 [-17]	4 [8]	24
14.	262	332	60	75	70 [27]	15 [25]	23
15.	307	223	59	81	-84 [-27]	22 [37]	18
16.	130	113	58	58	-17 [-13]	0 [0]	19
17.	250	250	54	60	0 [0]	6 [11]	7

and at the follow-up over a longer period. Two studies by Sultan *et al*., which included patients with first-generation MFMs, performed volumetric evaluation of aneurysm sacs over several months.[14,15] The first from 2013 reported an overall mean increase in sac volume of 3.3% in 55 patients[14], while the second study with 103 patients in 2014 reported an overall mean increase in sac volume of 5.1% and a mean volume change of 63 ml 12 months after implantation.[15] In our series, the mean volume increase was 33.4% and 91 ml, which is significantly more than noted by Sultan *et al*. This could be due to the longer observation period in our study, which is also supported by a significant positive correlation between the follow-up period and the change in volume and diameter of the aneurysm sac.

The proportion of aneurysm sac expansion in our study is consistent with the findings of Lowe *et al*.[11], who conducted a prospective study on patients treated with MFMs, characterized by a mean patient age of 74 years and a follow-up period of 22 months. Their findings revealed that none of the aneurysms demonstrated shrinkage. They reported a one-year all-cause survival rate of 79%, which dropped to 50% at two years. Remarkably, only two patients exhibited stable aneurysm sac diameters, with all others experiencing aneurysm sac volume expansion. While their conclusion does not support the continued use of MFMs, it is important to note that their study, conducted from 2011 to 2014, included only patients fitted with the first generation of the device. On the contrary, Vaislic *et al*. reported a very high proportion of stable aneurysm sac size (90%); however, the observational period was only 12 months.[16]

We found a strong correlation between aneurysm sac enlargement and follow-up period, which suggests that MFM implantation is probably not a lifelong stable solution as often advertised by the industry. Most of the aneurysm sacs became completely or partially obliterated by thrombus, which is consistent with the manufacturer's caution. However, despite that, in almost two-thirds of patients, the flow volume decreased, and the aneurysm sac size increased both in volume and diameter. We also noticed a strong positive correlation between aneurysm volume and diameter

change, suggesting that diameter measurements are probably sufficient for assessing aneurysm size at follow-up CT angiographies.

We observed that 41% of patients required re-intervention, a rate lower than the approximately 77% reported by Ibrahim *et al*. in 2018.[8] This discrepancy may stem from the urgency of treatments in the latter study, where most patients underwent emergency procedures. The selection of devices and implantation techniques in emergency scenarios differs markedly from those in elective procedures. Additionally, a notable proportion of reinterventions in our series was attributed to technical challenges associated with the first-generation MFMs, suggesting that device technology advancements may influence the need for subsequent interventions.

Existing literature suggests that aneurysm-related survival rates 18 months post-implantation of MFMs can be as low as 25.6% when these devices are used with deviations from the prescribed instructions.[15,17] However, our data indicates more favourable long-term survival outcomes, despite 29.4% of the procedures in our study deviating from the recommended usage guidelines. A significant factor contributing to these improved outcomes is likely the elective setting in which our procedures were conducted, suggesting that the context of the procedure may play an important role in patient survival post-implantation.

In our study, we observed three deaths attributed to aneurysm rupture: one occurring 9 months, another after 40 months, and the third nearly 6 years post-MFM implantation. Although the aneurysm-related mortality was relatively low, the overall mortality rate was notably high. This may be partly attributed to the inclusion of patients unsuitable for surgical intervention, often due to their suboptimal general health conditions. Notably, the instance of an aneurysm rupture 6 years after MFM implantation underscores the importance of prolonged follow-up in these patients, highlighting the need for ongoing monitoring even years after the initial treatment.

The primary limitation of our study, as with many others focusing on MFMs, is the relatively small patient sample size. However, our research holds distinct value due to its nature as a consecutive series conducted by a consistently trained team in a single centre. Furthermore, the extensive duration of our follow-up and the employment of volumetric measurements offer a significant contribution to the existing body of knowledge in the field of intravascular treatments.

Conclusions

MFMs present a viable treatment alternative for high-risk patients who are unsuitable for surgery and stent grafts. However, long-term real-life data show that while MFMs may not be as effective in preventing aneurysm expansion as originally thought, they can still provide a relatively stable haemodynamic solution over a prolonged period. Our study found a correlation between the duration of follow-up and the increase in aneurysm sac size. Although there are often no alternative treatment options for these patients and MFM insertion generally carries a low risk of periprocedural and long-term complications, careful, lifelong follow-up is essential to recognize early signs of deterioration and intervene appropriately.

Acknowledgments

The authors are thankful to dr. Chiedozie Kenneth Ugwoke for manuscript proofreading. This study was supported by University Medical Center Ljubljana tertiary funding. The author N.U. was supported by Slovenian Research and Inovation Agency, grant No. P3-0043.

Reference

1. Hicks CW, Mendes BC. The past, present, and future of fenestrated/branched endovascular aortic repair. *Semin Vasc Surg* 2022; **35:** 235. doi: 10.1053/j.semvascsurg.2022.08.003
2. Shuja F, Kwolek CJ. Treating the paravisceral aorta with parallel endografts (chimneys and snorkels). *Semin Vasc Surg* 2012; **25:** 200-2. doi: 10.1053/j.semvascsurg.2012.09.005
3. Mirza AK, Tenorio ER, Kärkkäinen JM, Hofer J, Macedo T, Cha S, et al. Learning curve of fenestrated and branched endovascular aortic repair for pararenal and thoracoabdominal aneurysms. *J Vasc Surg* 2020; **72:** 423-34. e1. doi: 10.1016/j.jvs.2019.09.046
4. Xiong Y, Wang X, Jiang W, Tian X, Wang Q, Fan Y, et al. Hemodynamics study of a multilayer stent for the treatment of aneurysms. *Biomed Eng Online* 2016; **15(S2):** 411-20. doi: 10.1186/s12938-016-0248-0
5. Benjelloun A, Henry M, Taberkant M, Berrado A, Houati R El, Semlali A. Multilayer flow modulator treatment of abdominal and thoracoabdominal aortic aneurysms with side branch coverage: outcomes from a prospective single-center Moroccan registry. *J Endovasc Ther* 2016; **23:** 773-82. doi: 10.1177/1526602816657087
6. Costache VS, Meekel JP, Costache A, Melnic T, Bucurenciu C, Chitic A, et al. One-year single-center results of the multilayer flow modulator stents for the treatment of Type B aortic dissection. *J Endovasc Ther* 2021; **28:** 20-31. doi: 10.1177/1526602820950720
7. Debing E, Aerden D, Gallala S, Vandenbroucke F, Van Den Brande P. Stenting complex aorta aneurysms with the cardiatis multilayer flow modulator: first impressions. *Eur J Vasc Endovasc Surg* 2014; **47:** 604-8. doi: 10.1016/j.ejvs.2014.02.020
8. Ibrahim W, Spanos K, Gussmann A, Nienaber CA, Tessarek J, Walter H, et al. Early and midterm outcome of multilayer flow modulator stent for complex aortic aneurysm treatment in Germany. *J Vasc Surg* 2018; **68:** 956-64. doi: 10.1016/j.jvs.2018.01.037

9. Pinto C, Garas G, Harling L, Darzi A, Casula R, Athanasiou T. Is endovascular treatment with multilayer flow modulator stent insertion a safe alternative to open surgery for high-risk patients with thoracoabdominal aortic aneurysm? *Ann Med Surg* 2017; **15:** 1–8. doi: 10.1016/j.amsu.2017.01.020

10. Sultan S, Kavanagh EP, Costache V, Sultan M, Elhali A, Dietrich E, et al. Streamliner multilayer flow modulator for thoracoabdominal aortic pathologies: recommendations for revision of indications and contraindications for use. *Vasc Dis Manag* 2017; **14**: E90-9.

11. Lowe C, Worthington A, Serracino-Inglott F, Ashleigh R, McCollum C. Multi-layer flow-modulating stents for thoraco-abdominal and peri-renal aneurysms: The UK pilot study. *Eur J Vasc Endovasc Surg* 2016; **51:** 225-31. doi: 10.1016/j.ejvs.2015.09.014

12. Ovali C, Şahin A, Eroğlu M, Balçin S, Dernek S, Sevin MB. Treatment of aortic and iliac artery aneurysms with multilayer flow modulator: single centre experiences. *Int J Vasc Med* 2018; **2018:** 7543817. doi: 10.1155/2018/7543817

13. Pane B, Spinella G, Perfumo C, Palombo D. A single-center experience of aortic and iliac artery aneurysm treated with multilayer flow modulator. *Ann Vasc Surg* 2016; **30:** 166-74. doi: 10.1016/j.avsg.2015.07.042

14. Sultan S, Hynes N. One-year results of the multilayer flow modulator stent in the management of thoracoabdominal aortic aneurysms and type B dissections. *J Endovasc Ther* 2013; **20:** 366-77. doi: 10.1583/12-4077MR-R.1

15. Sultan S, Sultan M, Hynes N. Early mid-term results of the first 103 cases of multilayer flow modulator stent done under indication for use in the management of thoracoabdominal aortic pathology from the independent global MFM registry. *J Cardiovasc Surg* 2014; **55:** 21-32. PMID: 24356043

16. Vaislic CD, Fabiani JN, Chocron S, Robin J, Costache VS, Villemot JP, et al. One-year outcomes following repair of thoracoabdominal aneurysms with the multilayer flow modulator: report from the STRATO trial. *J Endovasc Ther* 2014; **21:** 85-95. doi: 10.1583/13-4553R.1

17. Hynes N, Sultan S, Elhelali A, Diethrich EB, Kavanagh EP, Sultan M, et al. Systematic review and patient-level meta-analysis of the streamliner multilayer flow modulator in the management of complex thoracoabdominal aortic pathology. *J Endovasc Ther* 2016; **23:** 501-12. doi: 10.1177/1526602816636891

research article

Prognostic factors for overall survival and safety of trans-arterial chemoembolization (TACE) with irinotecan-loaded drug-eluting beads (DEBIRI) in patients with colorectal liver metastases

Maja Sljivic[1,2], Masa Sever[3], Janja Ocvirk[1,4,5], Tanja Mesti[1,4], Erik Brecelj[1,4], Peter Popovic[1,2]

[1] Faculty of Medicine Ljubljana, Ljubljana, Slovenia
[2] Clinical Institute of Radiology, University Medical Centre Ljubljana, Ljubljana, Slovenia
[3] Faculty of Medicine Belgrade, Serbia
[4] Institute of Oncology, Ljubljana, Slovenia
[5] University of Primorska, Faculty of Health Sciences, Isola, Slovenia

Radiol Oncol 2024; 58(2): 214-220.

Received 13 December 2023
Accepted 6 March 2024

Correspondence to: Assoc. Prof. Peter Popovič, M.D., Ph.D., University Medical Centre Ljubljana, Clinical Institute of Radiology, Zaloška cesta 7, SI-1000 Ljubljana, Slovenia. E-mail: peter.popovic@kclj.si

Maja Sljivic and Masa Sever contributed equally to this work.

Disclosure: No potential conflicts of interest were disclosed.

Background. Transarterial chemoembolisation with irinotecan-loaded drug-eluting beads (DEBIRI TACE) can be considered in patients with unresectable colorectal cancer liver metastases (CRLM) who progress after all approved standard therapies or in patients unsuitable for systemic therapy.

Patients and methods. Between September 2010 and March 2020, thirty patients (22 men and 8 women; mean age 66.8 ± 13.2) were included in this retrospective study. DEBIRI TACE was conducted in 43% of patients unsuitable for systemic therapy as a first-line treatment and 57% as salvage therapy after the progression of systemic therapy. All the patients had liver-limited disease. In the case of unilobar disease, two treatments were performed at four-week intervals, and in the case of bilobar disease, four treatments were performed at two-week intervals. All patients were premedicated and monitored after the procedure. Adverse events were graded according to the Cardiovascular and Interventional Radiological Society of Europe (CIRSE) classification system for complications.

Results. The median overall survival (OS) from the beginning of DEBIRI TACE in the salvage group was 17.4 months; in the group without prior systemic therapy, it was 21.6 months. The median overall survival of all patients was 17.4 months (95% confidence interval [CI]: 10.0–24.7 months), and progression-free survival (PFS) was 4.2 months (95% CI: 0.9–7.4 months). The one-year survival rate after the procedure was 61%, and the two-year rate was 25%. Univariate analysis showed better survival of patients with four or fewer liver metastases ($p = 0.002$). There were no treatment-related deaths or grade 4 and 5 adverse events. Nonserious adverse events (Grades 1 and 2) were present in 53% of patients, and Grade 3 adverse events were present in 6% of the patients.

Conclusions. DEBIRI TACE is a well-tolerated treatment option for patients with liver metastases of colorectal cancer. Patients with four or fewer liver metastases correlated with better survival.

Key words: colorectal cancer; liver metastases; irinotecan; drug-eluting beads; transarterial chemoembolization; survival

Introduction

Colorectal cancer (CRC) is Europe's second most frequently diagnosed malignancy and the second most common cause of death due to cancer (excluding skin carcinomas).[1,2] At the time of diagnosis, 25% of patients have already developed CRC metastases, and 25–35% of patients will develop metastases in the later stages of their disease.[3] The liver is the most common site of CRC metastases (CRLM). The disease progression in the liver is a significant source of complications and death.[4,5] The only curative treatment option for CRLM is surgical resection.[4,6] Unfortunately, most (approx. 70–80%) metastases are unresectable.[7,8] The therapy of choice for non-resectable CRLM is multiagent systemic chemotherapy, such as FOLFOX or FOLFIRI, and targeted agents, including epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) inhibitor.[7,8] The median overall survival of first-line chemotherapy ranges from 12 to 23 months, which is further increased by another two months with the addition of anti-VEGF agent bevacizumab. Median overall survival (OS) reaches around 30 months with a multi-line treatment plan.[7,8]

Conventional transarterial chemoembolisation (TACE) is a selective intraarterial administration of chemotherapeutic agents in combination with Lipiodol. The newer method uses drug-eluting beads (DEB) that cause embolisation and release chemotherapeutic agents into the targeted tissue. The most used chemotherapeutic agent in TACE for CRLM is irinotecan (transarterial chemoembolisation with irinotecan-loaded drug-eluting beads, DEBIRI TACE).[9] Based on current European Society for Medical Oncology (ESMO) guidelines, TACE should be considered a possible treatment option when patients with metastatic liver-limited disease do not respond to systemic chemotherapy.[7,8] Studies have shown that DEBIRI TACE is an effective and safe procedure, with serious high-grade adverse reaching up to 11%.[9-14] On the other hand, getting as much data as possible on how DEBIRI TACE works in real life and finding a group of patients who would benefit the most from this type of treatment is essential. Studies examining prognostic factors for determining survival and treatment efficacy in patients with CRLM treated with DEBIRI TACE are rare.[11,12,13] Research is ongoing, and most authors suggest that further research is needed. Therefore, additional clinical and radiological prognostic factors are required to choose the appropriate patient profile for treatment with DEBIRI TACE.

This retrospective study investigated the safety and prognostic factors in predicting overall survival in patients with CRLM treated with DEBIRI TACE.

Patients and methods

Study design and patient selection

This single-centre retrospective study was approved by the Republic of Slovenia National Medical Ethics Committee (0120-115/2020/9). The study complied with the protocol and principles in the Declaration of Helsinki. Between September 2010 and March 2020, 30 patients with unresectable liver metastases of colorectal cancer who did not respond to systemic therapy, had contraindication to systemic therapy or non-tolerance to systemic chemotherapy underwent treatment with DEBIRI TACE after a tumour board review. All the patients had liver-limited disease. The presence of metastases that exceeded 70% of the liver volume and the occurrence of metastases outside the liver were considered exclusion criteria. All patients had a life expectancy longer than three months and an Eastern Cooperative Oncology Group (ECOG) score equal to 2 or lower before the first DEBIRI TACE treatment.

Data analysis

All data were obtained by reviewing patient files. The following variables were collected – baseline demographic and clinical data (age, sex, ECOG performance status, tumour location), periprocedural complications, duration of hospital stay, previous cycles of systemic therapy, number of metastases, type of liver impairment (unilobar or bilobar), values of tumour markers carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9) before and after treatment, radiological tumour progression, and survival. Limit values for tumour markers were used based on estimated upper normal plasma levels (for CEA ≤ 5 μg/L, CA19-9 ≤ 37 kU/L). Variables assessed as possible prognostic factors were age, ECOG status, tumour location, previous systemic therapy, number of metastases, uni- or bilobar disease, CEA and CA 19-9 before the first treatment, and rise or fall of tumour markers after the first treatment.

All adverse events were graded according to the Cardiovascular and Interventional Radiological Society of Europe (CIRSE) classification system for complications.[15] Tumour response was assessed

TABLE 1. Patient demographics and clinicopathological features

Age in years	
Median (range)	68 (34–85)
Sex	**n (%)**
Male	22 (73)
Female	8 (27)
Primary tumour	
Colon	16 (53)
Rectum	14 (47)
ECOG performance status	
0	18 (60)
1	9 (30)
2	3 (10)
Liver metastases	
Unilobar	17 (57)
Bilobar	13 (43)
≤ 4 lesions	19 (63)
> 4 lesions	11 (37)
Previous chemotherapy	
Yes	17 (57)
No	13 (43)

using the Response Evaluation Criteria in Solid Tumours (RECIST) and modified RECIST (mRECIST) criteria.

Survival was calculated as the time from the first DEBIRI to death or to the end of follow-up (July 20, 2020). Survival analysis was performed by using the Kaplan-Meier method. Progression-free survival (PFS) was calculated from the date of the first DEBIRI to disease progression or death from any cause. Survival endpoints for each factor were estimated according to Kaplan-Meier analysis and compared with the log-rank test. The p-values are two-sided and considered statistically significant at ≤ 0.05. Data were analysed using the statistical software SPSS 25 for Windows (IBM Corp., NY, USA).

Treatment

Premedication included intravenous hydration, opioid analgesic, corticosteroid, antiemetic, and antibiotic prophylaxis. Intraprocedural pain was managed by a continuous intravenous infusion containing morphine (20 mg) combined with the nonsteroidal anti-inflammatory agent ketorolac (20 mg), starting two hours before the procedure for 24 hours. The procedure was performed in an angiography suite in local anaesthesia through the femoral approach. First, preliminary diagnostic angiography was performed to evaluate hepatic arterial supply. Then, a microcatheter (Progreat, Terumo Europe N.V, Belgium) was introduced into the left or right hepatic artery, followed by 2–4 mL intraarterial application of 1% lidocaine and 2 ml solution of microparticles loaded with 100 mg of irinotecan, respectively. Over time, the size of microparticles has changed noticeably. Initially, DC beads (Boston Scientific, Marlborough, Massachusetts) ranging between 100 and 300 micrometres in size were used. Later, there was a move towards using smaller particles, such as DC beads M1 ranging from 75 to 100 micrometres, and Tandem 100 micrometre beads (Tandem, Boston Scientific, Marlborough, Massachusetts) in the following years. The procedure was considered successful if at least 50% of the planned dose (50 mg of irinotecan-loaded beads) was delivered. In the case of unilobar disease, two treatments were performed at four-week intervals, and in the case of bilobar disease, four treatments were performed at two-week intervals. The patient's vital signs and femoral access site were monitored after the procedure.

Results

Between September 2010 and March 2020, 30 patients with histologically confirmed colorectal adenocarcinoma with liver-only metastases (22 men and 8 women; mean age 66,8 ± 13,2) were included in the study. DEBIRI TACE was conducted as a first-line treatment in 43% of patients unsuitable for systemic therapy and as salvage therapy after systemic therapy in 57%. In the second group, 100% (17 patients) were treated with the first line, 71% (12 patients) additionally with the second line and 47% (8 patients) with third-line systemic therapy. Eighty two percent of patients on systemic chemotherapy were treated in combination with targeted therapies. Patient characteristics are shown in Table 1.

Treatment compliance and safety

113 DEBIRI procedures were performed with a median of 4 treatments per patient (ranging from 2 to 8). All procedures were technically successful. After the procedure, patients were hospitalised

for a median of 4 days (ranging from 2 to 10 days). There were no treatment-related deaths or grade 4 and 5 adverse events. Non-serious adverse events were present in 53% of patients. Most of them were minor (grades 1 and 2). They contributed to post-embolic syndrome (PES) with significant abdominal pain in 43% of patients, vomiting in 6% of patients, nausea in 16%, diarrhoea in 3%, acute hypertension in 10%, and fever in 6% of patients. The PES symptoms were managed conservatively with hydration and non-steroidal anti-inflammatory drugs. The majority resolved in 48 hours. Seven (6%) high-grade adverse events (grade 3) occurred, including longer stay for pain management (n = 2), prolongation of hospitalisation due to the management of PES (n = 4) and PES requiring readmission (n = 1).

Survival and prognostic factors

During the follow-up time, 26 of the patients died, and 4 remained alive. The median OS from the first DEBIRI TACE procedure was 17.4 months (95% confidence interval [CI]: 10,0–24,7 months) (Figure 1). The 1-year survival rate from the first DEBIRI TACE procedure was 61%, and 2-year survival rate was 25%. The median PFS from the first DEBIRI TACE was 4.2 months (95% CI: 0,9–7.4 months) (Figure 2). The most common site of progression was the liver (20 patients), with the lungs being the second (6 patients). Other progression sites included adrenal glands, lymph nodes, the primary tumour site and the vertebrae.

In 17 patients treated with systemic therapy and DEBIRI TACE, median OS and PFS from the beginning of systemic therapy were 44.6 months and 37.0 months, respectively (Figures 3 and 4).

The median OS from the beginning of DEBIRI TACE in the 17 patients where DEBIRI TACE was used as salvage therapy was 17.4 months (95% CI: 11.0–23.7 months), and in the group without prior systemic therapy, the median OS was 21.6 months (95% CI: 3.8–39.4 months) (Table 2).

Results from the univariate analysis between 10 clinical and radiological characteristics and OS are reported in Table 2. There were no data on levels of CEA and CA 19-9 for three patients before DEBIRI TACE treatment. Further, four patients had no data on CEA and CA 19-9 levels after the treatment. Univariate analysis showed better survival of patients with four or fewer liver metastases (p = 0.002). Age (p = 0.284), ECOG status (p = 0.805), tumour location (p = 0.145), previous systemic chemotherapy (p = 0.472), uni- or bilobar disease (p = 0.106), CEA and CA 19-9 before (p = 0.591;0.393) and after (p = 0.037;0.583) the treatment did not prove to be statistically significant predictors of survival.

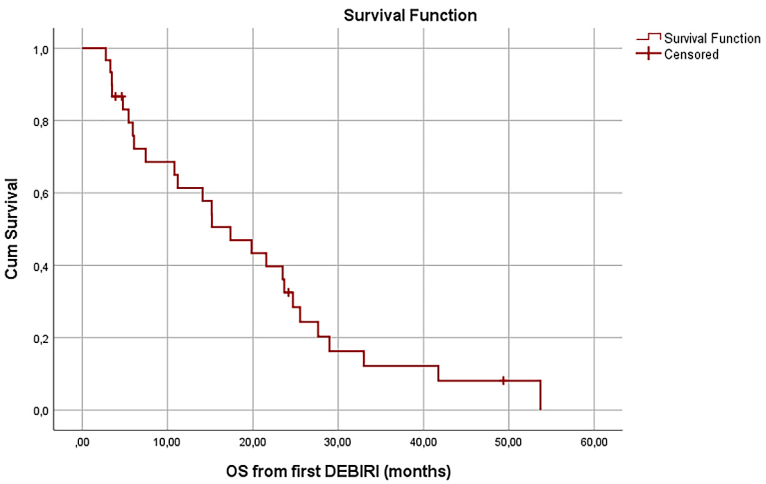


FIGURE 1. Overall survival (OS) from the beginning of irinotecan-loaded drug-eluting beads (DEBIRI) treatment.

Discussion

The first-line treatment for patients with unresectable CRLM is chemotherapy with consideration of additional targeted therapies, usually anti-VEGF or anti-EGFR antibodies – a regimen usually well tolerated, even in elderly patients.[8,16] When the liver is the sole or predominant site of metastases, and the response to systemic therapy is insufficient or systemic therapy is contraindicated or unsuitable, locoregional treatment options such as TACE should

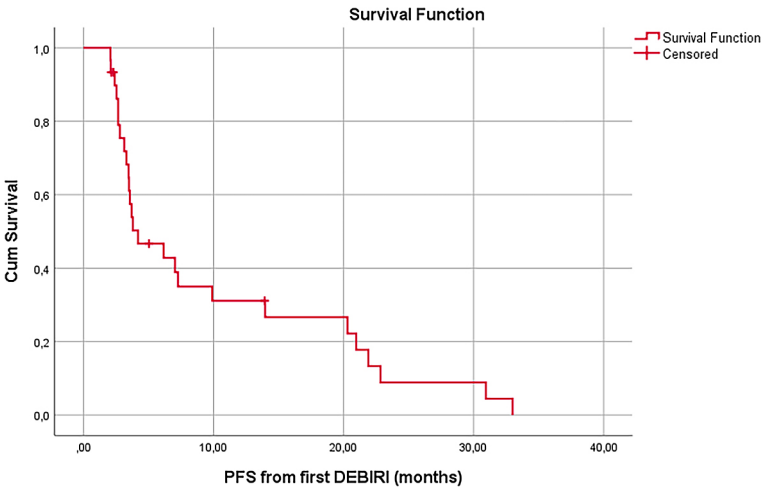


FIGURE 2. Progression-free survival (PFS) from the beginning of irinotecan-loaded drug-eluting beads (DEBIRI) treatment.

TABLE 2. Univariate analysis – influence of probable prognostic factors on overall survival

Characteristics	n (%)	OS	95 % CI	p-value
Age ≤ 65 years	11 (37)	15.2	8.5–21.8	0.284
Age > 65 years	19 (63)	21.6	4.7–38.4.	
Colon	16 (53)	23.7	16.8–30.5	0.145
Rectum	14 (47)	14.1	6.8–21.4	
ECOG 0	18 (60)	19.8	11.3–28.4	0.805
ECOG 1 or 2	12 (40)	17.4	10.0–29.7	
Previous chemotherapy	17 (57)	17.4	11.0–23.7	0.472
No previous chemotherapy	13 (43)	21.6	3.8–39.4	
Unilobar disease	17 (57)	23.5	7.4–39.6	0.106
Bilobar disease	13 (43)	15.2	1.9–28.4	
≤ 4 liver lesions	19 (63)	**23.5**	15.5–31.5	**0.002**
> 4 liver lesions	11 (37)	**10.8**	0.3–21.3	
CEA ≤ 5 µg/L before the first DEBIRI TACE	5 (27)	17.4	5.9–28.9	0.591
CEA > 5 µg/L before the first DEBIRI TACE	22 (73)	15.2	9.2–21.1	
Increase of serum CEA after first DEBIRI TACE	10 (33)	14.1	7.3–20.9	
Decrease of serum CEA after first DEBIRI TACE	12 (40)	24.7	7.0–42.4	0.037
CEA stayed the same	1 (3)	25.5		
CA 19-9 ≤ 37 kU/L before first DEBIRI TACE	16 (53)	15.2	10.1–20.2	0.393
CA 19-9 > 37 kU/L before first DEBIRI TACE	11 (47)	17.4	0.5–34.2	
Increase of serum CA 19-9 after first DEBIRI TACE	11 (48)	15.2	11.7–18.7	
Decrease of serum CA 19-9 after first DEBIRI TACE	10 (43)	19.8	0.0–44.9	0.583
CA 19-9 stayed the same	2 (9)	7.4		

CA 19-9 = cancer antigen 19-9; CEA = carcinoembryonic antigen; DEBIRI TACE = irinotecan-loaded drug-eluting beads transarterial chemoembolization; ECOG = Eastern Cooperative Oncology Group preformance status; OS = overall survival

be considered.[7,8] The introduction of DEBIRI TACE improved the ability to administer higher concentrations of irinotecan to liver metastases while reducing the systemic peaks of irinotecan, thus minimising side - effects. DEBIRI TACE has been proven safe and effective in treating CRLM and is more frequently used than in the past.[11,12,13]

In our study, DEBIRI TACE was conducted in 43% of patients as a first-line treatment and 57% as salvage therapy for patients who had received previous lines of systemic therapy (patients who did not tolerate more cycles of chemotherapy). Our study's median OS from the beginning of DEBIRI TACE was 17.4 months, with progression-free survival of 4.2 months. This aligns with the previously reported trials with median OS and PFS for DEBIRI TACE of 18 months (ranging from 7.3 to 25) and 6.7 months (ranging from 4 to 11), respectively.[9,11] In the salvage therapy group, the median OS was 44,6 months from the beginning of treatment with systemic therapy, confirming the usefulness of DEBIRI TACE as salvage therapy.

In our study, not all patients received systemic therapy before DEBIRI TACE treatment. Interestingly, the group without previous systemic therapy had longer OS from the start of DEBIRI TACE treatment than the previously treated group. Although the difference in survival is not statistically significant, it does raise a question as to whether TACE should be implemented sooner. One such study was done by Martin *et al*., comparing OS, PFS and tumour response between patients who underwent concurrent systemic therapy (*FOLFOX and bevacizumab*) and DEBIRI TACE and patients who were treated with systemic therapy alone. The group simultaneously

treated with TACE had better tumour response in the first six months and longer PFS (15.3 months in the TACE arm versus 7.6 months in the arm with chemotherapy alone).[14] These results require further exploration into the viability of DEBIRI TACE treatment not only as salvage therapy but also as consolidation treatment in combination with systemic therapy for unresectable CRLM. One of the potential reasons for better survival in patients without previous systemic therapy may be irinotecan resistance. Some authors report that DEBIRI TACE shows less efficacy if applied after previous systemic therapy due to irinotecan resistance.[11] The reason for irinotecan resistance could be increased expression of EGFR receptors or active efflux, reducing the drug's intracellular accumulation after the previously used chemotherapeutic agent irinotecan.[10,11,17]

Clinical and radiological factors that affect survival have yet to be determined. Our study is one of the first to ascertain prognostic factors affecting the survival of patients with CRLM treated with DEBIRI TACE. We found that patients with four or fewer liver metastases survived better than those with more. However, the size of lesions varies considerably; therefore, the number of lesions usually doesn't give an accurate assessment of liver impairment. Thus, the metastatic volume would probably be a better predictive factor in further studies. One study where they used DEBIRI and capecitabine in heavily pre-treated patients found a statistically significant correlation between the decrease in CEA after the first DEBIRI treatment and survival.[18,19] Another study found that patients with an ECOG of 0 had better survival than those with an ECOG of 1 or 2.[20] While no such correlation was found in our research, more extensive studies should be performed to confirm these findings.

Safety of the procedure is also a primary concern, and we have shown that the number of significant adverse events (grade 3) is low, with only 6%. This is similar to recently published evidence on the CIREL registry, with 10% of grade 3 and 4% of grade 4 adverse events.[21] The most common mild AE (grades 1 and 2) after the procedure is post-embolic syndrome (PES). The incidence of PES after DEBIRI TACE varies between studies, with a median incidence of 57%.[9] Our study shows that 53% of patients had nonserious mild adverse events that contributed to PES, which is comparable to other studies.[9,21] We assume that the low percentage of serious adverse effects is due to good premedication with antibiotics, antiemetic and intravenous hydration before and during the procedure and peri-procedural pain management with morphine and intra-arterial lidocaine.

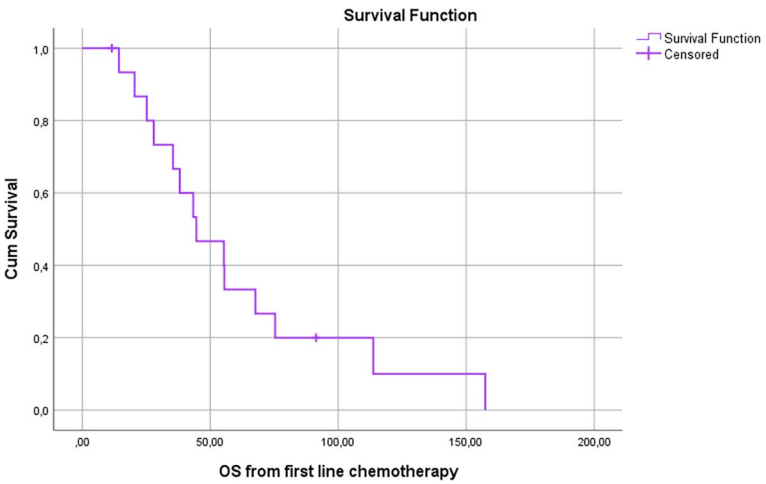


FIGURE 3. Overall survival (OS) of patients with prior systemic chemotherapy from the beginning of systemic treatment.

DEBIRI TACE is a relatively new procedure in interventional oncology, performed primarily in larger centres on a specific group of patients; therefore, data from the literature are usually based on small populations. Such studies have difficulty recognising any essential prognostic factors that could affect survival, yet the lack of such clinical and radiological markers makes patient selection difficult.

The limitations of this study were retrospective design, the small number of patients evaluated, data on biomarkers and molecular targets not be-

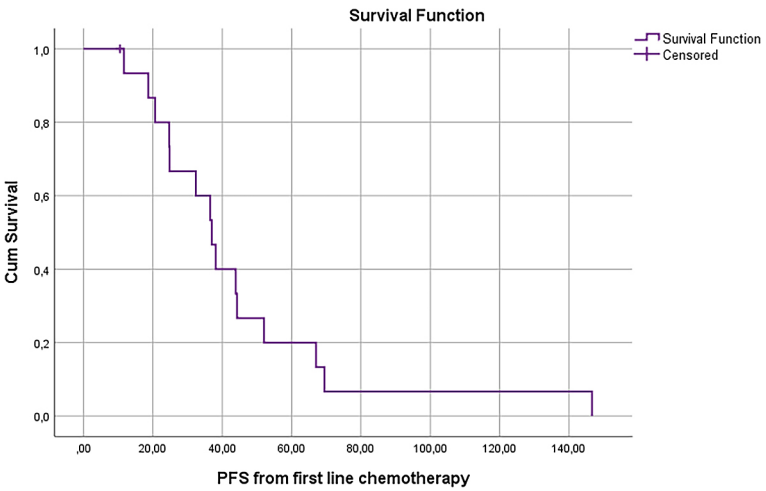


FIGURE 4. Progression-free survival (PFS) of patients with prior systemic chemotherapy from the beginning of systemic treatment.

ing collected, and the heterogeneity of the patient population.

In conclusion, DEBIRI TACE is a safe and effective treatment option for patients with CRLM refractory to systemic therapy. Our research found that four or fewer liver metastases correlated with better survival. Further studies are required to determine the role of DEBIRI TACE in treatment strategies for CRLM, as well as to recognise prognostic factors that would make patient selection easier.

References

1. Dyba T, Randi G, Bray F, Martos C, Giusti F, Nicholson N, et al. The European cancer burden in 2020: incidence and mortality estimates for 40 countries and 25 major cancers. *Eur J Cancer* 2021; **157:** 308-47. doi: 10.1016/j.ejca.2021.07.039
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; **71:** 209-49. doi: 10.3322/caac.21660
3. Van Cutsem E, Nordlinger B, Adam R, Köhne CH, Pozzo C, Poston G, et al. Towards a pan-European consensus on the treatment of patients with colorectal liver metastases. *Eur J Cancer* 2006; **42:** 2212-21. doi: 10.1016/j.ejca.2006.04.012
4. Hackl C, Neumann P, Gerken M, Loss M, Klinkhammer-Schalke M, Schlitt HJ. Treatment of colorectal liver metastases in Germany: a ten-year population-based analysis of 5772 cases of primary colorectal adenocarcinoma. *BMC Cancer* 2014; **14:** 810. doi: 10.1186/1471-2407-14-810
5. Engstrand J, Nilsson H, Strömberg C, Jonas E, Freedman J. Colorectal cancer liver metastases – a population-based study on incidence, management and survival. *BMC Cancer* 2018; **18:** 78. doi: 10.1186/s12885-017-3925-x
6. Brecelj E, Velenik V, Reberšek M, Boc N, Oblak I, Zadnik V, et al. [Recommendations for the diagnosis and treatment of patients with colorectal cancer]. [Slovenian]. *Onkologija* 2020; **6:** 60-92. doi: 10.25670/oi2020-012on
7. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol* 016; **27:** 1386-422. doi: 10.1093/annonc/mdw235
8. Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, et al. Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023; **34:** 10-32. doi: 10.1016/j.annonc.2022.10.003
9. Fiorentini G, Sarti D, Nani R, Aliberti C, Fiorentini C, Guadagni S. Updates of colorectal cancer liver metastases therapy: review on DEBIRI. *Hepatic Oncol* 2020; 21;**7:** HEP16. doi: 10.2217/hep-2019-0010
10. Scevola G, Loreni G, Rastelli M, Sposato S, Ramponi S, Miele V. Third-line treatment of colorectal liver metastases using DEBIRI chemoembolisation. *Med Oncol* 2017; **34:** 37. doi: 10.1007/s12032-017-0890-9
11. Szemitko M, Golubinska-Szemitko E, Sienko J, Falkowski A, Wiernicki I. Efficacy of liver chemoembolization after prior cetuximab monotherapy in patients with metastatic colorectal cancer. *Cancers* 2023; **15:** 541. doi: 10.3390/cancers15020541
12. Fereydooni A, Letzen B, Ghani MA, Miszczuk MA, Huber S, Chapiro J, et al. Irinotecan-eluting 75–150-mum embolics lobar chemoembolization in patients with colorectal cancer liver metastases: a prospective single-center Phase I study. *J Vasc Interv Radiol* 2018; **29:** 1646-53. doi: 10.1016/j.jvir.2018.08.010
13. Iezzi R, Marsico VA, Guerra A, Cerchiaro E, Cassano A, Basso M, et al. Transarterial chemoembolization with irinotecan-loaded drug-eluting beads (DEBIRI) and capecitabine in refractory liver prevalent colorectal metastases: a Phase II single-center study. *Cardiovasc Intervent Radiol* 2015; **6**:1523-31. doi: 10.1007/s00270-015-1080-9
14. Martin RCG, Scoggins CR, Schreeder M, Rilling WS, Laing CJ, Tatum CM, et al. Randomized controlled trial of irinotecan drug-eluting beads with simultaneous FOLFOX and bevacizumab for patients with unresectable colorectal liver-limited metastasis. *Cancer* 2015; **121:** 3649-58. doi: 10.1002/cncr.29534
15. Filippiadis DK, Binkert C, Pellerin O, Hoffmann RT, Krajina A, Pereira PL. Cirse quality assurance document and standards for classification of complications: the Cirse classification system. *Cardiovasc Intervent Radiol* 2017; **40:** 1141-6. doi: 10.1007/s00270-017-1703-4
16. Ocvirk J, Moltara ME, Mesti T, Boc M, Rebersek M, Volk N, et al. Bevacizumab plus chemotherapy in elderly patients with previously untreated metastatic colorectal cancer: single center experience. *Radiol Oncol* 2016; **50:** 226-31. doi: 10.1515/raon-2015-0030
17. Saletti P, Molinari F, De Dosso S, Frattini M. EGFR signaling in colorectal cancer: a clinical perspective. *Gastrointest Cancer Targets Ther* 2015; **5:** 21-38. doi: 10.2147/GICTT.S49002
18. Martin J, Petrillo A, Smyth EC, Shaida N, Khwaja S, Cheow H, et al. Colorectal liver metastases: current management and future perspectives. *World J Clin Oncol* 2020; **11:** 761-808. doi: 10.5306/wjco.v11.i10.761
19. Di Noia V, Basso M, Marsico V, Cerchiaro E, Rossi S, D'Argento E, et al. DEBIRI plus capecitabine: a treatment option for refractory liver-dominant metastases from colorectal cancer. *Future Oncol Lond Engl* 2019; **15:** 2349-60. doi: 10.2217/fon-2017-0025
20. Voizard N, Ni T, Kiss A, Pugash R, Raphael MJ, Coburn N, et al. Small particle DEBIRI TACE as salvage therapy in patients with liver dominant colorectal cancer metastasis: retrospective analysis of safety and outcomes. *Curr Oncol Tor Ont* 2022; **29:** 209-20. doi: 10.3390/curroncol29010020
21. Pereira PL, Iezzi R, Manfredi R, Carchesio F, Bánsághi Z, Brountzos E, et al. The CIREL cohort: a prospective controlled registry studying the real-life use of irinotecan-loaded chemoembolisation in colorectal cancer liver metastases: interim analysis. *Cardiovasc Intervent Radiol* 2021; **44:** 50-62. doi: 10.1007/s00270-020-02646-8

research article

The therapeutic effect of ultrasound targeted destruction of schisandrin A contrast microbubbles on liver cancer and its mechanism

Xiaohui Wang[1,2], Feng Wang[1], Pengfei Dong[3], Lin Zhou[4]

[1] Department of Interventional Therapy, First Affiliated Hospital of Dalian Medical University, Dalian Liaoning, China
[2] Department of Ultrasound, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, Henan, China
[3] Department of Traditional Chinese Medicine, the Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[4] Department of Pharmacology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, Henan, China

Radiol Oncol 2024; 58(2): 221-233.

Received 20 September 2023
Accepted 26 December 2023

Correspondence to: Wang Feng, Department of Interventional Therapy, First Affiliated Hospital of Dalian Medical University, Dalian Liaoning, China. Phone: +86 0371-57152565; E-mail: cjr.wangfeng@vip.163.com and Zhou Lin, Department of Pharmacology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, No.1 East Jianshe Rd. Zhengzhou, Henan, China, 450052. E-mail: 524734490@qq.com

Disclosure: No potential conflicts of interest were disclosed.

Background. The aim of the study was to explore the therapeutic effect of ultrasound targeted destruction of schisandrin A contrast microbubbles on liver cancer and its related mechanism.

Materials and methods. The Span-PEG microbubbles loaded with schisandrin A were prepared using Span60, NaCl, PEG-1500, and schisandrin A. The loading rate of schisandrin A in Span-PEG composite microbubbles was determined by ultraviolet spectrophotometry method. The Walker-256 cell survival rate of schisandrin A was determined by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide (MTT) assay. The content of schisandrin A in the cells was determined by high performance liquid chromatography. Ultrasound imaging was used to evaluate the therapeutic effect *in situ*. Enzyme linked immunosorbent assay (ELISA) was used to measure the content of inflammatory factors in serum. Hematoxylin-eosin (HE) staining was used to observe the pathological changes of experimental animals in each group. Immunohistochemistry was used to detect the expression of hypoxia inducible factor-1a (HIF-1a), vascular endothlial growth factor (VEGF) and vascular endothelial growth factor receptor 2 (VEGFR-2) in tumor tissues, and western blot was used to detect the protein expression of phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway in tumor tissues.

Results. The composite microbubbles were uniform in size, and the particle size distribution was unimodal and stable, which met the requirements of ultrasound contrast agents. The loading rate of schisandrin A in Span-PEG microbubbles was 8.84 ± 0.14%, the encapsulation efficiency was 82.24±1.21%. The IC50 value of schisandrin A was 2.87 μg/mL. The drug + microbubbles + ultrasound (D+M+U) group had the most obvious inhibitory effect on Walker-256 cancer cells, the highest intracellular drug concentration, the largest reduction in tumor volume, the most obvious reduction in serum inflammatory factors, and the most obvious improvement in pathological results. The results of immunohistochemistry showed that HIF-1a, VEGF and VEGFR-2 protein decreased most significantly in D+M+U group ($P < 0.01$). WB results showed that D+M+U group inhibited the PI3K/AKT/mTOR signaling pathway most significantly ($P < 0.01$).

Conclusions. Schisandrin A had an anti-tumor effect, and its mechanism might be related to the inhibition of the PI3K/AKT/mTOR signaling pathway. The schisandrin A microbubbles could promote the intake of schisandrin A in tumor cells after being destroyed at the site of tumor under ultrasound irradiation, thus playing the best anti-tumor effect.

Key words: ultrasonic targeted destruction; schisandrin A contrast microbubbles; liver cancer; mechanism of action

Introduction

Ultrasound contrast agent microbubbles were microparticles used to enhance the contrast of ultrasound images.[1] The latest generation of ultrasound contrast agents had started to use microbubbles to carry drugs or genes, which could improve the effect of ultrasound imaging and achieve the purpose of treating diseases.[2]

Liver cancer is a common malignant tumor of the digestive system and the second most common human malignant tumor after lung cancer.[3] At present, although the research on liver cancer had made a great breakthrough, it was still an important risk factor that seriously affected the life and quality of life of patients. At present, the treatment of liver cancer was mainly based on the stage and the age of the patient. The commonly used treatment methods include surgery, radiotherapy, chemotherapy, vaccines, and so on.[4] In the chemotherapy of liver cancer, cisplatin-based combination chemotherapy was commonly used in clinical practice.[5] Liver tumors were highly sensitive to platinum drugs and had good curative effect. However, the side effects and drug resistance of platinum drugs were also important factors affecting the application of platinum drugs.[6] Despite the continuous improvement of surgery, radiotherapy and technology, and the emergence of chemotherapy drugs, the survival time and quality of patients had not been fundamentally improved.

Ultrasound-targeted microbubble destruction (UTMD) was a technology that improved the efficacy of targeted drugs by increasing the absorption of targeted drugs into cells.[7] This technology mainly used microbubbles to localize "explosive" ultrasound irradiation and released the drugs they carried.[8] At the same time, the shock caused by ultrasound and microbubble rupture increased the local cell permeability, generates reversible sonopores, and promotes drug entry into the nucleus, which could improve the efficiency of drug intervention in tumor cells.[9] Secondly, the protection of microbubbles could prevent the drug from being metabolized and degraded by the body, thereby reducing the bioavailability of the drug, so that it could reach the target organ or tissue directly through the blood circulation.[10] Many preclinical studies and few clinical studies reported the use of microbubble-assisted ultrasound for the delivery of wide range of therapeutics into primary liver tumors or liver mets.[11] Schisandrin A is a bioactive lignan isolated from the traditional Chinese medicine *Fructus schisandrae chinensis*. Studies showed that schisandrin A had many pharmacological effects, such as anticancer, hepatoprotection, antiinflammation, which was worthy of further research and development in the future.[12] Our previous study found that Schisandrisin A significantly reduced the inflammation level of HepG2 cells; improved the oxidative stress state; downregulated transforming growth factor beta 1 (TGF-β1), vascular endothlial growth factor (VEGF), phosphoinositide 3-kinase (PI3K), and Akt mRNA levels; inhibited the expression of the PI3K-Akt signaling pathway, and had a significant anti-tumor effect on tumor cells with high activity and small molecular weight, which was an ideal candidate for the production of contrast-enhanced ultrasound microbubbles.[13] Therefore, in this study, Schisandin A was loaded into Span-PEG microbubbles to make ultrasound contrast agent and to play an anti-tumor effect on the lesions of liver cancer, which was rarely reported. This study would provide a new reference for the treatment of liver cancer.

Materials and methods

Instrumentation

Ultrasonic cell crushing instrument (Ningbo Xinzhi Biological Technology Co., LTD.), Doppler ultrasound diagnostic instrument (Kunshan Ultrasonic Instrument Co., LTD.), Zeta potential/particle size instrument (British Malvern Instrument Co., LTD.), SW-CJ-1D single side vertical air supply purification table (Suzhou Zhijing Purification Equipment Co., LTD., Jiangsu Province), HZQA Constant temperature incubator (Jintan Shenglan Instrument Manufacturing Co., LTD.), LX-C50L vertical automatic electric heating pressure steam sterilizer (Beijing Sibo Shengda Technology Co., LTD.), scanning electron microscope (Japan Electronics Co., LTD.), Ultrasound imaging instrument (mindray M9cv, Superficial probe), Bio-Rad 680 iMark Microplate reader(American Bio-Rad Co., LTD.)

Reagents

Span60 (Tianjin BASF Chemical Co., LTD.), PEG1500 (Tianjin Guangfu Fine Chemical Research Institute), NaCl (Tianjin Beilian Fine Chemical Development Co., LTD.), schisandrin A (Chengdu Manst Biotechnology Co., LTD. HPLC ⩾ 98%), phenol and sulfuric acid (Tianjin Kemio Chemical Reagent Co., LTD.), Walker-256 (number: 399-88-2) was obtained from Shanghai Hongshun

Biotechnology Co., LTD. MEM (containing NEAA) basal medium (Procell PM150410) and fetal bovine serum (Procell 164210) was obtained from Pricella Biotechnology Co., LTD. Fixative solution (4% Paraformaldehyde, P1110) was obtained from Shanghai solarbio Bioscience & TechnologyCo., LTD. Enzyme linked immunosorbent assay (ELISA) kit tumor necrosis factor-α (TNF-α) (ab236712), interleukin-1β (IL-1β) (ab255730) and interleukin-6 (IL-6) (ab234570) was obtained from abcam Bioscience & TechnologyCo.,LTD. Hematoxylin-eosin (HE) Stain Kit (G1120) was obtained from Solarbio Bioscience & TechnologyCo.,LTD. Immunohistochemistry kit hypoxia inducible factor-1α (HIF-1α) (IHC0103715), VEGF(IHC0100011) and vascular endothelial growth factor receptor 2 (VEGFR-2) (IHC0102817) was obtained from Shanghai CaiYOU industrial Co., LTD. WB kit: Primary antibody p-PI3K (bs-6417R), PI3K(20584-1-AP), p-Akt (bs-0876R) was obtained from Bioss Co., LTD, AKT (60203-2-Ig), p- mammalian target of rapamycin (mTOR) (67778-1-Ig), mTOR (66888-1-Ig), was obtained from Proteintech Group, GAPDH was obtained from Hangzhou Hua 'an Biotechnology Co. LTD, Secondary antibody (SA00001-1) was obtained from Bioss Co., LTD.

Experimental cells

Walker-256 cell (Free of mycoplasma infection, cells were derived from ascites of liver cancer in rats) were cultured in RPMI 1640 medium containing 10% fetal bovine serum and incubated at 37°C in 5%CO_2 incubator. The cells were routinely digested and subcultured with 2.5 g/L trypsin, and the logarithmic growth phase cells were used for experiments.

Experimental animals

36 SD (Sprague Dawley, male, 6 weeks, 180–200 g, wide type rats) rats were obtained from Henan Laboratory Animal Center. Animal experiment ethics was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (No. KY2023-006).

Preparation and analytical characterization of Span-PEG microbubbles loaded with schisandrin A

450 mg Span60, 900 mg NaCl, 450 mg PEG-1500 and 300 mg schisandrin A were weighed, placed in a mortar and thoroughly ground, dissolved in 40 mL PBS phosphate buffer solution, and heated to 80°C in a magnetic heating mixer, and stirred and dispersed evenly. Then, the solution was continuously sonicated at 570 W power for 6 min using an ultrasonic cell disruptor by acoustic cavitation method, while nitrogen gas was continuously injected into the above solution. A uniform milky yellow liquid mixture was prepared and centrifuged in an ultracentrifier at 2 000 g for 8 min. After centrifugation, a stratified solution was obtained. The upper and middle layers were removed and placed in a 250 mL separating funnel, washed with an equal volume of PBS phosphate buffer, and left to stand. The middle layer microbubbles were collected and freeze-dried to obtain Span-PEG ultrasound contrast agent microbubbles loaded with schisandrin A.[14] The size and shape of the microbubbles were observed by scanning electron microscopy (SEM). Detailed operation details were as follows: A small amount of microbubble powder was coated to one side of the double-sided glue and the other side was fixed on the stage of the scanning electron microscope. The surface morphology of the drug microbubbles was observed and photographed by scanning electron microscopy under a high voltage of 15 kV at a magnification of 5000. The particle size distribution and Zeta potential of the microbubbles were determined by ZS90 laser particle size analyzer. Detailed operation steps were as follows: Added pure water into the sample tank as the dispersing agent, turned on the ultrasonic disperser and set the intensity to 7, turned on pump switch after 2 minutes, adjusted the pump speed to 2680 r/min, specified water as the dispersing agent in the TAB, and other parameters were determined, clicked "Start", measured the sample, and saved the results. Then changed the measurement conditions and re-measured until the next measurement results were basically in line with the last measurement results, then the last measurement results are the particle size measurement results of the sample. This experiment was independently repeated three times with consistent results.

Determination of loading rate of schisandrin A in Span-PEG composite microbubbles

The loading rate of schisandrin A in Span-PEG microbubbles was determined by ultraviolet spectrophotometric method. The standard of schisandrin A was prepared at concentrations of

2.5 μg·mL^{-1}, 5 μg·mL^{-1}, 10 μg·mL^{-1}, 20 μg·mL^{-1}, 30 μg·mL^{-1}, 40 μg·mL^{-1} and 50 μg·mL^{-1}, respectively. The absorbance value was measured at 254 nm, the standard curve was drawn, and the regression equation was calculated. Span-PEG microbubbles loaded with schisandrin A were weighed 10 mg, dispersed in distilled water for ultrasonic release for 2 h, filtered, and constant volume to a 10 mL volumetric flask. The absorption wavelength of schisandrin A in Span-PEG composite microbubbles at 254 nm was determined by the same method. The schisandrin A loading rate was calculated according to the standard curve. This experiment was independently repeated three times with consistent results.

Determination of the cell survival rate of schisandandin A by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide (MTT) assay

The standard Schisandandin A was diluted in double dilutions in cell culture medium, so that the final concentrations in cell culture medium were 64 μg/mL, 32 μg/mL, 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL, 1 μg/mL and 0 μg/mL, respectively. Walker-256 cells with logarithmic growth were seeded in 96-well plates and divided into eight groups. And culture medium containing the corresponding concentration of drug was added to each well (1×10^4/mL), the cells and the drug were incubated in an incubator for 72 h. Then, 10 μL of freshly prepared 5 mg/mL MTT solution was added to each well and continue to culture for 4 hours. The supernatant was discarded and dissolved by adding 200 μL dimethylsulfoxide (DMSO), and the absorbance value was measured at 490 nm by microplate reader. The drug concentration in the control group was zero. In the blank group, only culture medium, MTT and DMSO were added. The cell survival rate = (Experimental group OD - blank group OD)/(Control group OD - blank group OD). The 50% inhibitory concentration (IC50) was calculated by IC50 software to define the concentration range of schisandrin A in this experiment.[16] Fitting and calculation of cell survival curves were calculated with GraphPad Prism 9.0.

The above MTT assay was also used to detect following cell survival rate in control (C), microbubbles (M), ultrasound (U), drug (D), drug + ultrasound group (D+U), drug + microbubbles + ultrasound group (D+M+U) group. This experiment was independently repeated three times with consistent results.

Anti-tumor cell experiment assessment in different groups

The cells in logarithmic growth phase were used for the experiment and randomly divided into 6 groups: simple microbubbles group (M): the cells were resuspended in cell culture medium containing 5% microbubbles; simple ultrasound group (U): the cells were exposed to 300 kHz, 0.25 W ultrasound for 10 s; simple drug group (D): the cells were resuspended in cell culture medium containing 2.5 μg/mL schisandrin A; Drug + ultrasound group (D+U): The cells were incubated with 2.5 μg/mL schisandin A and then exposed to 300 kHz, 0.25 W ultrasound for 10 s; Drug + microbubbles + ultrasound group (D+M+U): After routine digestion and centrifugation, the cells were added with cell culture medium containing 2.5 μg/mL Schisandandin A and 5% microbubbles, and then ultrasound irradiation was performed under the same conditions as the previous group. Control group (C): The digested and centrifuged cells were resuspended and cultured in conventional culture medium.[17] Cell survival rate was measured by MTT assay. This experiment was independently repeated three times with consistent results.

The intracellular content of schisandrin A for quantitative determination

Sample collection: The treated cells of each group were cultured in 24-well plates with 6 multiple wells in each group. After 24 hours of culture, the cells of each group were routinely digested, centrifuged and collected, rinsed 3 times with PBS, and the last time was fixed in 1 mL double distilled water. Cells were destroyed using an ultrasonic cell morcellator to obtain a double distilled aqueous solution of intracellular fluid in each group. The chromatographic conditions were as follows: Kro-masil C18(250 mm×4.6 mm, 5 μm) column; The mobile phase was methanol-0.1% glacial acetic acid aqueous solution (82:18). Flow rate :1mL/min; Detection wavelength: 254 nm; The column temperature was 30°C. The chromatogram of schisandrin A samples and intracellular liquid standard was drawn under the selected chromatographic conditions.[18] This experiment was independently repeated three times with consistent results.

Establishment and grouping of rat liver cancer model

After 7 days of adaptive feeding, all groups of animals were inoculated with Walker-256 cells in the liver under ultrasound guidance to establish an orthotopic liver cancer model. When the tumor gradually grew to 35mm^2, the drug treatment was started. The rats were divided into 6 groups with 6 rats in each group: control group (injected with equal volume of normal saline), microbubble group (injected with microbubbles via the tail vein without ultrasound), ultrasound group (injected with equal volume of normal saline with ultrasound), ultrasound + schisandrin A group (injected with schisandrin A via the tail vein), ultrasound + microbubble group (injected with microbubbles via the tail vein), and ultrasound + schisandrin A microbubble group (injected with schisandrin A microbubble via the tail vein). The liver parts of all animals were subjected to low-frequency ultrasound treatment at 300 kHz, 2.0 W/cm^2, PRF(Pulse Repetition Frequency) 1 kHz, DC(Duty Cycle) 20%, PNP(Peak Negative Pressure) 0.5 MPa, with 10 s irradiation, 10 s interval, and a total of 20 min. The injection dose of schisandrin A was 20 mg/kg, and the microbubble injection dose was 0.3 mL/kg. The treatment was given every 3 days for a total of 16 days. Tumor observation: Ultrasound was used to observe the tumor growth at the inoculation site 7 days and 16 days after treatment. This experiment was independently repeated three times with consistent results.

The treatment evaluation effect *in situ* by ultrasound imaging

In this study, mindray M9cv Doppler ultrasound was used to collect the *in situ* tumor images of animals in each group before and after treatment. The acquisition method was as follows: under the guidance of ultrasound, the tumor site was determined, and the longest and shortest diameters of the tumor were measured at the same time. This experiment was independently repeated three times with consistent results.

Serum inflammatory factors detection

Twenty-four hours after the last treatment, blood samples were collected from the abdominal aorta of rats and stored in a test tube without anticoagulant. The samples were placed at 37°C for coagulation, and after blood coagulation, the samples were equilibrated and centrifuged (4°C, 2000 g, centrifugal radius was 7.5 cm), and the final obtained supernatant was the serum. The contents of TNF-α, IL-1β and IL-6 in serum were determined by enzyme-linked immunosorbent assay. The specific operation steps are as follows: 10 μL standards and 10 μL samples were added into the wells of the corresponding reaction plates. 40 μL TNF-α/IL-1β/IL-6 Biotin and 40 μL TNF-α/IL-1β/IL-6 POD were added to each well. The plates were mixed gently for 30 seconds, the wells were sealed, and the plates were incubated at room temperature for 45 minutes. Washed the plate: dump all the liquid in the plate, washed the reaction plate with washing solution (add 350 μL of washing solution to each well), and removed water droplets (pat dry on thick absorbent paper): washed 5 times repeatedly. 100 μL of chromogenic solution was added to each well, gently mixed for 10 seconds, and incubated at room temperature for 20 minutes. Added 100 μL stop solution to each well. Gentle mixing for 30 s: OD values were read at 50 nm within 30 min. This experiment was independently repeated three times with consistent results.

Histopathological examination

Fresh liver tumor tissues were taken and fixed with fixative solution for more than 24 hours. The tissues were removed from fixative solution and trimmed in a fhood with a scalpel. Then, the tissues were dehydrated and immersed in wax, embedded, sectioned, hematoxylin staining, eosin staining, dehydrated and sealed. Finally, microscopic examination and image acquisition and analysis were performed. Pathological evaluation was made by pathologists under a microscope. Sample preparation was performed as follows: sampling, fixation, dehydration, transparency, wax immersion, embedding, and sectioning. The procedure for HE staining was as follows: The sections were successively washed in xylene I 10 min, xylene II 10 min, absolute ethanol I 5 min, absolute ethanol II 5 min, 95% alcohol 5min, 90% alcohol 5 min, 80% alcohol 5 min, 70% alcohol 5 min, distilled water. The sections were stained with Harris hematoxylin for 3–8 min, washed with tap water, differentiated in 1% hydrochloric acid alcohol for 7 seconds, rinsed with tap water, returned to blue in 0.6% ammonia water, and rinsed with running water. Sections were stained in eosin staining solution for 1-3min. The slices were successively placed in 95% alcohol I 5, min, 95% alcohol II 5 min, absolute ethanol I 5 min, absolute

ethanol II 5 min, xylene I 5 min, xylene II 5 min for dehydration and transparency. The slices were taken out of xylene to dry slightly and sealed with neutral gum. This experiment was independently repeated three times with consistent results.

The expression of HIF-1α, VEGF and VEGFR-2 by immunohistochemistry

The paraffin sections of liver tumor tissue were deparaffinized to water, and then underwent antigen repair, endogenous peroxidase blocking, serum blocking, primary antibody, secondary antibody, DAB staining, nucleus counterstain, dehydration and sealing. Finally, the sections were taken out of xylene to dry slightly, and sealed with sealing glue for microscopic examination. Specific IHC procedures are as follows: Paraffin sections were routinely deparaffinized to water. 3% hydrogen peroxide was used for 10 min at room temperature to inactivate endogenous enzymes, and the cells were washed twice with distilled water. The sections were immersed in 0.01 mol/L citrate buffer solution (pH 6.0), heated to boiling in microwave oven, and then turned off. After an interval of 5 min, the sections were cooled repeatedly and washed twice x 3 min with PBS buffer (pH 7.2–7.6). The blocking solution was added droppers at room temperature for 20min. Toss off any excess liquid without washing. Appropriate primary antibodies (Rat Anti-VEGF 1:300; Rat Anti-VEGFR2 1:200; Rat Anti-HIF-1a 1:200), incubated at 37°C for 2 h. Washed 3 min x 3 times with PBS buffer (pH 7.2–7.6). The working solution of biotinylated secondary antibody was added at 20~37°C for 20min. Washed 3 min x 3 times with PBS buffer (pH 7.2–7.6). Working solution of horseradish enzyme-labeled streptavidin was dropped and washed 3 min x 3 times with 20~37°C, 20 min, PBS buffer (pH 7.2–76). The reaction time was controlled under the DAB chromoscope at room temperature, generally between 5 and 30 min. Light counterstained with hematoxylin, dehydrated, sealed with transparent neutral gum, and observed under microscopy. This experiment was independently repeated three times with consistent results.

Protein expression levels detection of PI3K-AKT-mTOR signaling pathway

The tumor tissue was taken and detected by Western blottting. About 200 mg of fresh tumor tissue was weighed, then liquid nitrogen was added while grinding in a mortar, the ground tissue was weighed in a peeled and precooled centrifuge tube. Then, the tumor tissue was fully lysed with RIPA (Radio Immuno Precipitation Assay) lysate containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and inhibitors containing sodium orthovanadate, sodium fluoride, EDTA, leupeptin. The total protein was extracted and the protein content was determined by BCA method. The protein samples (50 μg) were denatured, subjected to 12% polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride (PVDF) films, and blocked with 5% skim milk for 2 h at 4°C. Then, antibodies against PI3K, p-AKT, AKT, p-AKT, mTOR and p-mTOR proteins were added (diluted 1:1 000) and incubated at 4°C overnight. The membranes were washed with TBST buffer, the secondary antibody (dilution 1:10 000) was added, and the membranes were incubated for 2 hours at room temperature. The membranes were washed with TBST (Tris Buffered Saline Tween20) buffer, and after adding ECL (Electro Chemi Luminescence) solution, the membranes were exposed and developed in an automatic chemiluminescence gel imaging analysis system. This experiment was independently repeated three times with consistent results.

Statistical analysis

SPSS 22.0 statistical software was used for all statistical analyses of this study. The measurement data were expressed as mean ± standard deviation. Comparison between groups was performed using analysis of variance. $P < 0.05$ for the difference was considered statistically significant.

Results

Span-PEG microbubbles loaded with schisandrin A exhibited a stable physical and chemical properties

Figure 1A shows the structure SEM of Span-PEG composite microbubbles loaded with schisandrin A. The prepared microbubbles have smooth surface and uniform particle size. Figure 1B and 1C show the particle size distribution and Zeta potential of Span-PEG composite microbubbles, respectively. From the measurement results and distribution curves, it can be seen that the composite microbubbles have uniform size and single peak particle size distribution. The average particle size is 595.3 nm, less than 700 nm, and the Zeta poten-

TABLE 1. Loading rate of schisandrin A in Span and polyethylene glycol (Span-PEG) composite microbubbles

Number of experiments	Absorbance value	Rate of load/%
1	0.524	8.68
2	0.536	8.88
3	0.541	8.96
Mean	0.534	8.84
SD	0.009	0.14

tial is -18.8 mV, which is relatively stable. It meets the requirements as an ultrasound contrast agent.

Span-PEG composite microbubbles showed a satisfactory loading rate of schisandrin A

According to the experimental method, the standard curve of schisandrin A was drawn. The standard curve equation of schisandrin A was: Y = 0.0121X-0.0012, R^2 = 0.9992.

The absorbance value of schisandrin A in Span-PEG composite microbubbles was measured and substituted into the standard curve to calculate the loading rate of schisandrin A. The results are shown in Table 1. The loading rate of schisandrin A in Span-PEG composite microbubbles was 8.84 ± 0.14%, the encapsulation efficiency was 82.24 ± 1.21%.

Schisandrin A could reduce cell survival rate

The results of MTT assay showed that the cell survival was inhibited to varying degrees after treatment with different concentrations of schisandrin A for 24h. The cell survival rates of 0 μg/mL, 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL, 64 μg/mL schisandrin A on Walker-256 cells after 24 hours were 100.00%, 72.36%, 64.52%, 45.90%, 33.39%, 24.78%, 21.97%, 17.44%, respectively. The IC50 value was 2.87 μg/mL. The concentration of schisandrin A selected for this experiment was 2.5μg/mL, as shown in Figure 2.

U+M+D treatment could effectively inhibit cell survival

Compared with the control group, the cell survival rate of each experimental group decreased significantly: group C (100%) > group U (98.31%) > group M (95.68%) > group D (53.14%) > group U+D (42.53%) > group U+M+D (32.17%). There were significant differences among the groups (F = 626.5, P < 0.0001), group D *vs.* group D+U (MD = -0.32587, P < 0.001), group D *vs.* group D+M+U (MD = -0.52608, P < 0.001), group D+U *vs.* group D+M+U (MD = -0.32673, P < 0.001), as shown in Figure 3.

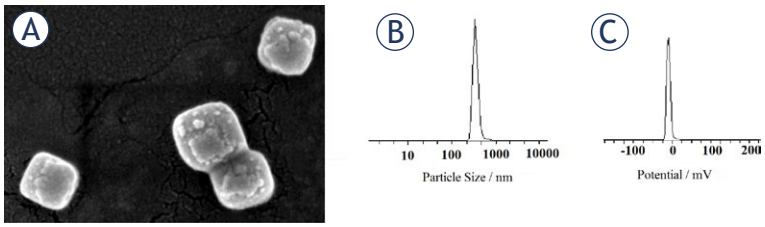


FIGURE 1. Analytical characterization of Span and polyethylene glycol (Span-PEG) microbubbles loaded with schisandrin A **(A)** The structure SEM of schisandrin A microbubbles; **(B)** The particle size distribution; **(C)** Zeta potential of schisandrin A microbubbles, (n = 6).

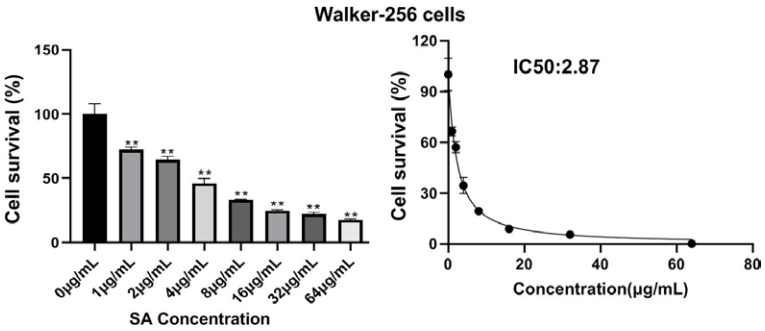


FIGURE 2. The Walker-256 cell survival rate of schisandrin A (n = 6; compared with control group [0 μg/mL], **P < 0.01).

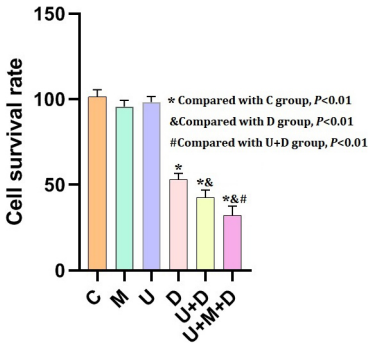


FIGURE 3. Anti-tumor cell experiment assessment in different groups (n = 6; *Compared with C group, P < 0.01; &Compared with D group, P < 0.01; #Compared with U+D group, P < 0.01)

U+M+D treatment could significantly increase the intracellular content of schisandrin A

Under the selected chromatographic conditions, the chromatographic profiles of schisandrin A standard, blank intracellular liquid, blank in-

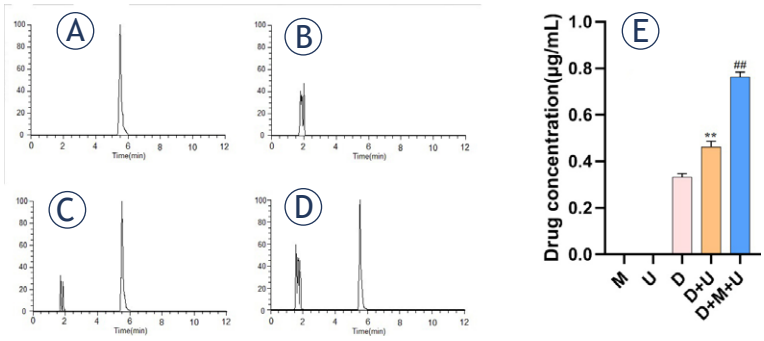


FIGURE 4. The content of schisandrin A in the cells of each group (A. Chromatogram of standard schisandrin A; B. Chromatogram of blank intracellular fluid; C. Chromatogram of blank intracellular solution + standard schisandrin A; D. Chromatogram of the tested intracellular liquid; E. Comparison of drug concentrations between different groups, n = 6; **Compared with C group $P < 0.001$; ##Compared with D group $P < 0.001$)

tracellular liquid + schisandrin A standard and the tested intracellular liquid were obtained (Figure 4A–D). The standard curve of schisandrin A was drawn to obtain the linear regression equation Y = 0.0544X+0.0128 ($R^2 = 0.9997$), and the linear range was 0–6.4 μg/mL. The average concentration of schisandrin A in each group was calculated by taking the peak area of schisandrin A into the equation: group M (0 μg/mL); group U (0 μg/mL); Group D 0.33 μg/mL; Group D+U 0.46 μg/mL; Group D+M+U 0.76 μg/mL. There were significant differences in intracellular drug concentrations among the groups (F = 587.5, $P < 0.0001$), Group D *vs.* Group D+U (MD = -0.13667, $P < 0.001$), Group D *vs.* Group D+M+U (MD = -0.43500, $P < 0.001$), Group D+U *vs.* Group D+M+U (MD = -0.29833, $P < 0.001$), shown in Figure 4E.

U+M+D treatment showed the best anti-tumor effect

The experimental results showed that the tumor in the control group (without treatment drugs), M (microbubbles) group and U (ultrasound) group had progressed and enlarged, and the other different treatment groups had significant effects before and after drug treatment ($P < 0.05$). Among them, the tumors in the ultrasound + schisandrin A group and the ultrasound + microbubble group showed a certain degree of atrophy ($P < 0.05$). The most significant effect was in the ultrasound + schisandrin + A microbubble group ($P < 0.01$). The results suggest that schisandrin A has a certain anti-tumor effect, and the microbubbles loaded schisandrin A can promote the intake of schisandrin A in tumor cells after blasting at the tumor site under ultrasound irradiation, thus playing the best anti-tumor effect, as shown in Figure 5.

Serum inflammatory factors (TNF-α, IL-6, IL-1β) decreased obviously in U+M+D group

The experimental results showed that the levels of inflammatory factors in the control group (without therapeutic drugs) were higher, and there was no significant improvement for the levels of inflammatory factors in M group and U group. However, there were significant differences among the groups for TNF-α, (F = 73.698, $P < 0.001$), Group C *vs.* Group D+U (MD = 0.745, $P < 0.01$), Group C *vs.* Group M+U (MD = 1.228, $P < 0.01$), Group C *vs.* Group D+M+U (MD = 2.060, $P < 0.01$), Group D+U *vs.* Group M+U (MD = 0.483, $P < 0.01$), Group D+U *vs.* Group D+M+U (MD = 1.315, $P < 0.01$), Group M+U

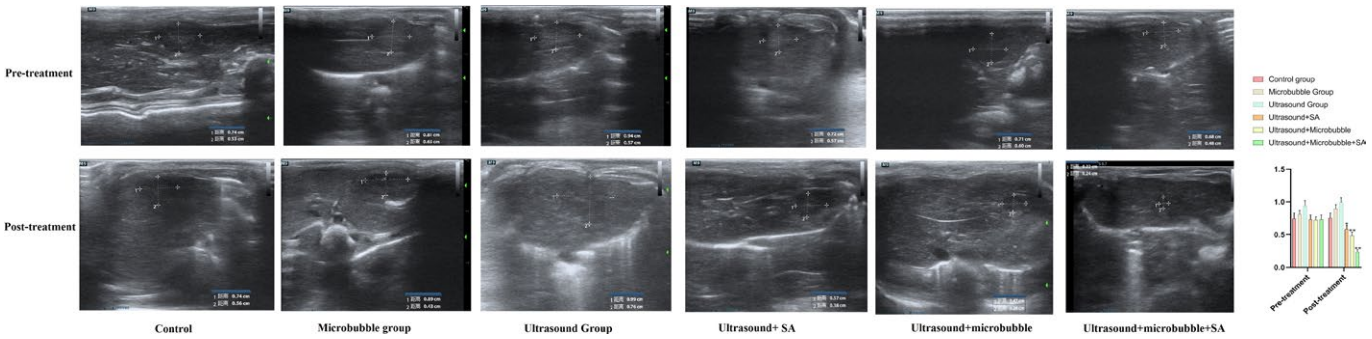


FIGURE 5. The changes in tumor size before and after drug treatment detected by Ultrasound images (n = 6; *Compared with Pre-treatment group, $P < 0.05$; **Compared with Pre-treatment group, $P < 0.01$).

vs. Group D+M+U (MD = 0.831, $P < 0.01$). There were significant differences among the groups for IL-6, (F = 828.16, $P < 0.001$), Group C *vs.* Group D+U (MD = 47.280, $P < 0.01$), Group C *vs.* Group M+U (MD = 68.040, $P < 0.01$), Group C *vs.* Group D+M+U (MD = 74.818, $P < 0.01$), Group D+U *vs.* Group M+U (MD = 20.760, $P < 0.01$), Group D+U *vs.* Group D+M+U (MD = 27.539, $P < 0.01$), Group M+U *vs.* Group D+M+U (MD = 6.78, $P < 0.01$). There were significant differences among the groups for IL-1β, (F = 230.955, $P < 0.001$), Group C *vs.* Group D+U (MD = 9.99, $P < 0.01$), Group C *vs.* Group M+U (MD = 17.54, $P < 0.01$), Group C *vs.* Group D+M+U (MD = 27.14, $P < 0.01$), Group D+U *vs.* Group M+U (MD = 7.56, $P < 0.01$), Group D+U *vs.* Group D+M+U (MD = 17.15, $P < 0.01$), Group M+U *vs.* Group D+M+U (MD = 9.60, $P < 0.01$). Compared with the control group, the levels of inflammatory factors in the ultrasound + schisandrin A (U+D) group and the ultrasound + microbubble group (U+M) showed a downward trend. The level of decrease was most pronounced in the ultrasound + schisandrin A + microbubble (U+M+D) group, as shown in Figure 6.

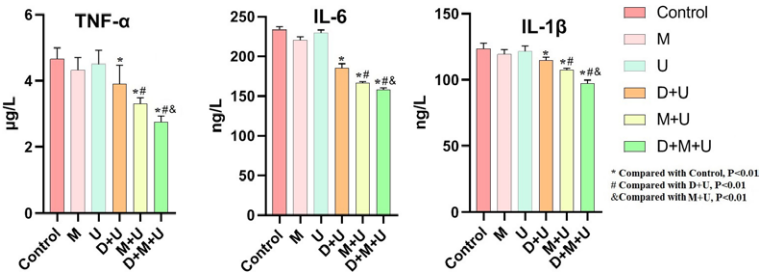


FIGURE 6. Detection of serum inflammatory factors (tumor necrosis factor-α [TNF-α], interleukin-6 [IL-6], interleukin-1β [IL-1β]) in different group (n = 6, *Compared with C group, P < 0.01; #Compared with D+U group, P < 0.01; &Compared with M+U group, P < 0.01).

The pathological improvement was most obvious in U+M+D group

In the control group, the tumor cells were closely arranged and the intercellular space was small, the nuclear showed atypia, the nuclei were large, the nuclear to cytoplasmic ratio was high, and mitotic figures were visible (red arrows), necrosis of tumor cells was occasionally observed (yellow arrow), the nuclei were pyknotic and hyperchromatic, there was a small amount of thin collagen fiber proliferation between the tumor cells (green arrow). There was no improvement in pathology for microbubble and ultrasound group. Compared with control group, in the ultrasound + schisandrin A group, the mitosis of tumor cell nuclei decreased (red arrows), there was a decrease in collagen fibers between tumor cells (green arrow), balloon-like swelling of tumor cells was observed (blue arrow). Compared with control group, in the ultrasound + microbubble group, tumor necrosis was increased (yellow arrow), the mitosis of tumor cell nuclei was decreased (red arrows), there was a decrease in collagen fibers between tumor cells (green arrow). Compared with control group, in the ultrasound + schisandrin A + microbubble group, tumor cell necrosis was significantly increased (yellow arrow), the mitosis of tumor cell nuclei was significantly decreased (red arrows), balloon-like swelling of tumor cells was significantly observed (blue arrow), there was a decrease in collagen fibers between tumor cells (green arrow), as shown in Figure 7.

The immunohistochemical improvement was most obvious in U+M+D group

The results of immunohistochemistry showed that the control group (without treatment drugs) had a large staining area and strong staining intensity. There was no improvement in immunohistochemical result for microbubble and ultrasound group. Compared with the control group, the staining area of ultrasound + schisandrin A group was smaller and the staining intensity was weakened. Compared with the ultrasound + schisandrin A group, the staining area of the ultrasound + microbubble group and the ultrasound + schisandrin A microbubble group gradually became smaller,

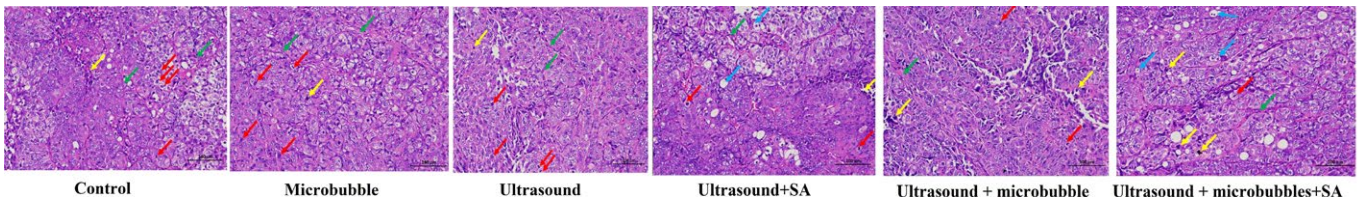


FIGURE 7. Histopathological changes in different groups (division of the tumor cell nucleus [red arrows], hyperplasia of collagen fibers [green arrows], necrosis of tumor cells [yellow arrows], and balloon-like swelling of tumor cells [blue arrows]).

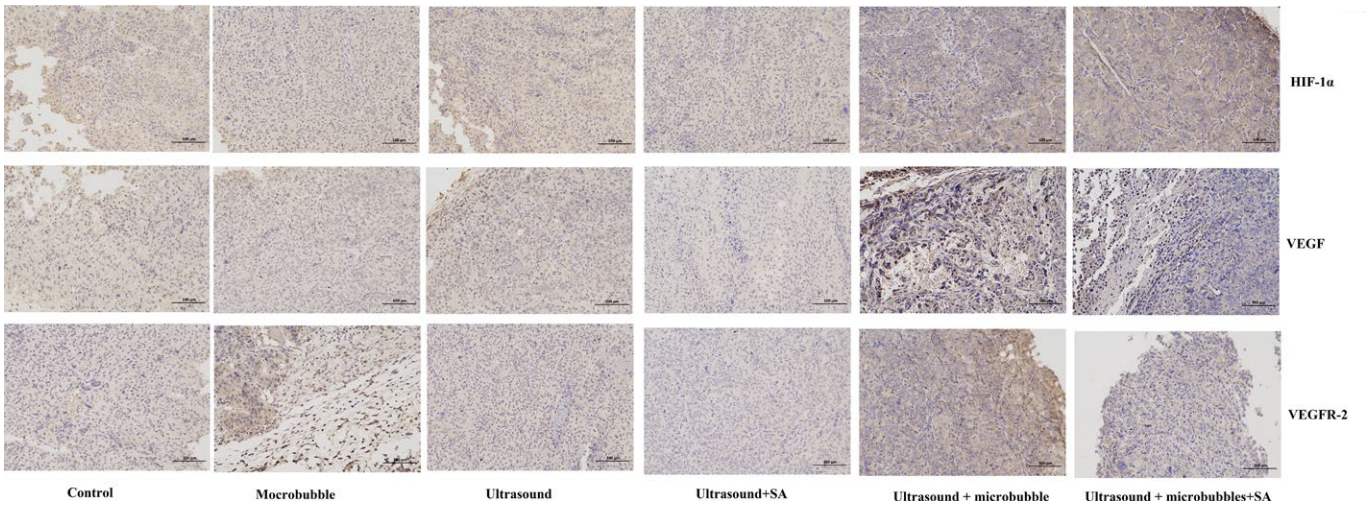


FIGURE 8. Immunohistochemical changes in different groups.

as shown in Figure 8. The above results showed that the expression of HIF-1α, VEGF and VEGFR-2 proteins in the tumor tissues of the ultrasound + schisandrin A group, the ultrasound + microbubble group and the ultrasound + schisandrin A microbubble group showed a weakening trend ($P < 0.01$), as shown in Figure 9.

PI3K/AKT/mTOR signaling pathway was most inhibited in U+M+D group

Compared with the control group, the relative protein expression levels of p-PI3K, PI3K, p-Akt, AKT, p-mTOR, mTOR proteins in the ultrasound + schisandrin A group and the ultrasound + schisandrin A microbubble group were significantly decreased ($P < 0.05$), and the ultrasound + schisandrin A microbubble group had the most obvious effect. It was suggested that schisandrin A can inhibit the PI3K/AKT/mTOR signaling pathway in tumor tissues. After ultrasonograph-assisted microbubble destruction, the uptake of schisandrin A by tumor cells was further promoted, so the tumor inhibition effect was more obvious, as shown in Figure 10.

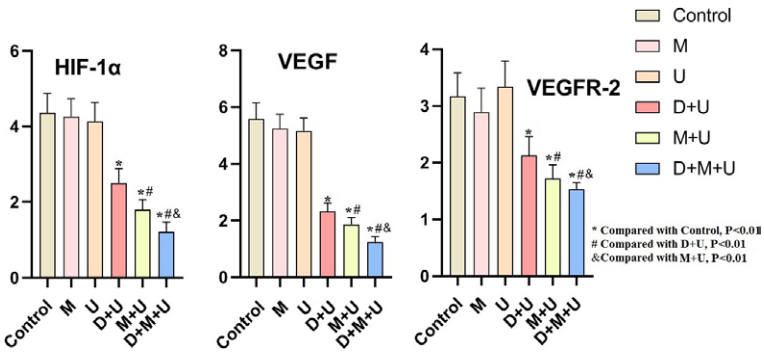


FIGURE 9. The expression of hypoxia inducible factor-1a (HIF-1a), vascular endothlial growth factor (VEGF) and vascular endothelial growth factor receptor 2 (VEGFR-2) proteins in the tumor tissues (n = 6, *Compared with C group, P < 0.01; #Compared with D+U group, P < 0.01; &Compared with M+U group, P < 0.01)

Discussion

Microbubbles (MBs) combined with ultrasound appeared to be an alternative therapeutic strategy for many diseases, and showed good clinical results.[19] The combination of microbubbles and ultrasound had emerged as a promising method for local drug delivery. Microbubbles could be locally activated by a targeted ultrasound beam, which could result in several bio-effects, this was essential for targeted tumor therapy.[20] In addition to the development of new types of ultrasound contrast agents, various imaging methods dedicated to contrast agents had been introduced, and some of them were now commercially available.[21]

This study found that the ultrasound irradiation microbubble contrast agent could increase the concentration of schisandrin A in Walker-256 cells, and the cytotoxic of schisandrin A on Walker-256 cells could be enhanced by the ultrasound irradiation microbubble contrast agent. The combination of ultrasonic microbubble contrast agent with low dose of cytotoxic drugs could achieve the killing effect of high dose of drugs on tumor cells when the drug was used alone, and it could reduce the toxic side effects on normal cells and tissues. It could not only improve the efficacy of chemother-

apy, but also improve the tolerance of patients to chemotherapy.

On the basis of the cell experiment, this study carried out the verification experiment of the orthotopic rat liver cancer model. Imaging showed that the tumor volume of the D+M+U group was the smallest, and there was necrosis and liquefaction in the tumor. The pathological results of HE staining in each group showed that the tumor cells in the D+M+U group were loose, and a large number of necrotic cells appeared in the tissues. Although different degrees of cell necrosis and hemorrhage were observed in all groups except the control group, the changes in D+M+U group were more significant ($P < 0.05$). Studies had shown that HIF-1α, a transcription factor of VEGF, could regulate the expression of its downstream target gene VEGF through a variety of pathways.[22-23] VEGFR-2 was the main receptor of VEGF for angiogenesis.[24] The combination of VEGF and VEGFR-2 could promote the proliferation and extension of vascular endothelial cells and induce the formation of new blood vessels, which was conducive to the proliferation, invasion and metastasis of tumors. Therefore, HIF-1α/VEGF/VEGFR pathway was a key signal pathway to regulate tumor angiogenesis.[25] In this study, immunohistochemical results showed that the expressions of HIF-1α, VEGF and VEGFR-2 in the D+M+U group were significantly decreased ($P < 0.01$), indicating that ultrasound combined with schisandrine A microbubbles could effectively inhibit the HIF-1α/VEGF/VEGFR pathway, thereby acting as an anti-tumor agent.

Inflammatory cytokines refer to the various cytokines involved in the inflammatory response. Among the many inflammatory cytokines, TNF-α, IL-1β, IL-6 and so on played a major role. In general, the content of cytokines in body was very low, and it participated in anti-inflammatory and anti-tumor effects with a variety of factors. Cytokines was highly expressed in liver cancer. After treatment, the drug and microbubble destroyed the microenvironment involved in tumor occurrence, inhibited the enzymes that promote tumor growth and proliferation, blocked the continued growth and metastasis of tumors, and then reduced the production of tumor necrosis factor in the body's immune response, so that the serum level of tumor body after schisandrine A microbubbles treatment became low ($P < 0.01$).

PI3K/AKT/mTOR signaling pathway played an important role in the proliferation, metastasis, energy metabolism, autophagy and drug resistance of liver cancer.[26] In recent years, the relationship between autophagy and tumors had attracted more and more attention from scholars. Studies had found that autophagy could inhibit tumor formation by reducing the accumulation of useless or damaged organelles and proteins, inhibiting oxidative stress and other processes. That was, promoting the autophagic activity of tumor cells and even inducing autophagic death could play an anti-tumor role. PI3K belonged to the lipid kinase family and could be activated by many cytokine receptors.[27] The activation of PI3K led to the phosphorylation of Akt and further activates downstream signaling molecules such as mTOR, thereby inhibiting autophagy. mTOR was usually highly expressed in tumor cells.[28] Inhibition of mTOR function and inactivation of PI3K/Akt/mTOR signaling pathway could induce autophagy.[29] The results of western blot analysis in the present study showed that the p-PI3K, PI3K, p-Akt, Akt, p-mTOR, mTOR were significantly decreased in liver cancer tissues treated with schisandrin A combined with ultrasound ($P < 0.01$). These results suggested that schisandrin A combined with ultrasound might have an inhibitory effect on PI3K/Akt /mTOR signaling pathway in liver cancer.

In this study, we conducted a preliminary experiment on the selection of ultrasound parameters. For the intensity of ultrasound, when the intensity was lower than 1.5 W/cm^2, the therapeutic

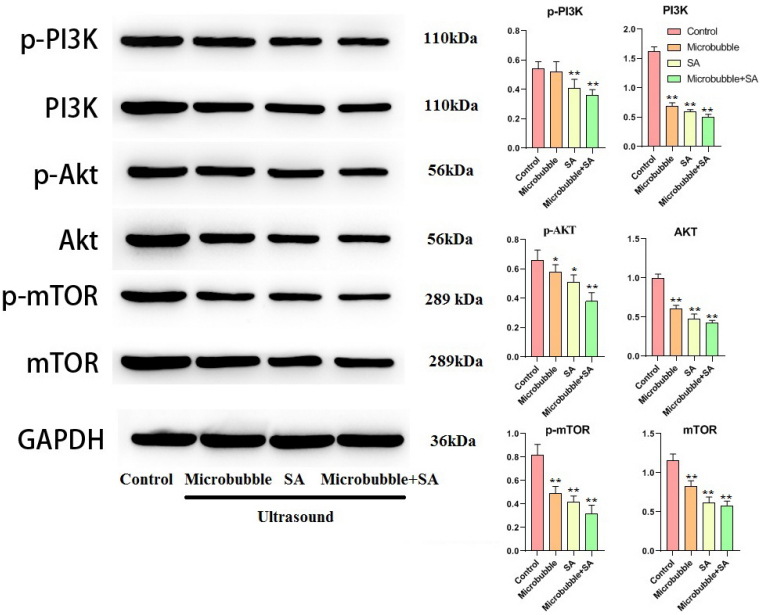


FIGURE 10. Protein expression results of phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway in tumor tissues (n = 6, *Compared with Control, $P < 0.05$; **Compared with Control, $P < 0.01$).

effect was not good, and the microbubbles could not be fully burst to exert the anti-tumor effect. When the intensity was higher than 2.5 W/cm^2, it would cause damage to the body's own tissues, and the side effects would increase significantly. After repeated attempts, we found that 2.0 W/cm^2 could reduce the toxic and side effects of drugs on normal tissues and organs, reduce the deposition of drugs in non-targeted sites, improve the tumor selectivity of drugs, and improve the tumor efficacy. Therefore, we had selected 2.0 W/cm^2 as the optimal ultrasonic intensity parameters.

In addition, there were other ultrasound parameters that played a crucial role in the effect of treatment. PRF (Pulse Repetition Frequency) was a key parameter affecting blood flow effect. Higher PRF could significantly enhance the blood supply of the tumor area, produce stronger blood perfusion effect and obtain higher tissue drug concentration. The PNP (Peak Negative Pressure) of ultrasound was one of the parameters most related to the cavitation effect. The higher the peak negative pressure, the stronger the cavitation effect and the more serious the pathological changed. Therefore, it was necessary to choose moderate parameters to prevent damage to normal tissues. The ultrasonic cavitation effect occurred during the ultrasonic signal transmission. Therefore, increasing the duty cycle (DC) could prolong the actual ultrasonic signal transmission time, thereby enhancing the cavitation effect, accelerating the release of drugs and enhancing the therapeutic effect. We had systematically investigated the above parameters to ensure that the efficacy could be improved while the side effects could be reduced.

Conclusions

In this study, the imaging microbubbles loaded with schisandrin A were prepared and their significant inhibitory effect on tumor cells was verified. The results showed that ultrasound combined with drug-loaded microbubbles could significantly increase the drug uptake in tumor cells, which was an effective way to improve the anti-tumor effect of drugs. The results in the orthotopic animal model of liver cancer showed that ultrasound combined with Schisandin A contrast microbubbles could significantly reduce the tumor volume, reduce the level of inflammatory factors in the animal body, and effectively inhibit the HIF-1α/VEGF/VEGFR pathway and PI3K-AKT-mTOR signaling pathway, which was a potential important therapeutic mechanism. This study would lay a scientific foundation for the improvement of the diagnosis and treatment of liver cancer.

Acknowledgement

This research was supported by the fund from the Key Scientific Research Project of Colleges and Universities in Henan Province (23A320050; 23A360018), Medical Science and Technology Project of Henan Province (LHGJ20210283; LHGJ20210348).

Reference

1. Szabo TL. *Diagnostic ultrasound imaging: inside out*. 2nd edition. Academic Press; Elsevier Inc. 2014. doi: 10.1016/C2011-0-07261-7
2. Sennoga CA, Kanbar E, Auboire L, Dujardin PA, Fouan D, Escoffre JM, et al. Microbubble-mediated ultrasound drug-delivery and therapeutic monitoring. *Expert Opin Drug Deliv* 2017; **14:** 1031-43. doi: 10.1080/17425247.2017.1266328
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021; **71:** 209-49. doi: 10.3322/caac.21660
4. Liu CY, Chen KF, Chen PJ. Treatment of liver cancer. *Cold Spring Harb Perspect Med* 2015; **5:** a021535. doi: 10.1101/cshperspect.a0215
5. Yamashita T, Kaneko S. [Liver Cancer]. [Japanese]. *Rinsho Byori* 2016; **64:** 787-96. PMID: 30695467
6. Makovec T. Cisplatin and beyond: molecular mechanisms of action and drug resistance development in cancer chemotherapy. *Radiol Oncol* 2019; **53:** 148-58. doi: 10.2478/raon-2019-0018
7. Kooiman K, Roovers S, Langeveld SAG, Kleven RT, Dewitte H, O'Reilly MA, et al. Ultrasound-responsive cavitation nuclei for therapy and drug delivery. *Ultrasound Med Biol* 2020; **46:** 1296-325. doi: 10.1016/j.ultrasmedbio.2020.01.002
8. Liu S, Zhang Y, Liu Y, Wang W, Gao S, Yuan W, et al. Ultrasound-targeted microbubble destruction remodels tumour microenvironment to improve immunotherapeutic effect. *Br J Cancer* 2023; **128:** 715-25. doi: 10.1038/s41416-022-02076-y
9. Chen H, Hwang JH. Ultrasound-targeted microbubble destruction for chemotherapeutic drug delivery to solid tumors. *J Ther Ultrasound* 2013; **1:** 10. doi: 10.1186/2050-5736-1-10
10. Schoen S Jr, Kilinc MS, Lee H, Guo Y, Degertekin FL, Woodworth GF, et al. Towards controlled drug delivery in brain tumors with microbubble-enhanced focused ultrasound. *Adv Drug Deliv Rev* 2022; **180:** 114043. doi: 10.1016/j.addr.2021.114043
11. Escoffre JM, Sekkat N, Oujagir E, Bodard S, Mousset C, Presset A, et al. Delivery of anti-cancer drugs using microbubble-assisted ultrasound in digestive oncology: from preclinical to clinical studies. *Expert Opin Drug Deliv* 2022; **19:** 421-33. doi: 10.1080/17425247.2022.2061459
12. Fu K, Zhou H, Wang C, Gong L, Ma C, Zhang Y, et al. A review: pharmacology and pharmacokinetics of schisandrin A. *Phytother Res* 2022; **36:** 2375-93. doi: 10.1002/ptr.7456
13. Wang X, Zhou L, Zhang T, Chen H, Song X, Wang F. Effect and mechanism of schizandrin A in the treatment of liver cancer using network pharmacology, molecular docking, and target validation. *Nat Prod Commun* 2023; **18:** 1-12. doi: 10.1177/1934578X231176916

14. Omata D, Munakata L, Kageyama S, Suzuki Y, Maruyama T, Shima T, et al. Ultrasound image-guided gene delivery using three-dimensional diagnostic ultrasound and lipid-based microbubbles. *J Drug Target* 2022; **30:** 200-7. doi: 10.1080/1061186X.2021.1953510

15. Zhang J, Yang J, Zhang H, Hu M, Li J, Zhang X. New Span-PEG-composited Fe3O4-CNT as a multifunctional ultrasound contrast agent for inflammation and thrombotic niduses. *RSC Adv* 2020; **10:** 38592-600. doi: 10.1039/d0ra05401a

16. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicol Lett* 2006; **160:** 171-77. doi: 10.1016/j.toxlet.2005.07.001

17. Liu Y, Jiang J, Liu C, Zhao W, Ma Y, Zheng Z, et al. Synergistic anti-tumor effect of anti-PD-L1 antibody cationic microbubbles for delivery of the miR-34a gene combined with ultrasound on cervical carcinoma. *Am J Transl Res* 2021; **13:** 988-1005. PMID: 33841635

18. Yu B, Sheng D, Tan Q. Determination of schisandrin A and schisandrin B in traditional chinese medicine preparation huganpian tablet by RP-HPLC. *Chem Pharm Bull* 2019; **67:** 713-6. doi:10.1248/cpb.c18-00968

19. Auboire L, Sennoga CA, Hyvelin JM, Ossant F, Escoffre JM, Tranquart F, et al. Microbubbles combined with ultrasound therapy in ischemic stroke: a systematic review of in-vivo preclinical studies. *PLoS One* 2018; **13:** e0191788. doi: 10.1371/journal.pone.0191788

20. Lammertink BH, Bos C, Deckers R, Storm G, Moonen CT, Escoffre JM. Sonochemotherapy: from bench to bedside. *Front Pharmacol* 2015; **6:** 138. doi: 10.3389/fphar.2015.00138

21. Bouakaz A, Versluis M, Borsboom J, de Jong N. Radial modulation of microbubbles for ultrasound contrast imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* 2007; **54:** 2283-90. doi: 10.1109/tuffc.2007.532

22. Rashid M, Zadeh LR, Baradaran B, Molavi O, Ghesmati Z, Sabzichi M, et al. Up-down regulation of HIF-1α in cancer progression. *Gene* 2021; **798:** 145796. doi: 10.1016/j.gene.2021.145796

23. Frezzetti D, Gallo M, Maiello MR, D'Alessio A, Esposito C, Chicchinelli N, et al. VEGF as a potential target in lung cancer. *Expert Opin Ther Targets* 2017; **21:** 959-66. doi: 10.1080/14728222.2017.1371137

24. Liu XJ, Zhao HC, Hou SJ, Zhang HJ, Cheng L, Yuan S, et al. Recent development of multi-target VEGFR-2 inhibitors for the cancer therapy. *Bioorg Chem* 2023; **133:** 106425. doi: 10.1016/j.bioorg.2023.106425

25. Kim KW, Lee SJ, Kim JC. TNF-α upregulates HIF-1α expression in pterygium fibroblasts and enhances their susceptibility to VEGF independent of hypoxia. *Exp Eye Res* 2017; **164:** 74-81. doi: 10.1016/j.exer.2017.08.008

26. Wang W, Dong X, Liu Y, Ni B, Sai N, You L, et al. Itraconazole exerts anti-liver cancer potential through the Wnt, PI3K/AKT/mTOR, and ROS pathways. *Biomed Pharmacother* 2020; **131:** 110661. doi: 10.1016/j.biopha.2020.110661

27. Liu X, Zhou Q, Hart JR, Xu Y, Yang S, Yang D, et al. Cryo-EM structures of cancer-specific helical and kinase domain mutations of PI3Kα. *Proc Natl Acad Sci U S A* 2022; **119:** e2215621119. doi: 10.1073/pnas.2215621119

28. Xiang X, Zhao J, Xu G, Li Y, Zhang W. mTOR and the differentiation of mesenchymal stem cells. *Acta Biochim Biophys Sin* 2011; **43:** 501-10. doi:10.1093/abbs/gmr041

29. Tian LY, Smit DJ, Jücker M. The Role of PI3K/AKT/mTOR Signaling in hepatocellular carcinoma metabolism. *Int J Mol Sci* 2023; **24:** 2652. doi: 10.3390/ijms24032652

research article

Correlation of laminin subunit alpha 3 expression in pancreatic ductal adenocarcinoma with tumor liver metastasis and survival

Yueyi Xing[1], Xue Jing[2], Gong Qing[1], Yueping Jiang[2]

[1] Qingdao University, Qingdao, Shandong Province, China
[2] Gastroenterology Department, the Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China

Radiol Oncol 2024; 58(2): 234-242.

Received 30 October 2023
Accepted 14 January 2024

Correspondence to: Dr. Yueping Jiang, Gastroenterology Department, the Affiliated Hospital of Qingdao University, Qingdao, Shandong Province, China. E-mail: yuepingmd@hotmail.com

Disclosure: No potential conflict of interest were disclosed.

Background. The high mortality rate of pancreatic ductal adenocarcinoma (PDAC) is primarily attributed to metastasis. Laminin subunit alpha 3 (LAMA3) is known to modulate tumor progression. However, the influence of LAMA3 on liver metastasis in PDAC remains unclear. This study aimed to elucidate whether LAMA3 expression is increased in PDAC with liver metastasis.

Patients and methods. We extracted information related to LAMA3 expression levels and associated clinicopathological parameters from The Cancer Genome Atlas (TCGA) and four Gene Expression Omnibus (GEO) datasets. Clinicopathological analysis was performed; the Kaplan-Meier Plotter was used to evaluate LAMA3's prognostic effect in PDAC. We retrospectively collected clinicopathological data and tissue specimens from 117 surgically treated patients with PDAC at the Affiliated Hospital of Qingdao University. We assessed LAMA3 expression and investigated its correlation with the clinicopathological traits, clinical outcomes, and hepatic metastasis.

Results. Amplified expression of LAMA3 was observed in PDAC tissue compared with normal tissue in the TCGA and GEO databases. High LAMA3 expression was associated with poor overall survival (OS) and relapse-free survival (RFS) in patients with PDAC. LAMA3 expression was significantly enhanced in PDAC tissues than in adjacent tissues. Tumor tissues from patients with PDAC exhibiting liver metastasis showed higher LAMA3 expression than those without liver metastasis. High LAMA3 expression correlated with large tumor size and TNM stage. LAMA3 expression and liver metastasis were independent predictive factors for OS; the former was independently associated with liver metastasis.

Conclusions. LAMA3 expression is elevated in patients with PDAC with liver metastasis and is a predictor of prognosis.

Key words: pancreatic ductal adenocarcinoma; laminin subunit alpha 3; liver metastasis; prognosis

Introduction

Pancreatic ductal adenocarcinoma (PDAC), a disease that is prevalently observed within the digestive system, is distinguished by its severe malignancy and exhibits a disconcerting confluence of incidence and mortality.[1] The 5-year survival rate of patients with PDAC is < 10%, with an extremely poor prognosis. If this trend is sustained, the impending decade may witness pancreatic cancer ascending to the rank as the second most lethal cancer.[2] Most patients with PDAC remain asymp-

tomatic until the disease reaches advanced stages. Ninety percent of patients with PDAC diagnosed only after metastasis have a poor prognosis, with 50% developing systemic metastasis.[3,4] The potential for enduring survival among patients with PDAC considerably depends on tumor size and disease stage. Therefore, early detection of potentially curable cancers is crucial for reducing mortality rates among patients with PDAC. The elucidation of key molecular mechanisms and prospective intervention targets associated with pancreatic cancer metastasis will aid in deciphering the genetic and molecular underpinnings of this disease, provide biomarkers for preliminary warning and metastasis surveillance, and pave the way for enhancing the survival prospects of patients with pancreatic cancer.

Laminin, a heterotrimeric molecule consisting of α, β, and γ subunits, is the primary constituent of the extracellular matrix while collagen and fibronectin form the basement membrane. Among the three subunits of laminin, the α subunit is involved in tissue-specific distribution and biological activity.[5] Laminin subunit alpha 3 (LAMA3), which encodes for the laminin α subunit, enables its globular carboxyl-terminal domain to engage with integrins at the plasma membrane, thereby participating in intracellular signal transduction.[6] Currently, LAMA3 contributes to cell proliferation and apoptosis in diverse malignant tumors and modulates tumor progression through signal transduction pathways, such as focal adhesion plaques.[7-9] The aberrant expression of LAMA3 in various tumors is inextricably associated with the clinical stage, tumor size, and pathological manifestations of patients.[10] However, the influence of LAMA3 on liver metastasis in PDAC remains unclear. This study aimed to clarify LAMA3 expression in PDAC and investigate the relationship between LAMA3 expression and liver metastasis in patients with unresectable PDAC.

Patients and methods

Procurement of bioinformatics analysis data

RNA sequencing expression traits, along with their associated clinical data pertaining to LAMA3, were procured from the The Cancer Genome Atlas (TCGA) dataset (https://portal.gdc.com). The current-release GTEx datasets were accessed from the GTEx data portal website (https://www.gtexportal.org/home/datasets). The data comprised 179 tumor samples and 4 normal samples sourced from the TCGA, in addition to 328 normal mRNA expression data points from GTEx. Corresponding platform annotation files were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) to validate the expression levels of LAMA3 in PDAC. Finally, we identified four datasets: GSE28735 (n = 90), GSE62452 (n = 130), GSE101448 (n = 43), and GSE62165 (n = 131). To perform a clinicopathological analysis of LAMA3, we used University of ALabama at Birmingham CANcer (UALCAN) (http://ualcan.path.uab.edu). Survival curves were generated using the Kaplan-Meier Plotter database (http://kmplot.com/analysis/).[11]

Acquisition of human pancreatic ductal adenocarcinoma (PDAC) specimens and clinicopathological data

Our study included 117 patients with PDAC who underwent pancreatic surgery at the Affiliated Hospital of Qingdao University. These patients had not received any anticancer treatment before surgery, and the diagnosis of pancreatic carcinoma was confirmed by postoperative pathology. Paraffin-embedded tumor tissues were obtained from each patient, and the corresponding para-carcinomatous tissues were obtained from 60 patients. All patients provided informed consent, and the investigation was conducted in accordance with the Declaration of Helsinki with the endorsement of the Medical Ethics Committee of the Affiliated Hospital of Qingdao University (QYFYWZLL27485 and QYFYWZLL27608).

Clinicopathological data were obtained from retrospective medical records, which consisted of age, sex, tumor size, tumor location, histological grade, perineural invasion, lymph node metastasis, vascular invasion, liver metastasis, tumor-node-metastasis (TNM) stage, preoperative serum carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) concentrations. Overall survival (OS) was calculated as the interval between surgery and either death or last follow-up appointment. The dates of death were ascertained from hospital records or follow-up telephone interviews.

Immunohistochemistry

Paraffin-embedded PDAC and para-cancerous tissues underwent sequential sectioning at a thickness of 4 μm. After baking, deparaffinizing, and

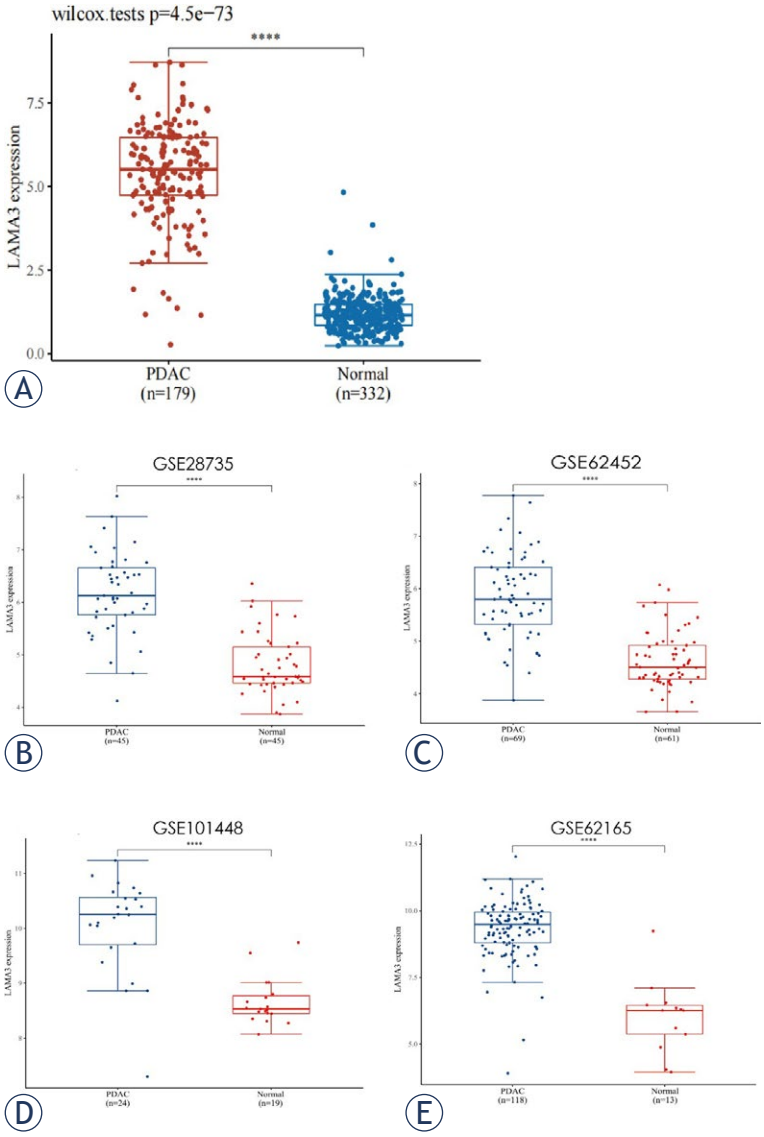


FIGURE 1. Expression of laminin subunit alpha 3 (LAMA3) in pancreatic ductal adenocarcinoma (PDAC) and normal tissues from the Cancer Genome Atlas (TCGA) database **(A)** and the Gene Expression Omnibus (GEO) database **(B-E)**. Expression of LAMA3 in 179 PDAC and 332 normal tissues from TCGA **(A)**; Expression of LAMA3 in 45 PDAC and 45 normal tissues from GSE28735 cohort **(B)**; Expression of LAMA3 in 69 PDAC and 61 normal tissues from GSE62452 cohort **(C)**; Expression of LAMA3 in 24 PDAC and 19 normal tissues from GSE101448 cohort **(D)**; Expression of LAMA3 in 118 PDAC and 13 normal tissues from GSE62165 cohort **(E)**.

****P<0.001.

hydrating, the paraffin sections were ensconced in a pressure cooker for 10 min for antigen repair. Subsequently, the antigen repair box was relocated to an ice box for a 25-min interval, permitting cooling to room temperature. To curb endogenous peroxidase activity, the tissue sections were immersed in a concoction of 3% hydrogen peroxide and methanol for 15 min. Each section received a blockade of 10% sheep serum and incubated at 37°C for half an hour. This was followed by an overnight incubation at 4°C with primary antibodies (1:100 L, no. ab242197; Abcam Inc.), followed by incubation with secondary antibodies (no. ab242197; Abcam Inc.) at 37°C for 30 min. The tissue sections were then stained with 3, 3-diaminobenzidine (Roche) for 5–10 min at room temperature. Hematoxylin (Roche) was used for counterstaining for 25 s before proceeding with dehydration, clarification, and sealing. Microscopic visualization was performed to record the images. An independent duo of pathologists evaluated all samples.

The cytoplasmic staining score (CF) was defined as follows: 0 (0–20%), 1 (21–50%), 2 (51–75%), and 3 (>75%). Moreover, the cytoplasmic staining intensity (CI) was categorized as 0 (negative), 1–2 (weak), and 3 (strong). The cytoplasmic composite score was calculated as CF×CI.

Statistical analyses

For all the TCGA and GEO databases, we used the Wilcox test to perform differential expression analysis between tumor and normal tissues. Categorical variables are expressed as frequencies and percentages, and significance was determined using the χ^2 or Fisher's exact test. Quantitative variables are expressed as means±standard deviations, and significance was determined using Student's t-test. Non-normally distributed variables are expressed as medians and interquartile ranges, and significance was determined using the Mann–Whitney U test. Multivariate logistic regression analyses were performed to identify the independent risk factors for PDAC. We used the cutoff points of the test variables produced on receiver operating characteristic curves. Survival analysis was performed using Kaplan–Meier analysis and assessed using the log-rank test. Cox regression analysis was performed to analyze the effect of OS on the survival of patients with PDAC. All analyses were performed using SPSS version 24.0, GraphPad Prism version 8.0.1, and R software version 4.0.3. $P < 0.05$ was considered statistically significant.

Results

Elevation of laminin subunit alpha 3 in PDAC and its correlation with prognosis using bioinformatics analysis

Using the TCGA database, we identified a prominent divergence in LAMA3 expression between PDAC tissues (n = 179) and normal tissues (n = 332)

($P < 0.001$) (Figure 1A). Four datasets (GSE28735, GSE62452, GSE101448, and GSE62165) were obtained from the GEO database and used as validation sets. The results showed that LAMA3 was significantly upregulated in PDAC tissues (all $P < 0.001$) (Figure 1B-E).

To further elucidate the role of LAMA3 in PDAC, we investigated its expression using various clinicopathological parameters. LAMA3 expression displayed no remarkable correlation with age (Supplementary Figure 1A), sex (Supplementary Figure 1B), and drinking habits (Supplementary Figure 1C) in patients with PDAC. Grade 1 indicated a well-differentiated (low-grade) tumor, grade 2 denoted a moderately differentiated (intermediate-grade) tumor, grade 3 indicated a poorly differentiated (high-grade) tumor, and grade 4 indicated an undifferentiated (high-grade) tumor. The grade of patients with PDAC influenced LAMA3 expression, and heightened expression was observed in grades 2 and 3 (Supplementary Figure 1D). However, there was no significant difference in LAMA3 expression with respect to nodal metastasis (Supplementary Figure 1E) or diabetes (Supplementary Figure 1F).

Survival curves were generated using the Kaplan–Meier Plotter database. Elevated LAMA3 expression was positively associated with poorer OS (Figure 2A, hazard ratio [HR] = 3.86, $P < 0.001$) and relapse-free survival (RFS) (Figure 2B, HR = 406726946.65, $P < 0.001$). These results demonstrate that high LAMA3 expression is associated with an unfavorable prognosis in patients with PDAC.

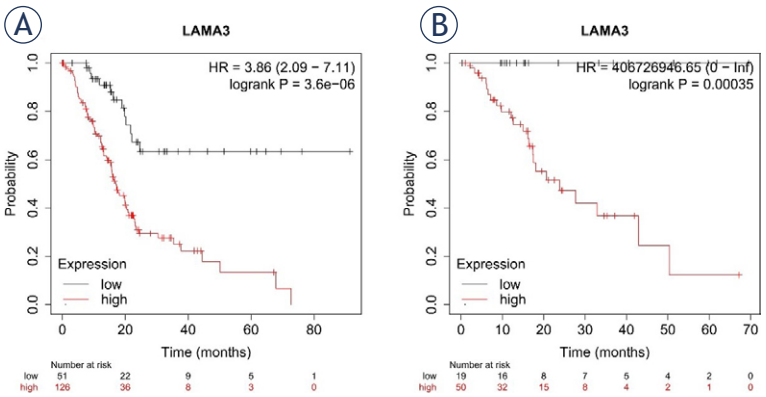


FIGURE 2. The expression of laminin subunit alpha 3 (LAMA3) for prediction of overall survival (OS) and relapse free survival (RFS) in patients with pancreatic ductal adenocarcinoma (PDAC). OS **(A)**, RFS **(B)**.

Clinical characteristics of patients with PDAC

After meticulous filtering according to the inclusion and exclusion criteria, 117 patients with PDAC were included in this study. The baseline characteristics of the patients are summarized in Table 1. The mean age of all patients was 62.43 ± 9.33 years, with males accounting for 73 (62.3%) of the total population. Pancreaticoduodenectomy and distal pancreatectomy were performed in 69 and 48 patients, respectively. In our cohort, 61 postsurgical patients received chemotherapy, 3 patients received radiotherapy, 6 patients received immunotherapy, and 4 patients received interventional therapy. Based on the immunohistochemical results of LAMA3 expression levels, the patients were categorized into groups with high or low expression. Notably, 62 patients showed elevated LAMA3 expression. The univariate analyses of the two cohorts are presented in Table 1.

Increased LAMA3 expression correlated with large tumor size (P = 0.007), and the degree of LAMA3 expression was associated with different TNM stages (P = 0.002). In addition, LAMA3 expression was higher in tumor tissue from patients with PDAC and liver metastases than those without liver metastases (P = 0.005). In the two groups, the surgical modalities used were significantly different (P = 0.036), but there were no significant differences in age, gender, tumor location, histological grade, perineural invasion, vascular inva-

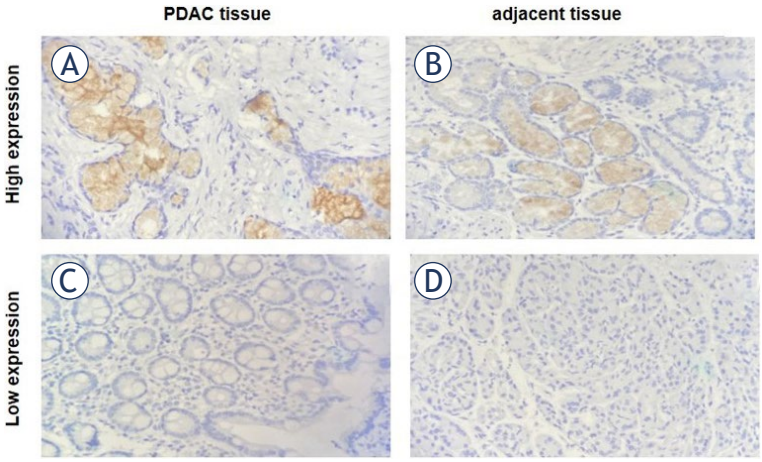


FIGURE 3. Representative immunohistochemical staining of laminin subunit alpha 3 (LAMA3) in pancreatic ductal adenocarcinoma (PDAC) and adjacent normal tissue. High expression of LAMA3 in PDAC tissue **(A)** compared with adjacent tissue **(B)**. Low expression of LAMA3 in PDAC tissue **(C)** compared with adjacent tissue **(D)**. Magnification, x400

TABLE 1. Characteristics of all patients

Characteristics	All	LAMA3 expression		P-value
		High (n = 62)	Low (n = 55)	
Age(year), mean±SD	62.43 ± 9.33	62.19 ± 9.38	62.69 ± 9.36	0.775
Sex, n (%)				0.615
Male	73 (62.4)	40	33	
Female	64 (37.6)	22	22	
Tumor location, n (%)				0.054
Head	70 (59.8)	32	38	
Body and tail	47 (40.2)	30	17	
Tumor size, n (%)				**0.007**
≤ 2 cm	5 (4.3)	2	3	
> 2 cm and ≤ 4 cm	81 (69.2)	37	44	
> 4 cm	31 (26.5)	23	8	
Histological grade, n (%)				0.810
G1	31 (26.5)	17	14	
G2–3	86 (73.5)	45	41	
TNM stage, n (%)				**0.002**
I–II	92 (78.6)	42	50	
III–IV	25 (21.4)	20	5	
Perineural invasion, n (%)				0.921
Yes	91 (77.8)	48	43	
No	26 (22.2)	14	12	
Vascular invasion, n (%)				0.340
Yes	37 (31.6)	22	15	
No	80 (68.4)	40	40	
Lymph node metastasis, n (%)				0.255
Yes	49 (41.9)	29	20	
No	68 (58.2)	33	35	
Liver metastasis, n (%)				**0.005**
Yes	45	30	15	
No	72	29	43	
CEA (ng/ml)				0.392
≤ 12	108 (92.3)	56	52	
> 12	9 (7.7)	6	3	
CA19-9 (U/ml)				0.395
≤ 282	74 (63.2)	37	37	
> 282	43 (36.8)	25	18	
Surgical modalities, n (%)				**0.036**
Pancreaticoduodenectomy	69 (59)	31	38	
Distal pancreatectomy	48 (41)	31	17	
Postoperative chemotherapy, n (%)				0.77
Yes	60	40	20	
No	57	41	16	
Postoperative radiotherapy, n (%)				0.063
Yes	3	2	1	
No	114	79	35	
Immunotherapy, n (%)				0.068
Yes	6	4	2	
No	111	77	34	
Interventional therapy, n (%)				0.374
Yes	4	4		
No	114	77	36	

CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; LAMA3 = laminin subunit alpha 3

sion, lymph node metastasis, CEA and CA19-9 levels, and adjuvant systemic therapy (P > 0.05).

Heightened expression of LAMA3 in PDAC tissues relative to adjacent tissues

Immunohistochemistry was performed to measure LAMA3 expression in PDAC and adjacent normal tissues. LAMA3 staining was almost undetectable in normal tissues, and protein intensity was negative (Supplementary Table 1, Figure 3). Conversely, moderate staining and a robust intensity of LAMA3 protein expression were observed in PDAC tissues. The results demonstrated that LAMA3 expression was significantly higher in carcinoma specimens than in the adjacent tissues ($P < 0.001$).

Superior expression of LAMA3 in PDAC tumor tissues exhibiting liver metastasis

All patients with PDAC were categorized into two groups based on the emergence or absence of liver metastasis postoperatively (Table 2). Univariate analysis showed that histological grade ($P = 0.001$), TNM stage ($P = 0.013$), and vascular invasion ($P = 0.018$) were significantly associated with liver metastasis. Immunohistochemistry was used to assess LAMA3 expression in patients with PDAC with or without liver metastasis. LAMA3 expression in tumor tissues from patients with PDAC and liver metastasis was significantly higher than in those without liver metastasis ($P = 0.005$). Representative immunohistochemical images are shown in Figure 4. Age, sex, tumor location, tumor size, lymph node metastasis, perineural invasion, and serum CEA and CA19-9 levels were not associated with the development of liver metastasis. Significant factors from the univariate analysis, as shown in Table 2, were incorporated into the multivariate logistic regression analysis (Supplementary Table 2). The results showed that histological grade and LAMA3 expression were independently associated with liver metastasis.

High LAMA3 expression correlates with poor PDAC prognosis

The median survival times of patients with low and high LAMA3 expressions were 29 and 14 months, respectively. The 1-, 2-, and 3-year survival rates of the high-expression group (n = 62) were 58.1%, 14.5%, and 4.8%, respectively. Conversely, those in the low-expression group (n = 55) were 90.9%, 47.2%, and 16.4%, respectively. Using

Kaplan–Meier curves, high LAMA3 expression in PDAC was associated with poor OS, suggesting an unfavorable prognosis (P < 0.001) (Figure 5).

Univariate analysis suggested that tumor size, TNM stage, liver metastasis, and LAMA3 expression had a significant prognostic influence on OS (Table 3). Multivariate survival analysis revealed that LAMA3 expression (HR, 2.016; 95% confidence interval [CI], 1.257–3.234; *P* = 0.004) and liver metastasis (HR, 2.284; 95% CI, 1.426–3.657; *P* = 0.001) were independent predictive factors of OS.

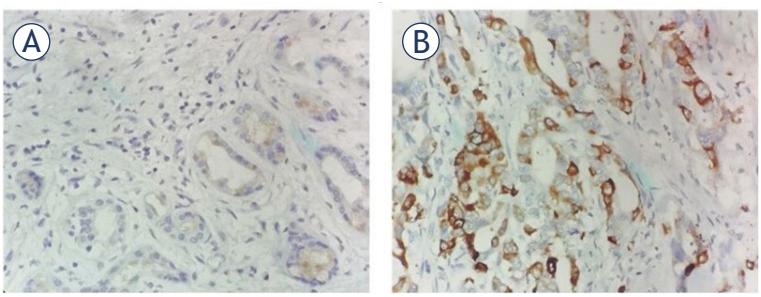


FIGURE 4. Representative immunohistochemical staining of laminin subunit alpha 3 (LAMA3) in pancreatic ductal adenocarcinoma (PDAC) with and without liver metastasis. Low expression of LAMA3 in PDAC tissues **(A)** without liver metastatic. High expression of LAMA3 in PDAC tissues **(B)** with liver metastasis. Magnification, x400.

Discussion

Pancreatic cancer is a highly aggressive neoplasm of the digestive system and is characterized by a mortality rate equal to its incidence rate. Its strong invasiveness and early metastasis render approximately 80% of patient ineligible for surgical intervention at the time of diagnosis. This results in a 5-year survival rate < 10%. Current treatment approaches for PDAC include surgical resection combined with chemotherapy, radiation therapy, interventional therapy, and immunotherapy. Even in patients with resectable localized tumors, the postoperative 5-year survival rate remains approximately 20%.[12] The major contributor to the high mortality rate of PDAC is its propensity for early metastasis, which poses a significant challenge in clinical management. Consequently, there is an urgent need to identify additional predictive biomarkers to enhance the risk stratification of patients with PDAC.

LAMA3, a member of the laminin family, plays a pivotal role in cellular processes by interacting with integrins on the cell membrane and participating in the intracellular signal transduction pathways. Recent studies have implicated elevated LAMA3 expression in various types of tumors, where it appears to promote cell proliferation, apoptosis, and tumor progression by modulating signal transduction pathways.[7-9] Zboralski *et al.* demonstrated the simultaneous functional inactivation of the tumor suppressor mothers against decapentaplegic homolog 4 (SMAD4) and invasive growth of tumors in rectal and pancreatic cancers. Laminin 332 (LM-332) is the target of SMAD4, a positive transcriptional regulator that promotes the transcription of LAMA3, LAMB3, and LAMC2 genes encoding LM-332. SMAD4 mediates transcriptional activity through distinct molecular mechanisms associated with the LAMA3, LAMB3, and LAMC2 promoters.[13] Additionally, Huang *et al.* highlighted the increased expression of LAMA3 protein in PDAC tumor cells relative to that in normal pancreatic cells. They further showed that high LAMA3 expression promoted the proliferation, migration, and invasion of PDAC tumor cells.[5] However, the effect of LAMA3 on liver metastasis in PDAC has not been fully elucidated. This study aimed to clarify the expression profile of LAMA3 in PDAC and investigate its potential association with liver metastasis in patients diagnosed with unresectable PDAC.

In this study, we integrated data from the TCGA database with four independent datasets from the GEO database as validation cohorts and found that LAMA3 expression was upregulated in PDAC, validating previous study findings.[14] Furthermore, through the analysis of clinical samples, we ob-

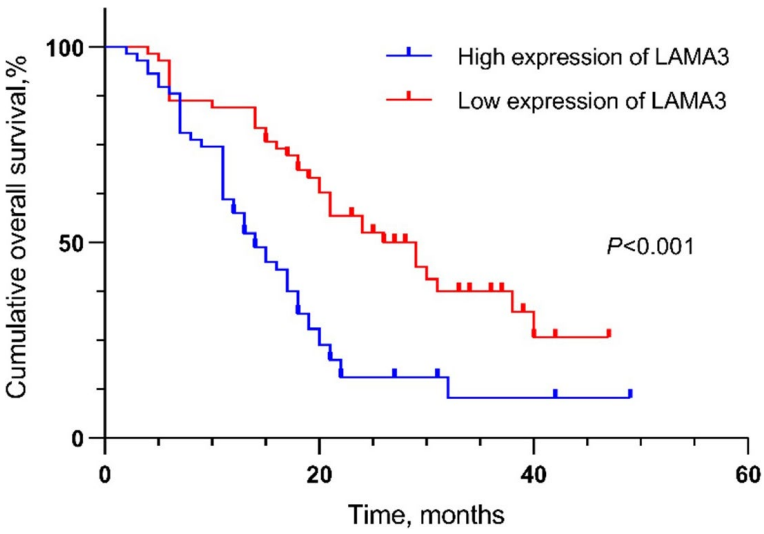


FIGURE 5. The expression of laminin subunit alpha 3 (LAMA3) for prediction of overall survival (OS) in patients with Pancreatic ductal adenocarcinoma (PDAC). Survival analysis was carried out with Kaplan-Meier and checked by log-rank test.

TABLE 2. Univariate analysis of clinicopathological characteristics in patients with pancreatic ductal adenocarcinoma with and without liver metastasis

Characteristics	Liver metastasis (n = 45)	Without liver metastasis (n = 72)	*P*-value
Age (year), M (IQR)	63 (58–69)	63 (56–68)	0.814
Sex, n			0.717
Male	29	44	
Female	16	28	
Tumor location, n			0.456
Head	25	45	
Body and tail	20	27	
Tumor size, n			0.240
≤ 2 cm	2	3	
> 2 cm and ≤4 cm	28	53	
> 4 cm	15	16	
Histological grade, n			**0.001**
G1	20	11	
G2-3	25	61	
TNM stage, n			**0.013**
I–II	30	62	
III–IV	15	10	
Perineural invasion, n			0.170
Yes	38	53	
No	7	19	
Vascular invasion, n			**0.018**
Yes	20	17	
No	25	55	
Lymph node metastasis, n			0.110
Yes	23	26	
No	22	46	
CEA (ng/ml)			0.701
≤ 12	41	67	
> 12	4	5	
CA19-9 (U/ml)			0.832
≤ 282	29	45	
> 282	16	27	
LAMA3 expression, n			**0.005**
High	30	29	
Low	15	43	

CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9; LAMA3 = laminin subunit alpha 3

served that LAMA3 was overexpressed in pancreatic carcinoma tissues compared with adjacent noncancerous tissues. When we analyzed clinicopathological data from the UALCAN database, we found that LAMA3 expression levels correlated with the histological grade of tumors in patients with PDAC. However, our analysis of clinical data revealed that high LAMA3 expression was associated with larger tumor size, advanced TNM stage, and liver metastasis. This discrepancy may be due to the inherent bias from our relatively small sample size. Jun *et al.* demonstrated that overexpression of the α3, β3, and γ2 chains of LM-332 might play a crucial role in the progression and prognosis of PDAC.[15] Based on these findings, we evaluated the prognostic value of LAMA3 expression in patients with PDAC. Using the Kaplan–Meier Plotter dataset, which incorporates data from the GEO, European Genome-phenome Archive, and TCGA databases, we found that high LAMA3 expression was strongly associated with worse OS and RFS in patients with PDAC. Additionally, we followed up 117 patients with PDAC and observed that high LAMA3 expression in PDAC was correlated with poor OS, indicating an overall poor prognosis. Univariate and multivariate Cox regression analyses further demonstrated that LAMA3 was an independent predictive factor for mortality in patients with PDAC. In conclusion, these results strongly support that the expression of LAMA3 can serve as a robust prognostic biomarker of PDAC.

Metastasis, the primary cause of cancer-related mortality, continues to be an area of limited understanding regarding its cellular and molecular mechanisms.[16] Various studies have implicated LAMA3 in different mechanisms of metastasis. Shu *et al.* demonstrated that the overexpression of LINC00936 hindered ovarian cancer progression by competitively binding to miR-221-3p and modulating LAMA3 expression.[7] Moreover, Xu *et al.* reported that LINC00628 could obstruct cell proliferation, invasion, migration, and apoptosis while reducing drug resistance in lung adenocarcinoma cells by downregulating the methylation of the LAMA3 promoter[8]. Kinoshita *et al.* demonstrated that miRNA-218 modulated the focal adhesion pathway, thereby impeding tumor cell invasion and metastasis.[9] The liver, which serves as the primary blood-borne drainage site for related organs, such as the portal vein system, colon, and pancreas, is crucial for distant metastasis in patients with PDAC.[17] To gain a deeper understanding of the correlation between LAMA3 and liver metastasis in PDAC, we examined independent risk factors associated with liver metastasis. Univariate analysis revealed that histological grade, TNM stage, vascular invasion, and high LAMA3 expres-

TABLE 3. Univariate and multivariate Cox proportional hazard regression analyses of overall survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR 95% CI)	*P*-value
Age	1.009 (0.985–1.035)	0.459	1.009 (0.983–1.035)	0.512
Sex	1.346 (0.850–2.131)	0.205	0.696 (0.435–1.112)	0.696
Tumor location (head vs. body and tail)	0.895 (0.573–1.399)	0.628	0.780 (0.474–1.283)	0.327
Tumor size				
≤ 2 cm vs. > 4 cm	0.543 (0.186–1.583)	0.263	0.626 (0.191–2.054)	0.440
> 2 cm and ≤ 4 cm vs. > 4 cm	0.607 (0.373–0.987)	**0.044**	0.637 (0.337–1.204)	0.165
Histological grade (G1 vs. G2–3)	1.263 (0.778–2.049)	0.345	1.378 (0.797–2.383)	0.251
TNM stage (I–II vs. III–IV)	0.374 (0.227–0.615)	**< 0.001**	1.505 (0.855–2.647)	0.157
Perineural invasion (yes vs. no)	0.803 (0.464–1.389)	0.432	1.158 (0.611–2.196)	0.652
Vascular invasion (yes vs. no)	0.693 (0.442–1.085)	0.109	0.912 (0.529–1.572)	0.741
Lymph node metastasis (yes vs. no)	0.796 (0.513–1.235)	0.308	0.883 (0.548–1.423)	0.609
Liver metastasis (yes vs. no)	0.364 (0.231–0.574)	**< 0.001**	2.284 (1.426–3.657)	**0.001**
CEA (≤12 vs. >12)	0.512 (0.256–1.026)	0.059	1.622 (0.788–3.340)	0.189
CA19-9(≤282 vs. >282)	0.969 (0.613–1.533)	0.894	0.963 (0.560–1.655)	0.891
LAMA3 (low vs. high)	0.407 (0.259–0.641)	**< 0.001**	2.016 (1.257–3.234)	**0.004**

CA19-9 = carbohydrate antigen 19-9; CEA = carcinoembryonic antigen; CI = confidence interval; HR = hazard ratio; LAMA3 = laminin subunit alpha 3

sion were significantly associated with liver metastasis. Multivariate logistic regression analyses demonstrated that LAMA3 and histological grade were independent predictive factors for liver metastasis in patients with PDAC. These findings indicate that poor differentiation and high LAMA3 expression are correlated with an increased risk of metastasis.

This study has both strengths and limitations, necessitating further investigations to confirm and expand our findings. One of the strengths of this study is the use of data from public databases combined with bioinformatics analysis. This approach allows the utilization of large amounts of data, thus increasing the reliability and statistical power of our findings. We complemented this database analysis with clinical data to verify our results by adding another validation layer. However, this study has some limitations. This study was entirely based on data from public databases; although we used clinical data to confirm our findings, future studies with larger sample sizes and varied population groups are required to further validate our results. We evaluated LAMA3 expression in PDAC tissues using immunohistochemistry, which, although a common and reliable technique, only provides a snapshot of LAMA3 expression and does not provide functional information. Therefore, additional functional experiments are required to better understand the role of LAMA3 in PDAC. Finally, our study did not fully explore the mechanism by which LAMA3 promotes liver metastasis in PDAC. Understanding these mechanisms requires a series of in-depth molecular and cellular biology studies involving in vitro and in vivo models. By identifying and understanding the precise mechanisms involved, new potential therapeutic targets for PDAC can be identified.

This retrospective analysis suggests that LAMA3 may serve as a potential biomarker for predicting the prognosis of patients with PDAC. The increase in LAMA3 expression in PDAC tissues and its association with liver metastasis further underscore its potential role in disease progression. While these findings provide important insights, they also highlight the need for further studies. Understanding the specific mechanisms by which LAMA3 contributes to PDAC progression and liver metastasis may help uncover new therapeutic targets, potentially leading to more personalized treatment strategies.

Increased LAMA3 expression is associated with poor prognosis and liver metastasis in patients with PDAC. Our results indicate that LAMA3 can be a novel predictor of poor prognosis in patients with PDAC and liver metastasis, and LAMA3 may be a promising candidate for targeted therapy for PDAC liver metastasis.

Acknowledgements

Funding statement

This study was supported by the Natural Science Foundation of Shandong Province (No. ZR2020MH059)

Author contributions

All authors contributed to the study conception and design. Material preparation, data and specimen collection and analysis were performed by YyX and GQ. The first draft of the manuscript was written by YyX. The institutional review board approval and the direction of experiments by XJ. YpJ is the guarantor of the study and revised the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are openly available from The Cancer Genome Atlas database (https://portal.gdc.cancer.gov/) and the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo). The clinical data that support the findings of this study are available from the author, upon reasonable request.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; **71:** 209-49. doi: 10.3322/caac.21660
2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74:** 2913-21. doi: 10.1158/0008-5472.Can-14-0155
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics. *CA Cancer J Clin* 2021; **71:** 7-33. doi: 10.3322/caac.21654
4. Kommalapati A, Tella SH, Goyal G, Ma WW, Mahipal A. Contemporary management of localized resectable pancreatic cancer. *Cancers* 2018; **10:** 24. doi: 10.3390/cancers10010024
5. Huang C, Chen J. Laminin-332 mediates proliferation, apoptosis, invasion, migration and epithelial-to-mesenchymal transition in pancreatic ductal adenocarcinoma. *Mol Med Rep* 2021; **23:** 11. doi: 10.3892/mmr.2020.11649
6. Li R, Ochs MF, Ahn SM, Hennessey P, Tan M, Soudry E, et al. Expression microarray analysis reveals alternative splicing of LAMA3 and DST genes in head and neck squamous cell carcinoma. *PLoS One* 2014; **9:** e91263. doi: 10.1371/journal.pone.0091263
7. Shu C, Wang W, Wu L, Qi C, Yan W, Lu W, et al. LINC00936/microRNA-221-3p regulates tumor progression in ovarian cancer by interacting with LAMA3. *Recent Pat Anticancer Drug Discov* 2023; **18:** 66-79. doi: 10.2174/1574892817666220316152201
8. Xu SF, Zheng Y, Zhang L, Wang P, Niu CM, Wu T, et al. Long non-coding RNA LINC00628 interacts epigenetically with the LAMA3 promoter and contributes to lung adenocarcinoma. *Mol Ther Nucleic Acids* 2019; **18:** 166-82. doi: 10.1016/j.omtn.2019.08.005
9. Kinoshita T, Hanazawa T, Nohata N, Kikkawa N, Enokida H, Yoshino H, et al. Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion through targeting laminin-332 in head and neck squamous cell carcinoma. *Oncotarget* 2012; **3:** 1386-400. doi: 10.18632/oncotarget.709
10. Bizama C, Benavente F, Salvatierra E, Gutiérrez-Moraga A, Espinoza JA, Fernández EA, et al. The low-abundance transcriptome reveals novel biomarkers, specific intracellular pathways and targetable genes associated with advanced gastric cancer. *Int J Cancer* 2014; **134:** 755-64. doi: 10.1002/ijc.28405
11. Nagy Á, Munkácsy G, Győrffy B. Pancancer survival analysis of cancer hallmark genes. *Sci Rep* 2021; **11:** 6047. doi: 10.1038/s41598-021-84787-5
12. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; **378:** 607-20. doi: 10.1016/s0140-6736(10)62307-0
13. Zboralski D, Böckmann M, Zapatka M, Hoppe S, Schöneck A, Hahn SA, et al. Divergent mechanisms underlie Smad4-mediated positive regulation of the three genes encoding the basement membrane component laminin-332 (laminin-5). *BMC Cancer* 2008; **8:** 215. doi: 10.1186/1471-2407-8-215
14. Atay S. Integrated transcriptome meta-analysis of pancreatic ductal adenocarcinoma and matched adjacent pancreatic tissues. *Peer J* 2020; **8:** e10141. doi: 10.7717/peerj.10141
15. Chen J, Zhang H, Luo J, Wu X, Li X, Zhao X, et al. Overexpression of α3, β3 and γ2 chains of laminin-332 is associated with poor prognosis in pancreatic ductal adenocarcinoma. *Oncol Lett* 2018; **16:** 199-210. doi: 10.3892/ol.2018.8678
16. Xue K, Zheng H, Qian X, Chen Z, Gu Y, Hu Z, et al. Identification of key mRNAs as prediction models for early metastasis of pancreatic cancer based on LASSO. *Front Bioeng Biotechnol* 2021; **9:** 701039. doi: 10.3389/fbioe.2021.701039
17. Zheng B, Ohuchida K, Yan Z, Okumura T, Ohtsuka T, Nakamura M. Primary recurrence in the lung is related to favorable prognosis in patients with pancreatic cancer and postoperative recurrence. *World J Surg* 2017; **41:** 2858-66. doi: 10.1007/s00268-017-4068-6

research article

Impact of early integrated rehabilitation on fatigue in 600 patients with breast cancer - a prospective study

Masa Auprih[1], Tina Zagar[2], Nina Kovacevic[3,4], Andreja Cirila Skufca Smrdel[5], Nikola Besic[1,4], Vesna Homar[4]

[1] Department of Surgical Oncology, Institute of Oncology, Ljubljana, Slovenia
[2] Slovenian Cancer Registry, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[3] Department of Gynaecological Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[4] Faculty of Medicine Ljubljana, Ljubljana, Slovenia
[5] Department of Psycho-Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

Correspondence to: Prof. Nikola Besic, M.D., Ph,D., Department of Surgical Oncology, Institute of Oncology Ljubljana, Zaloška 2, Si-1000 Ljubljana, Slovenia. E-mail: nbesic@onko-i.si

Radiol Oncol 2024; 58(2): 243-257.

Received 5 June 2023
Accepted 9 December 2023

Disclosure: No potential conflicts of interest were disclosed.

Background. Fatigue after breast cancer treatment is a common burden that is challenging to treat. The aim of this study was to explore if such integrated rehabilitation program reduces the prevalence of chronic fatigue compared to simple, non-integrated rehabilitation.

Patients and methods. The subjects of our prospective study were 600 female breast cancer patients (29–65 [mean 52 years] of age), who participated in the pilot study on the individualized integrated rehabilitation of breast cancer patients in 2019-2021 and were monitored for one year. The control group included 301 patients and the intervention group numbered 299 patients. The patients completed three questionnaires (EORTC QLQ-C30, -BR23 and NCCN): before cancer treatment, and then six and twelve months after the beginning of cancer treatment. The control group obtained the standard rehabilitation program, while the intervention group was part of the early, individualized multidisciplinary and integrated approach of rehabilitation. The rehabilitation coordinator referred patients for additional interventions (*e.g.*, psychologist, gynecologist, pain management team, physiotherapy, clinical nutrition team, kinesiologist-guided online training, vocational rehabilitation, general practitioner). Data on the patients' demographics, disease extent, cancer treatment and complaints reported in questionnaires were collected and analyzed.

Results. There were no differences between the control and the intervention group of patients in terms of age, education, disease extent, surgical procedures, systemic cancer treatment, or radiotherapy, and also no differences in the fatigue before the beginning of treatment. However, patients from the control group had a greater level of constant fatigue than patients from the intervention group half a year ($p = 0.018$) and a year ($p = 0.001$) after the beginning of treatment. Furthermore, a greater proportion of patients from the control group experienced significant interference with their usual activities from fatigue than from the intervention group, half a year ($p = 0.042$) and a year ($p = 0.001$) after the beginning of treatment. A multivariate logistic regression showed that one year after the beginning of treatment, the only independent factor correlated to fatigue was inclusion into the intervention group ($p = 0.044$). Inclusion in the intervention group was beneficial—patients from the control group were 1.5 times more likely to be fatigued.

Conclusions. Early individualized integrated rehabilitation is associated with a lower prevalence of chronic fatigue or fatigue interfering with usual activities in breast cancer patients in comparison to the control group of patients.

Key words: early integrated rehabilitation; fatigue; breast cancer; EORTC questionnaire

Introduction

Breast cancer is the most common malignancy in women worldwide.[1] New diagnostic options and treatments result in a survival rate as high as 85–90% after five years in developed countries which sets a new challenge for health care systems – how to successfully improve the quality of life of breast cancer patients during and after the treatment.[2-5] The most important tool in achieving a good quality of life is early, optimized, individualized and integrated rehabilitation adapted to the needs of each patient.[2]

Fatigue is one of a number of burdens for breast cancer patients, which is caused by the cancer itself or its treatment. Fatigue is characterized by persistent physical, emotional, and cognitive tiredness related to cancer and/or cancer treatment that is not proportional to recent physical activity, interferes with usual functioning and is not relieved by rest or sleep.[6,7] It can also be a barrier to cancer survivors' return to work.[8] A meta-analysis, which included 12,327 breast cancer survivors, reported that approximately one in four breast cancer survivors suffer from severe fatigue.[9] Fatigue usually improves after the treatment, but it can also have long-term effects and can progress to chronic fatigue.[9]

Rehabilitation can help persons with chronic disease or impairment to achieve and maintain the highest possible physical, social, psychological, and occupational functioning.[10] Rehabilitation is a dynamic process that starts with the diagnosis and continues to the end of life. Implementation of guidelines for fatigue evaluation and management is best accomplished by an interdisciplinary team who are able to tailor interventions to the needs of the individual patient.[11] Patients are therefore referred to an appropriate health care provider – survivorship, palliative care, integrative oncology, psychology, psychiatry, physical therapy, vocational therapy, and/or physical medicine. The results are best if rehabilitation starts early, ideally before the beginning of the treatment.[11]

Breast cancer patients in this study were offered an improved rehabilitation program, that started soon after diagnosis and was tailored to individual needs. The aim of this study was to explore if such integrated rehabilitation program reduces the prevalence of chronic fatigue compared to simple, non-integrated rehabilitation.

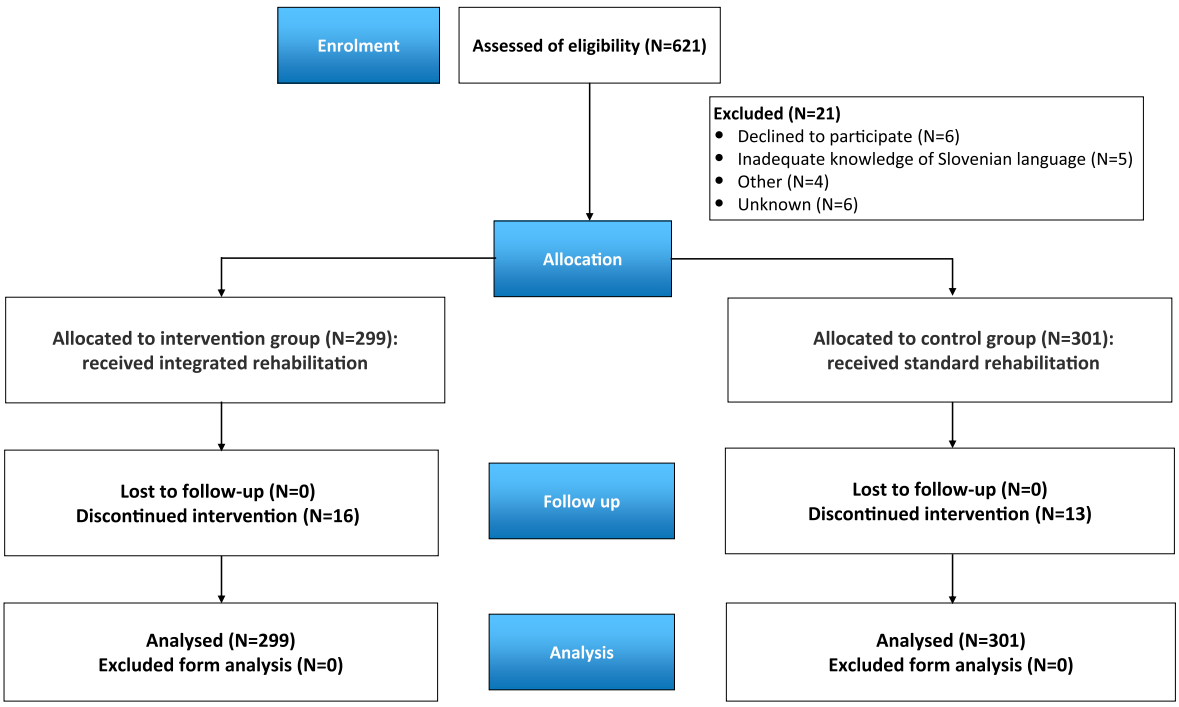


FIGURE 1. A flowchart of patients' inclusion in our study.

Patients and methods

Patients

A prospective pilot study included patients that were diagnosed and treated at the Institute of Oncology Ljubljana (IOL), Slovenia, from 2019 to 2022. Consecutively, 600 patients with all stages of invasive breast cancer and aged less than 65 years at the time of diagnosis were included. The exclusion criterion was if the patient refused to participate in the study or was unable to fill in the questionnaires. A flowchart of patients' inclusion in our study is presented in Figure 1. The study was reviewed and approved by the Protocol Review Board (ERID-KSOPKR-0086/2019) and the Ethics Committee of the Institute of Oncology Ljubljana (ERIDEK-0102/2019). The study was performed in accordance with the ethical standards laid down in the appropriate version of the 1964 Declaration of Helsinki and conducted with the understanding and consent of all the subjects involved.

All 600 planned patients were included in the study by December 2021. The control group consisted of 301 patients that were included in the study from December 2019 to the end of March 2021 and had already received existing routine non-integrated rehabilitation, *i.e.*, without a systematic patient needs evaluation and preemptive measures. Implementation of non-integrated rehabilitation began only if the individual patient specifically highlighted her problem in the outpatient clinic and/or when the attending physician noticed the need of the individual patient and directed her to appropriate treatment.

The inclusion of 299 patients in the intervention group started in September 2020 and ended in December 2021. In the intervention group, we included only those patients who live near the OIL, as we wanted them to be able to come twice a week to exercise in Ljubljana. The patients in the intervention group received integrated and individualized rehabilitation accordingly to the IOL's clinical guidelines and pathway of integrated rehabilitation developed specially for this study. Due to the COVID-19 pandemic, the study was prolonged with respect to the initial timeframe to reach the targeted number of participants.

Study protocol

During scheduled check-ups with the oncologist, each patient answered three standardized questionnaires (the European Organization for Research and Treatment of Cancer [EORTC] QLQ-C30, -BR23 and the National Comprehensive Cancer Network [NCCN]) before the treatment, half a year, and one year after the beginning of the treatment. The EORTC quality of life questionnaires (QLQ) and NCCN questionnaire are an integrated system for assessing the health-related quality of life of cancer patients.[11-13] The EORTC QLQ-C30 consists of a global health quality of life scale, five functional scales (physical, role, emotional, cognitive, and social function), and symptom scales (fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties).[12] The EORTC QLQ-BR23 consists of symptom scales of systemic therapy side effects (upset by hair loss, arm symptoms, breast symptoms) and functional scales (body image, future perspective, sexual functioning, and sexual enjoyment).[13] The NCCN questionnaire included questions about cardiac health, anxiety, depression, distress, cognitive function, fatigue, lymphedema, pain, hormone-related symptoms, sexual function, sleep disorder, healthy lifestyle (regular physical activity or exercise, diet, weight, use of vitamins or other supplements, smoking and consumption of alcohol), employment, and return to work.[11]

After completing all three standardized questionnaires, each patient also had an interview with a specialized registered nurse—a rehabilitation coordinator. The coordinator recorded the patient's most important needs and specific circumstances. The documentation of each patient from the intervention group was discussed at the multidisciplinary meeting for integrated rehabilitation before, half a year, and one year after the beginning of treatment. The multidisciplinary team consisted of an integrative rehabilitation coordinator, surgical oncologist, radiation oncologist, medical oncologist, psychologist, psychiatrist, general practitioner, physiotherapist, psychiatrist, specialist in medical rehabilitation and physical medicine, specialist in vocational medicine, and gynecologist.[14] The aim was to identify the patient's problems early, predict the late treatment consequences, implement measures to prevent or diminish the patient's problems and start rehabilitation as soon as needed. The mainstay of the patient's integrative rehabilitation was educating and empowering the patient to self-care and to be able to manage her symptoms and prevent undesired side effects of treatment, as already described in our recent publication.[14]

Management of a patient with fatigue

All patients were screened for fatigue with questionnaires as recommended in the NCCN clinical practice guidelines for survivorship, IOL's guidelines and clinical pathway of integrated rehabilitation.[11,15,16] The fatigue was graded in four grades (1 - without, 2 - mild, 3 - moderate, or 4 - severe) according to the EORTC QLQ-C30 questionnaire.[12] According to the NCCN questionnaire[11,15,16] the level of fatigue was assessed with a quantitative or semi-quantitative assessment on a 0 to 10 numeric rating scale (zero = no fatigue and 10 = worst fatigue imaginable). Mild fatigue had a score of 1 to 3, moderate fatigue 4 to 6, and severe fatigue 7 to 10.

According to the IOL guidelines and clinical pathway, the individualized integrated rehabilitation was carried out on three levels.[15,16] The first level was the treatment of all diseases and conditions that contribute to fatigue or may cause an increased baseline level of fatigue. The patients with moderate to severe fatigue (numeric scale from 4 to 10) were evaluated by the oncologist and/or general practitioner with regard to current disease status, history and physical examination, review of current medications, review of organ systems, and evaluation of other concurrent symptoms and contributing factors. The most important diseases that affect baseline fatigue such as heart failure or chronic kidney disease, thyroid malfunction, and/or anemia were ruled out clinically and by laboratory tests.

Secondly, all our patients from the control and intervention group were educated about a healthy lifestyle and were offered various techniques and training to help them cope with fatigue. All patients received written information about these topics and had information available on the website of the IOL dedicated to integrative rehabilitation. Prevention of fatigue is especially important before starting chemotherapy. Education and counseling are believed to be central to the effective management of fatigue[11] and the rehabilitation coordinator devoted a lot of time to patient education during each patient's visit. Cancer patients were encouraged to engage in regular moderate physical activity for at least 150 minutes per week and were educated about appropriate exercise to reduce fatigue. Patients were advised to be physically active each day by walking, cycling, doing resistance exercise, or a combination of aerobic and resistance exercise. All our patients from the intervention group who were treated with chemotherapy or reported fatigue had been asked to join a physical activity guided by a kinesiologist twice a week conducted online by a videoconference. On average more than 30 patients attended each videoconference. Advice on maintaining a healthy diet was given during a visit to the Clinical Nutrition and Dietotherapy outpatient clinic at our Institute as well as during online workshops guided by experienced clinical nutritionists. Since November 2021, the patients with fatigue from the intervention group were recommended to join the videoconferences with a yoga teacher once a week.

Thirdly, patients from the intervention group with moderate or severe fatigue were referred for consultations and treatment of fatigue to the oncologist, general practitioner, clinical psychologist or psychiatrist, pain relief clinic acupuncture, and/or yoga. All the interventions were covered by health insurance. Psychosocial interventions were recommended to all our patients with moderate to severe fatigue. These were available sooner for the first half of the intervention group than for the control group of patients as the COVID-19 pandemic prevented group therapies from taking place and enabled individual therapies from March 2020 onwards. However, because of a shortage of clinical psychologists in our country it was more difficult to obtain psychosocial intervention for the second part of the intervention group of patients. IOL's psycho-oncology department provided psychological counseling, crisis interventions, and cognitive behavioral psychotherapy. Evaluation at the psycho-oncology department was done during the first year after the beginning of oncological therapy in the intervention and control group of patients in 127 and 42 patients, respectively. Altogether 36 patients from the intervention group attended from one to eight (median 5.6) online group meetings with a clinical psychologist.

Depending on the patient's needs, the patient was referred also to other healthcare providers within the framework of the Slovenian health system. Anesthesiologists from the Institute of Oncology Ljubljana offered acupuncture as well as pharmacological therapy. General practitioners had the possibility to refer the patient to a number of workshops held at the Center for Health Promotion, which operates within the community health centers.

Statistical analysis

Data on the patients' demographics, disease extent, cancer treatment, fatigue, and other complaints re-

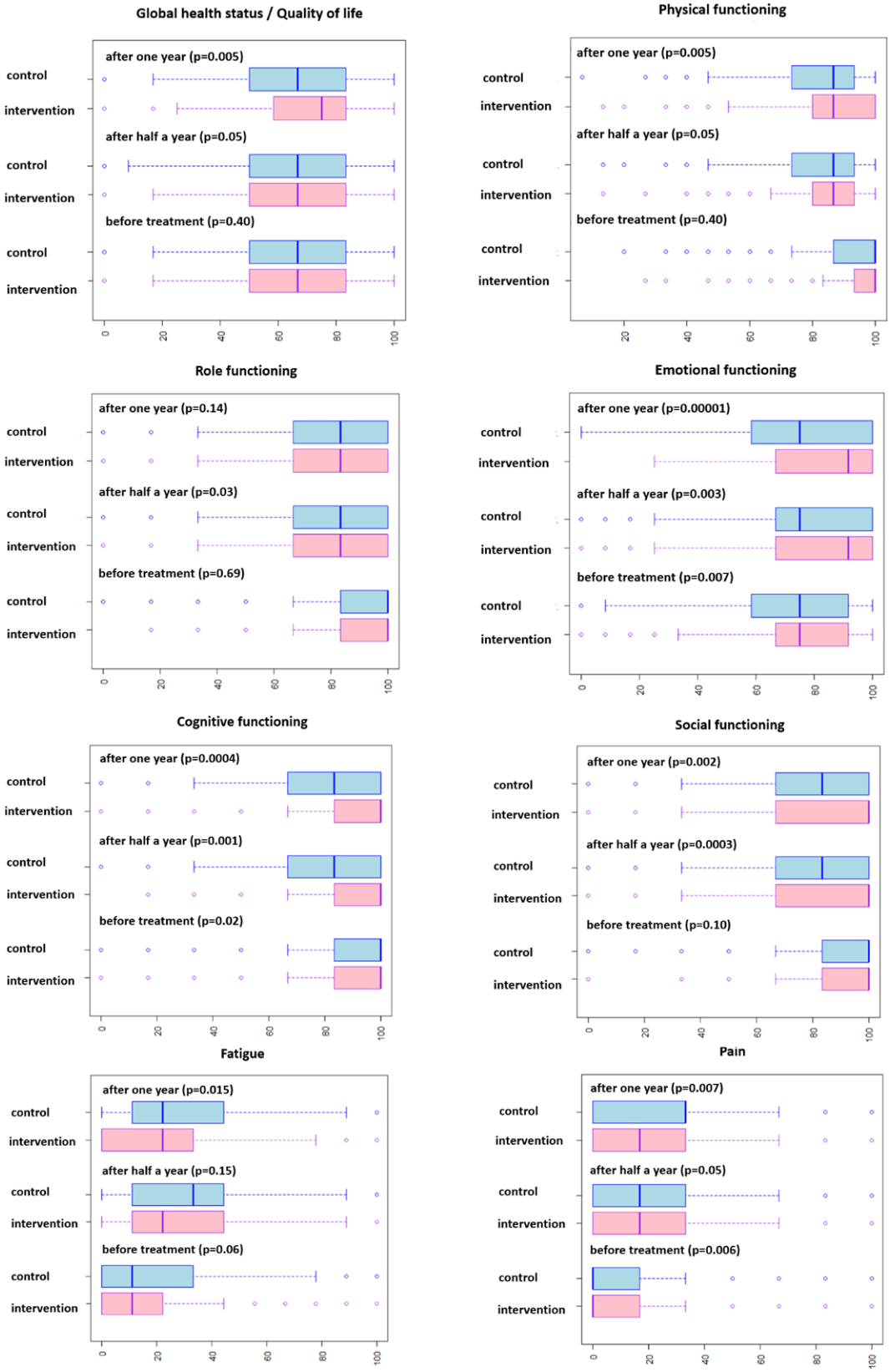


FIGURE 2. Data and statistical analysis from EORTC C30 questionnaires about the global health quality of life scale, physical, role, emotional, cognitive, and social function scale, symptom scales about fatigue, pain and insomnia in the intervention and control group of patients before treatment, half a year, and a year after the beginning of treatment.

TABLE 1. Demographic and clinical characteristics of patients, pathological characteristics of tumors and treatment. P-value refers to difference between control and intervention group; it is calculated by t-test in case of comparing means and by chi-squared test in case of counts

Factor	Subgroup	All patients (N = 600)	Control group (N = 301)	Intervention group (N = 299)	P-value
Mean age of patients (years)		50.78	50.59	50.97	0.601
Living areas	Urban	287	125	162	0.003
	Suburban	105	53	52	
	Rural	208	123	85	
Education (N = 599)	Primary school	66	39	27	0.290
	Secondary school	242	117	125	
	Higher	291	144	147	
Socioeconomic status	Low	71	36	35	0.940
	Middle	432	217	215	
	Higher	95	46	49	
With whom they live (N = 597)	Alone	58	24	34	0.600
	With partner only	145	71	74	
	Partner and children	289	147	142	
	With children only	42	22	20	
	Other	63	35	28	
Employment (N = 581)	Unemployed	54	35	19	0.067
	Employed	433	209	224	
	Retired	94	45	49	
Mean primary tumor size (mm)		26.3	25.5	27.2	0.285
Tumor stage	In situ	10	5	5	0.152
	I	260	133	127	
	II	214	97	117	
	III	81	50	31	
	IV	35	16	19	
Concomitant diseases	No	301	154	147	0.624
	Yes	299	147	152	
Neoadjuvant chemotherapy and/or anti-HER-2 therapy	No	465	227	237	0.241
	Yes	135	74	61	
Breast surgery	Mastectomy	252	135	117	0.357
	Tumorectomy	326	156	170	
	No surgery	22	10	12	
Lymph node surgery	Lymphadenectomy	151	83	86	0.337
	Sentinel node biopsy	417	204	213	
	No surgery	32	14	18	
Breast reconstruction	No	431	214	217	0.402
	Tissue expander	127	69	58	
	Free-flap	42	18	24	
Breast external beam radiotherapy	No	149	83	66	0.131
	Yes	451	218	233	
Chemotherapy	No	280	137	143	0.623
	Yes	320	164	156	
Anti-HER2 therapy	No	522	264	258	0.629
	Yes	78	37	41	
Hormone therapy	No	132	69	63	0.623
	Yes	468	232	236	

ported in questionnaires were collected and managed in REDCap (Research Electronic Data Capture) Version 12.4.22. Additional data processing was performed in Excel (Microsoft Office Professional Plus 2016). The average score of all answers to questions from EORTC questionnaires about different function scales and symptoms was standardized with a linear transformation on a scale from 0 to 100. Differences between scores between the intervention and control groups at the same time point were assessed with the Wilcoxon signed-ranks test. Differences measured at two time points (in the same persons) used the Wilcoxon signed-rank paired difference test. Distribution between cat-

TABLE 2. Mean values of psychological factors and pain reported by patients before, half a year and one year after the beginning of treatment

Factor	Time of assessment	Group	Mean value	Standard deviation	p-value
Depression level	Before therapy	Control Intervention	4.2 3.6	2.8 2.5	0.013
	After half year	Control Intervention	3.0 2.2	2.4 1.7	< 0.001
	After one year	Control Intervention	3.2 2.3	2.4 1.9	< 0.001
Anxiety level	Before treatment	Control Intervention	4.2 3.8	2.7 2.6	0.041
	After half year	Control Intervention	3.2 2.4	2.5 1.8	< 0.001
	After one year	Control Intervention	3.5 2.6	2.6 2.0	< 0.001
Level of difficulty concentrating	Before treatment	Control Intervention	3.2 3.1	2.5 2.3	0.96
	After half year	Control Intervention	3.2 2.8	2.4 2.1	0.02
	After one year	Control Intervention	3.6 2.7	2.4 2.1	< 0.001
Constant fatigue	Before treatment	Control Intervention	3.2 3.1	2.4 2.2	0.59
	After half year	Control Intervention	3.8 3.3	2.6 2.4	0.018
	After one year	Control Intervention	4.0 3.3	2.7 2.3	0.001
Disturbing fatigue	Before treatment	Control Intervention	2.8 2.9	2.3 2.2	0.50
	After half year	Control Intervention	3.9 3.3	2.5 2.3	0.003
	After one year	Control Intervention	3.8 3.2	2.5 2.2	< 0.001
Insomnia	Before treatment	Control Intervention	4.2 3.9	3.0 2.8	0.22
	After half year	Control Intervention	4.8 4.0	3.0 2.7	0.002
	After one year	Control Intervention	4.8 3.9	3.0 2.9	< 0.001
Pain	Before treatment	Control Intervention	2.8 2.3	2.4 2.0	0.005
	After half year	Control Intervention	3.4 2.9	2.4 2.1	0.006
	After one year	Control Intervention	3.7 2.7	2.5 1.8	< 0.001

egories was analyzed using the chi-square test. ANOVA was used to test for differences in the means of three or more groups. Differences between the answers from the intervention and control groups to questions from NCCN questionnaires at the same time point were assessed with the ANOVA test. All statistical analyses were done in Version 27 of the SPSS Statistical Software and Software R version 4.2.2. P-values under 0.05 were considered statistically significant.

Results

Data about patients, disease characteristics, and treatment are presented in Table 1. There were no differences between the control and the intervention group of patients in terms of age, education, disease extent, surgical procedures, systemic cancer treatment, or radiotherapy. As expected, both groups of patients differed in living areas. The majority of patients from the intervention group

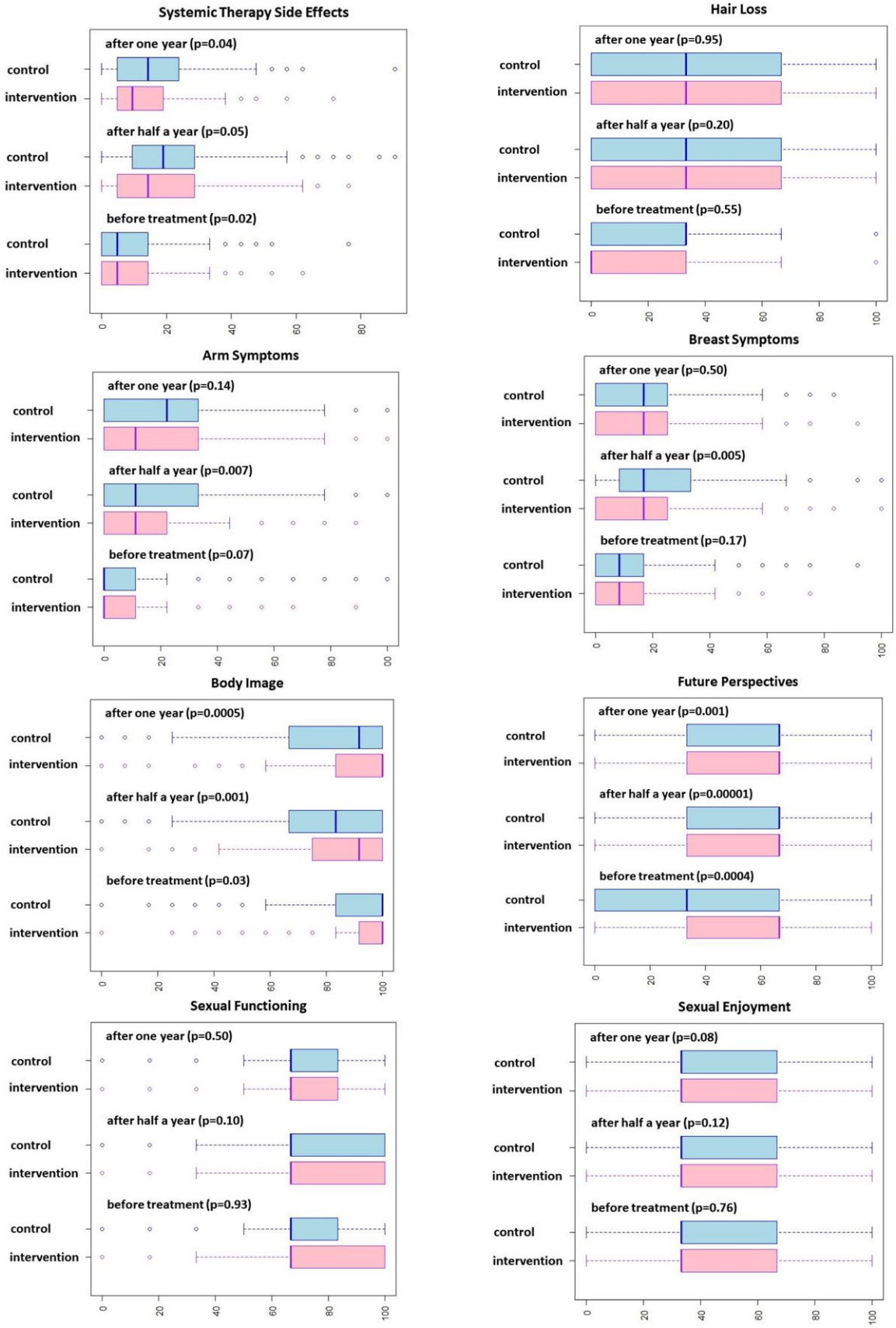


FIGURE 3. Data and statistical analysis from EORTC BR23 questionnaires.

lived in urban areas, while patients from the control group were more distributed between rural and suburban areas. Namely, it was planned that the patients from the intervention group would exercise under the supervision of a kinesiologist in the gym close to our Institute, so only the patients from central Slovenia were included in the intervention group. However, due to the COVID-19 pandemic, we could not do physical exercise in the gym, so it was done online instead.

EORTC questionnaires

Data and statistical analysis from EORTC C30 questionnaires about the global health quality of life scale, physical, role, emotional, cognitive, and social function scale, symptom scales about fatigue, pain, and insomnia in the intervention and control group of patients before treatment, half a year, and a year after the beginning of treatment are presented in Figure 2. Before the treatment, the patients from the intervention group reported significantly fewer problems in emotional and cognitive function scale and pain in comparison to the control group. Half a year after the beginning of treatment, the patients from the intervention group reported significantly fewer problems on the physical, role, emotional, cognitive, and social function scale, and pain in comparison to the control group. A year after the beginning of treatment, the patients from the intervention group reported significantly fewer problems on the global health quality of life scale, physical, emotional, cognitive, and social function scale, fatigue, and pain in comparison to the control group.

Data and statistical analysis from EORTC BR23 questionnaires are presented in Figure 3. Before the treatment, the patients from the intervention group reported significantly fewer problems with systemic therapy side effects but were more concerned about body image and future perspectives in comparison to the control group. Half a year after the beginning of treatment, the patients from the intervention group reported significantly fewer problems with systemic therapy side effects, arm symptoms, and breast symptoms, but were still more concerned about body image and future perspectives in comparison to the control group. A year after the beginning of treatment, the patients from the intervention group reported significantly fewer problems with systemic therapy side effects but were still more concerned about body image and future perspectives in comparison to the control group.

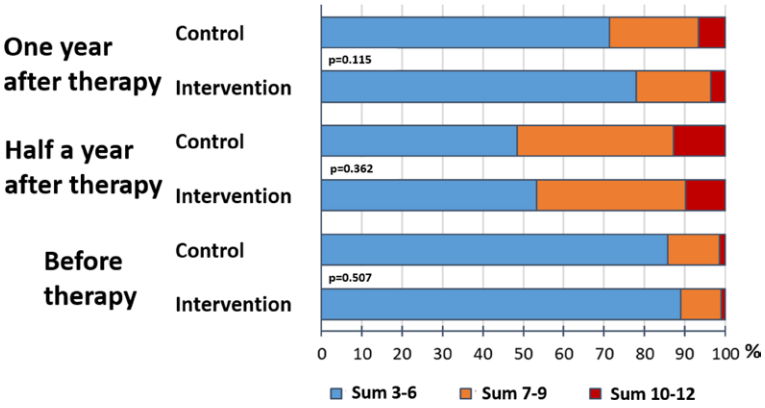


FIGURE 4. The sum of responses to EORTC questions 10, 12 and 18 in the intervention and control groups before, half a year, and a year after treatment.

NCCN questionnaires

Table 2 shows mean values of psychological factors and pain reported by patients and assessed on a 0 to 10 numeric rating scale (zero = no pain and 10 = worst imaginable) before, half a year, and one year after the beginning of treatment. Before the treatment, the patients from the intervention group reported a significantly lower level of depression, anxiety, and pain in comparison to the control group. Half a year and one year after the beginning of treatment, the patients from the intervention group reported a significantly lower level of depression, anxiety, difficulty concentrating, disturbing fatigue, insomnia, and pain in comparison to the control group.

Regarding the proportion of patients with physical activity of at least 150 minutes per week, there was no difference between the groups before treatment ($p = 0.73$), but after one year the difference was statistically significant ($p = 0.034$). Before the cancer treatment, smoking was present in the intervention and control group in 22% and 27% ($p = 0.27$), respectively. However, one year after the beginning of cancer treatment, smoking was less common in the intervention group in comparison to the control group of patients ($p = 0.001$).

Fatigue

Regarding question 18 from the EORTC C30 questionnaire, 50% of the patients answered that they were not tired when asked before the beginning of the treatment. After half a year and one year after the beginning of treatment the answer was no in only 32% and 34%, respectively.

TABLE 3. The influence of each individual variable on fatigue (univariate models) by individual logistic regressions before treatment, half a year and one year after the beginning of treatment. OR – odds ratio; CI – confidence interval

Before treatment	**OR**	**95% CI**	**p-value**
Control group	1.3	0.8–2.2	0.245
Half a year after the beginning of treatment	**OR**	**95% CI**	**p-value**
Control group	1.3	0.9–1.9	0.114
Age group 45–54 years	1.0	0.7–1.6	0.855
Age group 55–64 years	0.9	0.6–1.5	0.803
Chemotherapy – yes	1.3	0.9–1.9	0.135
Hormonal therapy – yes	0.9	0.6–1.4	0.689
Radiotherapy – yes	0.8	0.5–1.3	0.364
Neoadjuvant chemotherapy and/or anti-HER2 therapy	1.3	0.9–1.9	0.227
Surgery – not done	1.7	0.6–4.4	0.308
Surgery – Tumorectomy – yes	1.3	0.9–1.8	0.208
Axillary lymphadenectomy – yes	0.7	0.5–1.1	0.162
Breast reconstruction – yes	0.8	0.5–1.2	0.256
Presence of distant metastases	1.4	0.7–2.8	0.403
A year after the beginning of treatment	**OR**	**95% CI**	**p-value**
Control group	1.5	1.0–2.2	**0.046**
Age group 45–54 years	1.2	0.8–2.0	0.387
Age group 55–64 years	0.6	0.4–1.0	0.064
Chemotherapy – yes	1.4	0.8–1.7	0.493
Hormonal therapy – yes	1.3	0.8–2.2	0.246
Radiotherapy – yes	0.7	0.5–1.2	0.191
Neoadjuvant chemotherapy and/or anti-HER2 therapy	1.2	0.8–1.9	0.347
Surgery – not done	1.7	0.5–5.2	0.371
Surgery – Tumorectomy – yes	1.1	0.7–1.6	0.961
Axillary lymphadenectomy – yes	0.7	0.5–1.1	0.161
Breast reconstruction – yes	0.9	0.6–1.3	0.454
Presence of distant metastases	1.8	0.8–4.0	0.130

The symptom of fatigue was assessed with the sum of EORTC questions number 10, 12, and 18 on the Likert scale (1–without, 2–mild, 3–moderate, 4–severe). The sum of all three answers to EORTC questions before, after half a year, and after a year after treatment can be a minimum of 3 and a maximum of 12. Figure 4 shows the sum of responses to EORTC questions 10, 12, and 18 in the intervention and control groups before, half a year, and a year after treatment. We considered that the patient has moderate or severe fatigue when the sum of all three responses was equal to seven or more or at least one of the patient's responses was "4–severe". Fatigue was present in all our patients before treatment, half a year, and a year after treatment in 12.7%, 47.7%, and 24.2%, respectively.

The univariate association of each individual variable on fatigue by individual logistic regressions and multivariate models' logistic regression about the association of all included variables simultaneously and fatigue are presented in Table 3 and Table 4, respectively.

A multivariate logistic regression analysis showed that half a year after the beginning of treatment, fatigue was only associated with treatment with chemotherapy. Patients who received chemotherapy were 1.6 times more likely to be fatigued in comparison to those without chem-

TABLE 4. The influence of all included variables simultaneously on fatigue half a year and a year after the beginning of treatment

	Half a year after treatment			One year after treatment		
	OR	95% CI	p-value	OR	95% CI	p-value
Control group	1.4	0.9–2.0	0.100	1.5	1.0–2.2	**0.044**
Age group 45–54 years	1.1	0.7–1.7	0.822	1.1	0.7–1.9	0.617
Age group 55–64 years	1.0	0.6–1.6	0.931	0.6	0.3–1.0	0.052
Chemotherapy – yes	1.6	1.1–2.5	**0.025**	1.3	0.9–2.1	0.206
Hormonal therapy – yes	1.1	0.7–1.8	0.616	1.6	0.9–2.6	0.088
Radiotherapy – yes	0.7	0.4–1.2	0.196	0.7	0.4–1.3	0.277
Surgery – not done	1.2	0.3–4.4	0.745	0.9	0.2–3.9	0.879
Surgery – Tumorectomy – yes	1.6	0.9–2.6	0.080	1.4	0.8–2.4	0.256
Axillary lymphadenectomy – yes	0.7	0.4–1.2	0.232	0.7	0.4–1.2	0.148
Presence of distant metastases	1.4	0.5–3.7	0.491	2.1	0.8–5.9	0.145

OR = odds ratio; CI = confidence interval

otherapy. But, one year after the beginning of treatment, treatment with chemotherapy was no longer associated with fatigue. The only independent factor correlated to fatigue was inclusion into the intervention group. Inclusion into the intervention group was beneficial; patients from the control group were 1.5 times more likely to be fatigued.

Answers to the NCCN questionnaires show that there were no differences between the groups regarding constant fatigue before treatment ($p = 0.59$). However, patients from the control group had a greater level of fatigue than patients from the intervention group half a year ($p = 0.018$) and a year ($p = 0.001$) after the beginning of treatment. Furthermore, there were no differences in mean value between both groups regarding fatigue interfering with usual activities before therapy (0.50). Patients from the control group had more fatigue interfering with usual activities than from the intervention group half a year ($p = 0.003$) and a year ($p < 0.001$) after the beginning of treatment.

Discussion

Most published reports on oncological rehabilitation include patients who started rehabilitation after oncological treatment and a minority of reports focus on rehabilitation during oncological treatment. The purpose of our study was to improve the rehabilitation of our patients with breast cancer and to start implementing integrated rehabilitation. Breast cancer patients in this study were offered an improved rehabilitation program that started early after diagnosis and was tailored to individual needs. The aim of this study was to explore if such integrated rehabilitation program reduces the prevalence of chronic fatigue compared to simple, non-integrated rehabilitation. We expected that earlier rehabilitation would reduce the patients' difficulties and side effects of treatment, so our patients from the intervention group started with integrative oncological rehabilitation already at the beginning and it was carried on also during oncological treatment. Our results show that patients who received integrated rehabilitation reported significantly less fatigue and better quality of life compared to controls.

Before treatment, our two groups of patients did not differ in terms of fatigue, as the two groups did not differ in terms of risk factors for fatigue (age, education level, stage of disease, and extent of treatment). This is understandable, since we allocated the vast majority of patients to the two groups almost randomly according to the time of treatment; in one group there were patients who started treatment before the other group of patients. The essential reason for lower fatigue in the intervention group is that these patients received a number of measures that have been proven to reduce fatigue. The mainstay of our integrative rehabilitation was patient education about what they themselves can do to manage their symptoms, and to mitigate or even prevent the adverse effects of treatment. In contrast to the control group, the patients from the

intervention group had 3 interviews with the integrated rehabilitation coordinator, who during each interview educated patients about the prevention and treatment of fatigue. All patients were referred to a general practitioner for counseling on leading a healthy lifestyle. Additionally, patients from the intervention group were advised to be physically active and were provided with physical exercise guided by a kinesiologist twice per week, which was carried out online. They also had the possibility to practice yoga. Furthermore, some patients with fatigue had psychotherapy interventions and acupuncture.

Fatigue in breast cancer patients is a common symptom and varies between different phases of breast cancer treatment.[9,17,18] Reinertsen *et al*.[17] investigated levels of fatigue in women before, during chemotherapy and at a two-year follow-up. Chronic fatigue was reported before treatment, during chemotherapy and two years after the therapy in 8%, 12%, and 36% of patients, respectively. In our patients, fatigue was present before treatment, half a year, and a year after treatment in 13%, 48%, and 24%, respectively. The degree of difference between our and Norwegian patients regarding fatigue is probably related to the different tests that were used in our and the Norwegian study. Namely, we used EORTC questionnaires, while they also used a specific fatigue questionnaire. A meta-analysis showed that after completion of cancer treatment severe fatigue was present in 22% to 42% of 12,327 breast cancer survivors and that risk factors for chronic fatigue were demographic, the stage of disease, and the extent of oncological therapy.[9] The relatively low proportion of fatigue reported by our patients one year after the start of treatment (24%), compared to the data from the above-mentioned meta-analysis, could be attributed to the successful measures received by the intervention group of patients, which also reduced the proportion of fatigue in both groups together.

Many studies reviewed by Ruiz-Casado *et al*.[19] reported that younger and less educated women had greater fatigue. However, a higher level of education was significantly associated with moderate to severe fatigue in patients treated with aromatase inhibitors.[20] Patients with a partner were less susceptible to severe fatigue than those without a partner.[9] On the other hand, many patients have problems with fatigue even before starting treatment, and this problem may persist or even worsen during treatment.[18] Preexisting comorbid conditions or medications used to treat them may contribute to increased fatigue early during cancer treatment.[18,19] Such conditions include heart disease, hypertension, diabetes, anemia, obesity, arthritis, or psychiatric conditions.[18,19] Patients who experience psychosocial distress at baseline and patients who have a history of depression are prone to suffer from chronic fatigue.[18] Our patients from the intervention and control groups did not differ in terms of age, educational structure, or accompanying diseases, so the rate of fatigue between both groups of patients was not different before the beginning of treatment. However, these factors might have contributed to the difference in fatigue rate half a year and one year after the beginning of treatment. At the time of diagnosis, it is impossible to influence any of the studied independent risk factors for chronic fatigue. However, our results clearly show that integrated rehabilitation, although not able to influence individual risk factors, reduces the likelihood of developing chronic or severe fatigue compared to standard care.

Risk of fatigue is significantly higher in patients treated with chemotherapy.[9,18] Our univariate analysis showed that the patients treated with chemotherapy had increased risk for fatigue half a year and one year after the beginning of treatment. Furthermore, multivariate logistic regression showed that after half a year, fatigue was the only factor associated with treatment with chemotherapy. Patients who received chemotherapy were 1.6 times more likely to be fatigued than those without chemotherapy. This is much more common than the 1.12 times more reported in the meta-analysis by Abrahams *et al*.[9] However, one year after the beginning of therapy, treatment with chemotherapy was not an independent factor associated with fatigue and inclusion in the intervention or control group of patients was the only independent factor associated with fatigue. We assume that integrated oncological rehabilitation decreased fatigue in patients from the intervention group, while patients from the control group still experienced fatigue.

Conditions that are a consequence of cancer treatment such as insomnia or pain can also contribute to fatigue.[18] Integrated rehabilitation effectively decreased insomnia and pain in our intervention group of patients. Before treatment, there were no differences in the frequency of insomnia between both groups of patients. However, after half a year and a year, insomnia was more common in the control group of patients than in the intervention group. Furthermore, severe pain in patients from the control group one year after the beginning of treatment was significantly more

common than before the treatment. On the other hand, the proportion of patients with severe pain in the intervention group did not significantly change over time.

Several interventions could have positive effects on a specific symptom or a patient's problems, and timing of the intervention is important.[2] Proven interventions for prevention or treatment of fatigue are aerobic exercises, resistance training, yoga, psychological interventions (cognitive-behavioral therapy, psychoeducation, mindfulness), healthy lifestyle interventions, acupuncture, and pharmacotherapy.[18] Our intervention group of patients were advised and received many of these interventions, while the control group of patients did not receive these interventions to the same degree.

Aerobic exercise and resistance training is associated with an important reduction of fatigue in the majority of systematic reviews.[2] Longer duration, length, and frequency of physical activity has a stronger effect on reducing fatigue.[2] Furthermore, physical activity reduces fatigue if performed during or after chemotherapy and/or radiotherapy treatments.[21] Based on these findings, our patients from the intervention group who had chemotherapy or fatigue had been recommended to join a physical activity guided by a kinesiologist twice a week. One year after the beginning of treatment, a significantly larger proportion of patients from the intervention group became more physically active compared to those from the control group. Juvet *et al*.[21] in a meta-analysis of patients treated with chemotherapy found that fatigue was significantly lower in patients who received physical activity intervention in comparison to controls. In the group with physical activity intervention in comparison to controls during and after oncological treatment they reported a lower standard mean difference of fatigue of 0.19 and 0.52, respectively. Similarly, half a year and a year after the beginning of treatment, the rate of fatigue in our integrated rehabilitation group in comparison to the control group was 1.3 and 1.5 lower, respectively. Furthermore, early integrated rehabilitation helped smoking cessation in a significantly larger proportion of patients from the intervention group compared with the control group[14], adding to the healthier lifestyle of the patients.

Psychological interventions are the second most effective way to reduce fatigue after physical activity.[22] Moreover, the combination of physical activity and psychological interventions is even more effective than physical activity or psychological interventions per se.[22] During oncological treatment, fatigue may be effectively reduced with relaxation exercise, massage, cognitive-behavioral therapy, yoga, and different combinations of these.[23] Lack of clinical psychologists and psychotherapists in Slovenia makes it difficult to access psychotherapy. Although we had planned that all patients from the intervention group who needed it could receive psychological treatment or psychotherapy, we did not manage to reach this goal. During and after the COVID-19 pandemic access to psychological treatment or psychotherapy treatment became even more difficult than before the pandemic. The COVID-19 pandemic led to a sharp increase in demand for psychological treatment or psychotherapy in the general population. Despite these limitations in access to psychological treatment or psychotherapy, the intervention group had significantly fewer problems compared to the control group in the global health quality of life, physical, role, emotional, cognitive, and social function scale, fatigue, and pain one year after the beginning of treatment.

Another important factor which reduces fatigue is psychoeducation[24,25] helping participants cope with problems related to breast cancer, teaching stress management strategies, and teaching adaptive strategies improve patients' quality of life.[26] A Cochrane review by Bennett *et al*.[27] provided preliminary findings for the beneficial effect of educational interventions for reducing general cancer-related fatigue, fatigue intensity, fatigue distress, and fatigue interference compared with usual care. Yoga is associated with a significant improvement in quality of life and reduction of fatigue[2,18]; furthermore, acupuncture is effective for the management of fatigue particularly during anti-cancer treatment.[28] Based on these facts, we tried to include these interventions as much as possible in the rehabilitation of our patients and to provide psychoeducation for our patients. Group and individual behavioral psychotherapy and an individual interview with a psychologist and acupuncture were carried out. Furthermore, the patients from the intervention group attended nutrition workshops and yoga classes online. In addition, all patients had access to online publications about cancer diagnosis, treatment, and rehabilitation, available on our website. Patients also received written brochures. A rehabilitation coordinator provided patients with all the necessary information and was available to them throughout the entire time.

Our study has several limitations. One is that it was not randomized. We had planned to conduct an 'almost random' approach. Originally,

we intended that the first half of patients would be included in the control group, and the other half in the intervention group. But due to the COVID-19 pandemic, for some time we had to include patients simultaneously in the control or intervention groups based on their place of residence. Another limitation is a different place of residence, which may be associated with certain psychosocial characteristics which correlate to fatigue. Furthermore, the difference in distance from the hospital could influence the significant difference in fatigue between the two groups of patients (e.g., a more tiring drive to the hospital), so this must be considered when interpreting our results. Another limitation of the study is that targeted precision tests for the assessment of fatigue were not used, as we were also interested in other problems bothering patients. In addition, the number of included patients is still too small to enable a more detailed analysis of the connection between fatigue and other psychological factors. In addition, some patients decided to withdraw from the study and some patients did not respond to all parts of the questionnaires, therefore there are some missing data. Furthermore, different interventions were simultaneously implemented in order to achieve as much benefit for the patients as possible. Therefore, it was not possible to test the effect of a single intervention and to describe the contribution each intervention played in the treatment of fatigue. Due to waiting times for certain treatments in Slovenia, such as acupuncture or cognitive-behavioral therapy, some patients did not receive treatment immediately, or when they needed it most. But our study enables a realistic presentation of rehabilitation in our country and what possibilities exist for improving integrated rehabilitation. Finally, the number of patients included in the research is relatively small, but it represents 40% of all breast cancer patients detected annually in Slovenia, so we believe that the sample size is suitable for evaluating the effectiveness of non-integrated rehabilitation and integrative rehabilitation on fatigue in our country.

Conclusions

Early individualized integrated rehabilitation is associated with a lower prevalence of chronic fatigue or fatigue interfering with usual activities in breast cancer patients in comparison to the control group of patients. A year after the beginning of treatment, patients from the intervention group reported significantly fewer problems also in the global health quality of life scale, pain, physical, emotional, cognitive, and social function scale in comparison with the control group.

Acknowledgments

We are grateful to all our colleagues who participated in treatment of patients and all members of the V3-1907 research group. This research was funded by the Ministry of Education, Science and Sport of the Republic of Slovenia. Grant P3-0289 and V3-1907. Principal investigator: Nikola Bešić.

References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: an overview. *Int J Cancer* 2021; **149:** 778-89. doi: 10.1002/ijc.33588
2. Olsson Möller U, Beck I, Rydén L, Malmström M. A comprehensive approach to rehabilitation interventions following breast cancer treatment – a systematic review of systematic reviews. *BMC Cancer* 2019; **19:** 472. doi: 10.1186/s12885-019-5648-7
3. American Cancer Society. *Survival rates for breast cancer.* [Internet]. Atlanta: American Cancer Society. [cited 2022 Febr 14]. Available at: https://www.cancer.org/cancer/breast-ca ncer/understanding-a-breast-cancer-diagnosis/breast-cancer-survival-rates
4. Cancer Research UK: *Breast cancer survival.* [Internet]. London: Cancer Research UK. [cited 2022 Febr 14]. Available at: https://www.cancerresearchuk.org/about-cancer/breast-cancer/survival
5. Slovenian Cancer Registry. Cancer in Slovenia 2020. [Internet]. Ljubljana: Institute of Oncology Ljubljana, Epidemiology and Cancer Registry. [cited 2023 Oct 19]. Available at: https://www.onko-i.si/fileadmin/onko/datoteke/rrs/lp/letno_porocilo_2020.pdf
6. Bower JE, Bak K, Berger A, Breitbart W, Escalante CP, Ganz PA, et al. Screening, assessment, and management of fatigue in adult survivors of cancer: an American Society of Clinical oncology clinical practice guideline adaptation. *J Clin Oncol* 2014; **32:** 1840-50. doi: 10.1200/JCO.2013.53.4495
7. Bower JE. Cancer-related fatigue – mechanisms, risk factors, and treatments. *Nat Rev Clin Oncol* 2014; **11:** 597-609. doi: 10.1038/nrclinonc.2014.127
8. Dorland HF, Abma FI, Van Zon SKR, Stewart RE, Amick BC, Ranchor AV, et al. Fatigue and depressive symptoms improve but remain negatively related to work functioning over 18 months after return to work in cancer patients. *J Cancer Surviv* 2018; **12:** 371-8. doi: 10.1007/s11764-018-0676-x
9. Abrahams HJG, Gielissen MFM, Schmits IC, Verhagen CAHHVM, Rovers MM, Knoop H. Risk factors, prevalence, and course of severe fatigue after breast cancer treatment: a meta-analysis involving 12,327 breast cancer survivors. *Ann Oncol* 2016; **27:** 965-74. doi: 10.1093/annonc/mdw099
10 Nugraha B, Gutenbrunner C. Contribution of the scientific field of physical and rehabilitation medicine to improvements in health-related rehabilitation at all levels of the healthcare system: a discussion paper. *J Rehabil Med* 2021; **53:** jrm00155. doi: 10.2340/16501977-2773
11. *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Survivorship.* Version 1.2023 – March 24, 2023. National Comprehensive Cancer Network® (NCCN®). [cited 2022 March 31]. Available at: https://www.nccn.org/professionals/physician_gls/pdf/survivorship.pdf
12. *EORTC QLQ-C30 Scoring Manual*. European Organisation for Research and Treatment of Cancer (EORTC). [cited 2022 Febr 14]. Available at: https://www.eortc.org/app/uploads/ sites/2/2018/02/SCmanual.pdf
13. *EORTC QLQ-BR23 Scoring Manual*. European Organisation for Research and Treatment of Cancer (EORTC). [cited 2022 Febr 14]. Available at: https://www.eortc.be/qol/ScoringInstructions/BR23%20Summary.pdf

14. Cencelj-Arnez R, Besic N, Mavric Z, Mozetic A, Zagar T, Homar V, et al. Early integrated rehabilitation helps smoking cessation – A comparison between the intervention and control group in a prospective study. *Med Sci Monit* 2023; **29:** e942272. doi: 10.12659/MSM.942272

15. Besic N, Borstnar S, Kovacec Hermann T, Homar V, Kos N, Kurir-Borovcic M, et al. [*Guidelines for comprehensive rehabilitation of patients with breast cancer 2019*]. [Slovenian]. Ljubljana: Institute of Oncology Ljubljana. [Internet]. [cited 2022 Febr 14]. Available at: https://www.onko-i.si/fileadmin/onko/datoteke/Strokovna_knjiznica/smernice/Smernice_za_celostno_rehabilitacijo_bolnikov_z_rakom_dojk_2019.pdf

16. Besic N, Borstnar S, Homar V, Mlakar Mastnak D, Mavric Z, Mozetic A et al. [*Clinical pathway of comprehensive rehabilitation of patients with breast cancer*]. Version 4 2021. [Slovenian]. [Internet]. [cited 2022 Febr 14]. Available at: https://www.onko-i.si/fileadmin/onko/datoteke/Strokovna_knjiznica/klinicne_poti/Klinicna_pot_celostne_rehabilitacije_bolnikov_z_rakom_dojk_2021.pdf

17. Reinertsen KV, Engebraaten O, Loge JH, Cvancarova M, Naume B, Wist E, et al. Fatigue during and after breast cancer therapy – a prospective study. *J Pain Symptom Manage* 2017; **53:** 551-60. doi: 10.1016/j.jpainsymman.2016.09.011

18. Thong MSY, van Noorden CJF, Steindorf K, Arndt V. Cancer-related fatigue: causes and current treatment options. *Curr Treat Options Oncol 2020*; **21:** 17. doi: 10.1007/s11864-020-0707-5. Erratum in: *Curr Treat Options Oncol* 2022; **23:** 450-1. doi: 10.1007/s11864-021-00916-2

19. Ruiz-Casado A, Álvarez-Bustos A, de Pedro CG, Méndez-Otero M, Romero-Elías M. Cancer-related fatigue in breast cancer survivors: a review. *Clin Breast Cancer* 2021; **21:** 10-25. doi: 10.1016/j.clbc.2020.07.011

20. Mao H, Bao T, Shen X, Li Q, Seluzicki C, Im EO, et al. Prevalence and risk factors for fatigue among breast cancer survivors on aromatase inhibitors. *Eur J Cancer* 2018; **101:** 47-54. doi: 10.1016/j.ejca.2018.06.009

21. Juvet LK, Thune I, Elvsaas IKØ, Fors EA, Lundgren S, Bertheussen G, et al. The effect of exercise on fatigue and physical functioning in breast cancer patients during and after treatment and at 6 months follow-up: a meta-analysis. *Breast* 2017; **33:** 166-77. doi: 10.1016/j.breast.2017.04.003

22. Mustian KM, Alfano CM, Heckler C, Kleckner AS, Kleckner IR, Leach CR, et al. Comparison of pharmaceutical, psychological, and exercise treatments for cancer-related fatigue: a meta-analysis. *JAMA Oncol* 2017; **3:** 961-8. doi: 10.1001/jamaoncol. 2016.6914

23. Hilfiker R, Meichtry A, Eicher M, Nilsson Balfe L, Knols RH, Verra ML, et al. Exercise and other non-pharmaceutical interventions for cancer-related fatigue in patients during or after cancer treatment: a systematic review incorporating an indirect-comparisons meta-analysis. *Br J Sports Med* 2018; **52:** 651-8. doi: 10.1136/bjsports-2016-096422

24. Reif K, de Vries U, Petermann F, Görres S. A patient education program is effective in reducing cancer-related fatigue: a multi-centre randomised two-group waiting-list controlled intervention trial. *Eur J Oncol Nurs* 2013; **17:** 204-13. doi: 10.1016/j.ejon.2012.07.002

25. Corbett TK, Groarke A, Devane D, Carr E, Walsh JC, McGuire BE. The effectiveness of psychological interventions for fatigue in cancer survivors: systematic review of randomised controlled trials. *Syst Rev* 2019; **8:** 324. doi: 10.1186/s13643-019-1230-2

26. Setyowibowo H, Yudiana W, Hunfeld JAM, Iskandarsyah A, Passchier J, Arzomand H, et al. Psychoeducation for breast cancer: a systematic review and meta-analysis. *Breast* 2022; **62:** 36-51. doi: 10.1016/j.breast.2022.01.005

27. Bennett S, Pigott A, Beller EM, Haines T, Meredith P, Delaney C. Educational interventions for the management of cancer-related fatigue in adults. *Cochrane Database Syst Rev 2016*; **11:** CD008144. doi: 10.1002/14651858.CD008144.pub2

28. Zhang Y, Lin L, Li H, Hu Y, Tian L. Effects of acupuncture on cancer-related fatigue: a meta-analysis. *Support Care Cancer* 2018; **26:** 415-25. doi: 10.1007/s00520-017-3955-6

expert opinion

Advancing HER2-low breast cancer management: enhancing diagnosis and treatment strategies

Simona Borstnar[1], Ivana Bozovic-Spasojevic[2], Ana Cvetanovic[3], Natalija Dedic Plavetic[4], Assia Konsoulova[5], Erika Matos[1], Lazar Popovic[6], Savelina Popovska[7], Snjezana Tomic[8], Eduard Vrdoljak[8]

[1] Institute of Oncology Ljubljana, Slovenia
[2] Institute for Oncology and Radiology of Serbia, Medical Faculty, University of Belgrade, Serbia
[3] Department of Oncology, Medical Faculty University of Niš; Clinic of Oncology, University Clinical Centre Niš, Serbia
[4] University Hospital Centre Zagreb, School of Medicine, University of Zagreb, Croatia
[5] National Cancer Hospital, Sofia, Bulgaria
[6] Oncology Institute of Vojvodina, Faculty of Medicine, University Novi Sad, Novi Sad, Serbia
[7] Medical University Pleven, Bulgaria
[8] University Hospital of Split, University of Split - School of Medicine, Croatia

Radiol Oncol 2024; 58(2): 258-267.

Received 4 January 2024
Accepted 14 May 2024

Correspondence to: Simona Borštnar, M.D., Ph.D., Institute of Oncology Ljubljana, Zaloška 2, SI-1000 Ljubljana, Slovenia. E-mail: sborstnar@onko-i.si

All authors contributed equally to this paper.

Disclosure: Simona Borštnar declares support for present manuscript for medical writing and article processing charge from AstraZeneca; consulting fees from AstraZeneca, Eli Lilly, MSD, Novartis, Pfizer, Roche, and Swixx; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from AstraZeneca, Gilead, Eli Lilly, MSD, Novartis, and Pfizer. Ivana Božović-Spasojević declares support for present manuscript for medical writing and article processing charge from AstraZeneca; speaker and consulting fees from AstraZeneca, MSD, Novartis, PharmaSwiss, Pfizer, and Roche. Ana Cvetanović declares support for present manuscript for medical writing and article processing charge from AstraZeneca; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events and support from attending meetings and/or travel from AstraZeneca, Eli Lilly, Hemofarm, Merck, MSD, Novartis, Pfizer, and Roche; participation on a Data Safety Monitoring Board or Advisory Board from AstraZeneca, MSD, Novartis, Pfizer, and Roche; and leadership or fiduciary role in other board, society, committee, or advocacy group in Serbian Society of Medical Oncology (Vice President). Natalija Dedić Plavetić declares support for present manuscript for medical writing and article processing charge from AstraZeneca; grants or contracts for clinical trials from Novartis and Roche; consulting fees from AstraZeneca, Novartis, MSD, and Pfizer; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from AstraZeneca, Novartis, MSD, Pfizer, and Roche. Erika Matos declares support for present manuscript for medical writing and article processing charge from AstraZeneca; consulting fees from AstraZeneca, Eli Lilly, and MSD; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from AstraZeneca; Eli Lilly, MSD, and Novartis. Snježana Tomić declares support for present manuscript for medical writing and article processing charge from AstraZeneca; consulting fees from AstraZeneca, MSD, Novartis, and Roche Oncology; and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from AstraZeneca, MSD, Novartis, and Roche Oncology; and support for attending meetings and/or travel from AstraZeneca, MSD, and Roche Oncology. Eduard Vrdoljak declares support for present manuscript for medical writing and article processing charge from AstraZeneca; support for clinical trials and scientific projects from AstraZeneca, BMS, Pfizer, and Roche; speaker and consulting fees from Amgen, Astellas, AstraZeneca, Boehringer Ingelheim, Johnson & Johnson, MSD, Merck, Novartis, Pharmaswiss, Pfizer, Roche, and Sanofi. Assia Konsoulova, Savelina Popovska and Lazar Popović declare support for present manuscript for medical writing and article processing charge from AstraZeneca.

Background. Recent evidence brought by novel anti-human epidermal growth factor receptor 2 (HER2) antibody-drug conjugates is leading to significant changes in HER2-negative breast cancer (BC) best practices. A new targetable category termed 'HER2-low' has been identified in tumors previously classified as 'HER2-negative'. Daily practice in pathology and medical oncology is expected to align to current recommendations, but patient access to novel anticancer drugs across geographies might be impeded due to local challenges.

Materials and methods. An expert meeting involving ten regional pathology and oncology opinion leaders experienced in BC management in four Central and Eastern Europe (CEE) countries (Bulgaria, Croatia, Serbia, Slovenia) was held. Herein we summarized the current situation of HER2-low metastatic BC (mBC), local challenges, and action plans to prevent delays in patient access to testing and treatment based on expert opinion.

Results. Gaps and differences at multiple levels were identified across the four countries. These included variability in the local HER2-low epidemiology data, certification of pathology laboratories and quality control, and reimbursement conditions of testing and anticancer drugs for HER2-negative mBC. While clinical decisions were aligned to international guidelines in use, optimal access to testing and innovative treatment was restricted due to significant delays in reimbursement or limitative reimbursement conditions.

Conclusions. Preventing delays in HER2-low mBC patient access to diagnosis and novel treatments is crucial to optimize outcomes. Multidisciplinary joint efforts and pro-active discussions between clinicians and decision makers are needed to improve care of HER2-low mBC patients in CEE countries.

Key words: HER2-low; metastatic breast cancer; Balkans; testing; innovative treatment; access

Introduction

In the era of precision medicine, the diagnostic and treatment landscape in oncology has progressively become more biomarker driven.[1,2] In solid tumors, an early example of biomarkers with predictive value was the human epidermal growth factor receptor 2 (HER2), with positive results predicting response to targeted treatment with anti-HER2 monoclonal antibodies but no benefit for HER2-negative tumors.[1] In breast cancer (BC), HER2 overexpression/gene amplification determined by immunohistochemistry (IHC) and/or in situ hybridization (ISH) is found in 15–20% of all tumors.[3-5] Anti-HER2-directed therapies have significantly improved the survival of patients with both early and metastatic HER2-positive BC and consequently changed the treatment paradigm, being accepted as the standard of care throughout the world.[5,6] The current pathology guidelines define HER2-positive tumors when the IHC score is 3+ or 2+ with the HER2 encoding gene (erb-b2 receptor tyrosine kinase 2 [*ERRB2*]) amplification by ISH (ISH-positive), whereas HER2-negative tumors have IHC scores of 0+, 1+, or 2+/ISH-negative.[7]

In light of recent evidence brought by novel anti-HER2 antibody-drug conjugates (ADCs)[5,8–12], the current knowledge of HER2 expression range and its clinical applicability is changing since a significant proportion of HER2-negative tumors are in fact characterized by a spectrum of HER2 expression levels.[13,14] A new targetable category has been identified in patients whose tumors are scored IHC 1+ or 2+/ISH-negative[3], and this low level of HER2 expression has been termed 'HER2-low'.[15] HER2-low status is detected in 45–55% of all BC tumors: around two-thirds (65%) in hormone receptor-positive (HR+) BC and one-third (36%) in HR-negative (HR-) cancers.[6,16] Treatment paradigms for both HR+ and HR- BC with HER2-low expression are evolving at a fast pace, leading to a new 'revolution'.[17] The clinical trial DESTINY-Breast04 (DB-04), evaluating trastuzumab deruxtecan (T-DXd) in patients with HER2-low advanced BC previously treated with chemotherapy, showed significant and clinically meaningful progression-free survival (PFS) and overall survival (OS) improvements and a manageable safety profile as compared with conventional chemotherapy (PFS 10.1 months for T-DXd vs 5.4 months in the physician's choice of chemotherapy, hazard ratio=0.64, P=0.003, and OS 23.9 months for T-DXd vs 16.8 months in the physician's choice of chemotherapy, hazard ratio=0.64, P=0.001, respectively).[12] These results, together with the other ADC data, demonstrate the clinical relevance of HER2-low expression and are transforming the current understanding, and therefore management, of HER2-negative BC.[5,8-12,17-20]

Despite guideline recommendations for HER2-low diagnosis and European Society for Medical Oncology (ESMO) consensus reached for its treatment[5,7], implementing guidelines in a real-life setting is a lengthy and difficult process, partly due to the diverse accessibility of novel anticancer medicines. In countries in Central and Eastern Europe (CEE), significant delays in patient access to innovative oncology treatments have been previously described.[21-24] In an attempt to avoid such delays with ADCs in HER2-low BC, an expert meeting was held to identify the challenges and local unmet needs, and to find solutions to optimize access to diagnostics and adequate treatment of HER2-low metastatic BC (mBC) for patients from CEE. In this paper, we discuss the current situation pertaining to the overall diagnosis and management of HER2-low mBC and propose potential solutions to address the unmet needs in four CEE countries; we also consider similar situations, and solutions that may apply to many other former or current transitional countries throughout the world.

TABLE 1. Overview of cancer epidemiology across four CEE countries in 2020 (data extracted from GLOBOCAN 2020[25] and the European Cancer Information System[34])

Characteristics	Bulgaria	Croatia	Serbia	Slovenia
Total population	6 948 445	4 105 268	8 737 370	2 078 932
Number of new cancer cases (all cancer sites)	36 451	26 092	49 043	14 180
Incidence age-standardized rate per 100 000	100	120.3	145.3	121.2
Number of new BC cases in 2020, both sexes, all ages	4061	2894	6724[a]	1410
BC new cases – rank across all types of cancers	3	2	2	3
5-year prevalence, all ages (per 100 000)	425.45	523.4	549.32	560.03
Mortality age-standardized rate per 100 000	36.3	32.8	50.9	32.3
Number of BC deaths	1533	832	2342	405
BC deaths – rank across all types of cancers	3	3	2	5
Mortality-to-incidence ratio[b]	0.36	0.27	0.35	0.27

Methods

A panel of ten opinion leaders was organized as part of a virtual meeting logistically supported by AstraZeneca and held on June 12, 2023. Eight medical oncologists and two pathologists from academic centers and/or national institutes of oncology in Bulgaria, Croatia, Serbia, and Slovenia with experience in the diagnosis, management, and follow-up of mBC patients from the CEE region were individually approached and further agreed to participate in the panel discussion. A pre-meeting survey was developed specifically for this project and reviewed by experts. The experts responded in anonymized manner to the preliminary survey, which included 31 questions grouped in the following four topics: epidemiology, biology, pathologic diagnosis, and treatment of HR (+/-) HER2-low mBC. The average time to fill the survey was around 15 minutes. Data from the survey were retrieved in an excel sheet; all experts responded to the survey, with the difference that the specific treatment questions did not apply to the pathology experts. No formal statistical analysis was used. The responses grouped under the main topics were further discussed in detail during the meeting, while experts agreed that the structure of the manuscript will follow these topics. For each of these, the thought leaders discussed the institutional or national data versus literature, described the unmet needs across countries, and shared their independent views and experience. Relevant data discussed in the medical community with regard to the spectrum of the HER2-low in breast cancer were considered to firstly describe the general context and then, to a greater extent, elaborate on the local circumstances (no formal literature review). Experts identified local and/or regional challenges and constraints of clinical oncology and pathology daily practice and proposed action plans aimed at improving testing and access to treatment and, consequently, outcomes of HER2-low mBC for patients at the country and CEE level.

Results and discussion

Epidemiology of HER2-low breast cancer

Current status and challenges

The four CEE countries represented in this paper differ in terms of total population; however, in all four of these countries, BC ranks second or third in prevalence among all types of cancers and is one of the leading causes of death in women (Table 1).[25] BC incidence rates remain high in the region and are predicted to increase in the future due to the global trend of an increasingly aging population.[26,27] Early detection (eg screening programs) of BC is problematic in countries that have undergone economic transitions like those in CEE; many still lack clear policies and sustained investments in their medical healthcare systems.[28] Even so, the mortality-to-incidence ratio (MIR), which is an indicator of healthcare quality, with low values indicating better care (prevention, treatment, and overall management), varies slightly and is similar to the average European value (0.27) in Slovenia and Croatia.[29] As compared with data from 2012[28], we observe decreases in the MIR in all four countries, which might show that advances in cancer

care have been made to some extent in the last two decades. Despite these encouraging signs, recent data show trends of increase in BC mortality in Bulgaria and Croatia in women over 45 years.[30] In most Eastern European countries, patients with BC have a shorter OS following diagnosis compared with the rest of Europe[31]; however, in Slovenia, survival has been shown to be increasing over time.[32] Multiple challenges and gaps in receiving optimal cancer care by individuals with mBC have been described, especially in underserved patient populations from the CEE region where socioeconomic inequalities and educational or cultural status have a considerable impact on the quality of healthcare.[33]

Compared with reported rates for HER2-low cases, which range between 45% and 65% in HR+ tumors and 23% to 40% in HR- tumors[6,16,35], local reports indicate a similar or slightly lower percentage of HER2-low cases. In a sample of 11 234 cases from the Oncology Institute of Ljubljana (Slovenia), collected from 2011 to 2021, HER2-low (1+/2+ non-amplified) was identified in 52.8% of cases. The rate of HER2 IHC 0 decreased in the last 2 years of follow-up (2020, 2021), whereas the rate of HER2-low increased.[36,37] In Croatia, according to the National Pathohistological Breast Registry of newly diagnosed BC patients, in a sample of 8488 patients (early-stage, locally advanced, or metastatic BC), the HER2-low rate in the past 3 years was 42% (44% HER2-low in luminal A cancers, 54% in luminal B, and 36% in triple-negative BC) (unpublished data). In Serbia, in a sample of 500 patients from the Novi Sad registry, HER2-low status was identified in 50% of cases, irrespective of stage, whereas in mBC patients with testing performed only in primary tumors, the rate of HER2-low was 30% (unpublished data). For Bulgaria, no official data are available.

Unmet needs

Robust, more standardized data on incidence, prevalence, and mortality rates by type and stage of BC, and outcomes in specific groups that are usually underserved (i.e., men, patients with comorbidities, patients of cultural/racial/religious diversity) are scarce in the region and, consequently, very much needed. The difference between data from Western countries and those in the CEE region may be partly explained by lack of properly founded national cancer registries and clinical databases collecting systemized oncology data and, of course, variations of the healthcare systems in CEE. Progress has been made recently (for example, in January 2023 Slovenia opened the Clinical Breast Cancer Registry), and more changes are expected in the future.

Action plan

We outlined the following top priorities:

(1) to extract retrospective data from healthcare records in a centralized way in each country and use them as a benchmark for future studies;
(2) to expand existing registries/protocols to include all HER2-low BC patients.

These actions would more sufficiently explore the variability across countries and adequately inform diagnosis and management strategies for improving patient care in CEE countries based on recent and reliable real-world evidence. Most importantly, this would aid communication with health authorities to expedite access to effective anticancer drugs for patients in this region. Continuous monitoring and reporting of management of patients with BC on a national and potentially regional or, even better, European level, is necessary to inform healthcare policies and reforms. Exposing the weaknesses of general healthcare and/or oncology systems will help to improve outcomes by addressing similar issues.

Biology of HER2-low breast cancer

Current status and challenges

Whether HER2-low is a distinct biological entity or not is one of the key questions in the field of HER2-low biology.[16,38] While the spectrum of HER2 positivity expands, no robust evidence exists to consider HER2-low a clinically distinctive entity or a definite subtype[14,39,40], which has led some groups to conclude that such categorization remains to be clarified in the future.[17,20,41] At a local level, a recent report from Serbia including patients with early BC has shown a higher proportion of pathologic complete response after neoadjuvant chemotherapy in patients with HER2 IHC 0 as compared with HER2-low, indicating that new and improved treatment modalities are required for HER2-low patients.[42]

Tumor heterogeneity and tumor plasticity that traditionally characterize breast carcinomas also apply to HER2-expressing tumors.[5,43] HER2 intratumoral (spatial) heterogeneity is a well-known phenomenon reported in up to 40% of BC.[44,45] HER2-low expression was shown to be highly unstable during disease evolution, with a higher proportion of HER2-low rates in recurrent BC samples (temporal heterogeneity).[38,46,47]

Other matters of debate in the literature discussed were whether the efficacy of HER2-targeted treatments is higher in tumors with higher HER2 expression levels[48,49], and how HER2-low could be an escape mechanism displayed by tumors in case of HR+-directed treatments, leading researchers to believe that most cases of mBC will become HER2-low under the pressure of endocrine therapies.[50-52]

Unmet needs

Re-biopsy availability is related to our understanding of HER2-low biology. Yet, there is no specific strategy for re-biopsy at recurrence at the country or regional level, and computed tomography (CT)-guided biopsies are difficult to access and generally rarely performed. While financing for re-biopsies may not be an issue, Serbia, for example, is hindered by a scarcity of experts who can perform biopsies, such as interventional radiologists or pulmonologists.

Action plan

To optimize the management and subsequently the outcomes of HER2-low BC patients in future and to address spatial and temporal tumor heterogeneity, we proposed to:

(1) Foster HER2-low early diagnosis by developing and implementing local pathways for mBC patients, with mandatory checks of previous pathology reports;
(2) perform multiple rounds of re-biopsy at each relapse of locoregional or distant metastases;
(3) keep clinicians informed of new treatment options available in their countries, based on a possible different result of the re-biopsy compared with the primary tumor biopsy.

Pathologic diagnosis of HER2-low breast cancer

Current status and challenges

Historically, HER2 expression was classified in a binary way: positive or negative.[44,53] New evidence indicates that patients with low HER2 expression (IHC 1+ or 2+ and ISH-negative) represent a new targetable category of BC.[3] In light of these changes, HER2 testing and reporting has become more complex.[54]

The 2023 updated guidelines issued by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) for HER2 testing include no changes in prior (2018) terminology or traditional terminology of positive/equivocal/negative for HER2 IHC results but calls to increased awareness for IHC 1+ or 2+ non-amplified cases that deem patients eligible for treatment with T-DXd.[7,53] While pathology groups state that HER2-low is a qualitative term[55], medical oncologists use conflicting terminology for interpreting ASCO/CAP guidelines (i.e., HER2-0 with potential future categories HER2-null and HER2-ultralow, HER2-low, HER2-positive).[5] Pathology experts agreed that HER2-low is rather an operational term, with ASCO/CAP guidelines being currently followed in pathology clinical practice. No HER2-low term is currently included in reports; however, the term HER2-negative is recommended to be changed in "HER2-negative for protein overexpression/gene amplification" since non-overexpressed levels of the HER2 protein may be present in these cases.

Another challenge is related to the companion diagnostic tests for evaluating 1+ and 2+/ISH-negative disease.[3] Assays used are either those approved and currently available on the market (with Ventana HER2/neu 4B5 [F. Hoffmann-La Roche Ltd] being more frequently used in the CEE area) or ones developed in-house. Besides temporal and spatial tumor heterogeneity, many other factors are known to impact the IHC scoring – from pre- and post-analytical factors to test sensitivity, type of specimen, and laboratory and/or reader experience.[3,6,56-58]

Among the specific pathology challenges mentioned at local level, the following were underlined: in Bulgaria, lack of reimbursement for ISH (ISH tests are paid for by patients), lack of continuous medical education for pathologists to train on the changing paradigm of HER2 assessment, reporting and its relevance to treatment, lack of certification process of either pathology laboratories or clinical centers, and no quality control processes in place. In Serbia, previous discordance in IHC detection of HER2 between national pathology laboratories was reported (the overall agreement ranged between 79% and 89%), with discrepancies on chromogenic ISH indicating a misdiagnosis rate of almost 16%.[59] In Croatia, in a sample of 126 patients, discordance in HER2 scoring between central and local laboratories was 12% – results that are in line with the literature.[60,61] The sources of error in the local study were partly pre-analytical and partly analytical, thus emphasizing the need for rigorous application of standardized staining and scoring procedures for precise determination of HER2 protein level, which is particularly important in the HER2-low group. The experts from Bulgaria added that a high variability of HER2 testing results between pathology centers

TABLE 2. Status of reimbursement for anticancer drugs used for treatment of HER2-negative mBC in Bulgaria, Croatia, Serbia, and Slovenia, including the year of reimbursement of at least one representative of the class

Treatment	Bulgaria[a]	Croatia	Serbia[c]	Slovenia[d]
CDK4/6 inhibitors	2018	2018	2022	2018
Alpelisib	2023	2021	2023	2021
PARP inhibitors	2023	2022	2021	2021
Sacituzumab govitecan			2023	2022
Trastuzumab deruxtecan			2022	2023
Atezolizumab nab-paclitaxel		2022	2021	2020
Pembrolizumab	2023[b]		2022	2023
Everolimus	By >10 years		2021	2010
Fulvestrant	By >10 years	By >10 years	2019	2004
Aromatase inhibitors	By >10 years	By >10 years	2008	By >20 years

Green = reimbursed; orange = not reimbursed, but available through early access programs or out-of-pocket expenses; red = not reimbursed; CDK4/6 = cyclin-dependent kinase 4 and 6; HER2 = human epidermal growth factor receptor 2; mBC = metastatic breast cancer; PARP = poly(adenosine diphosphate ribose) polymerase;

[a]In Bulgaria, PARP inhibitors are available in early breast cancer and *BRCA*-positive tumors after lack of complete response in the neoadjuvant setting. In the metastatic setting, PARP inhibitors have been reimbursed since 2019, everolimus is reimbursed in metastatic estrogen receptor-positive (ER+) BC, and aromatase inhibitors are reimbursed in ER+ BC; [b] In Bulgaria, pembrolizumab is reimbursed only in triple-negative BC within HER2-negative BC; [c] In Serbia, medications in orange are registered and could be used in special circumstances but are not reimbursed/covered by public health insurance for HER2-negative mBC; [d] In Slovenia, sacituzumab govitecan is reimbursed for triple-negative BC only, and trastuzumab deruxtecan for HER2-positive BC only.

also applies in their country, leading to high number of retesting and second opinions.

Unmet needs

In terms of pathology diagnosis, the unmet needs identified in the four countries from the CEE region are broad and at multiple levels: specific medical education for pathologists, reimbursement of ISH testing in all countries, improved robustness of HER2 testing with current available techniques, standardization of and quality-controlled HER2 testing between centers, precise and accurate reporting systems, more homogenous inter-institutional procedures, and more certified laboratories.

Action plan

The action plan discussed included the following proposals:

(1) to improve pre-analytical and analytical phases of HER2 testing and to reduce false-negative/false-positive reports, a rigorous internal and external quality control is required at every institutional level;
(2) to increase awareness of HER2-low testing and scoring and to improve reporting, virtual meetings, and live workshops for pathology specialists, as well as multidisciplinary meetings of all specialists involved in the management of HER2-low BC patients, should be formally organized in each country;
(3) to improve HER2-low score accuracy and reduce inter-laboratory variability, participation in ring studies is highly encouraged;
(4) to increase comparability across various geographies and build best practices, center-, country-, and regional-level monitoring and reporting of pathology results is recommended.

Treatment of HER2-low breast cancer

Current status and challenges

CEE countries are characterized by a variable reimbursement status of anticancer drugs for HER2-negative mBC (Table 2). In Slovenia the majority of treatments are reimbursed, irrespective of line of treatment; however, T-DXd is not yet reimbursed for HER2-low BC. In Croatia, despite innovative treatments being reimbursed, their use in later line (third line [3L]+) depends on budgetary decisions at the institutional level, potentially introducing disparity in the treatment of mBC patients in need. By contrast, in Bulgaria, reimbursement is granted in general in any line for all drugs approved at the European level for HER2-positive mBC, which facilitates treatment sequencing; however, this does not apply for HER2-low mBC. Serbia faces the biggest challenges in the region, with innovative drugs being available for first-line (1L) and second-line (2L)/3L HER2-positive mBC, while treatment options for metastatic triple-negative and HR+ BC are

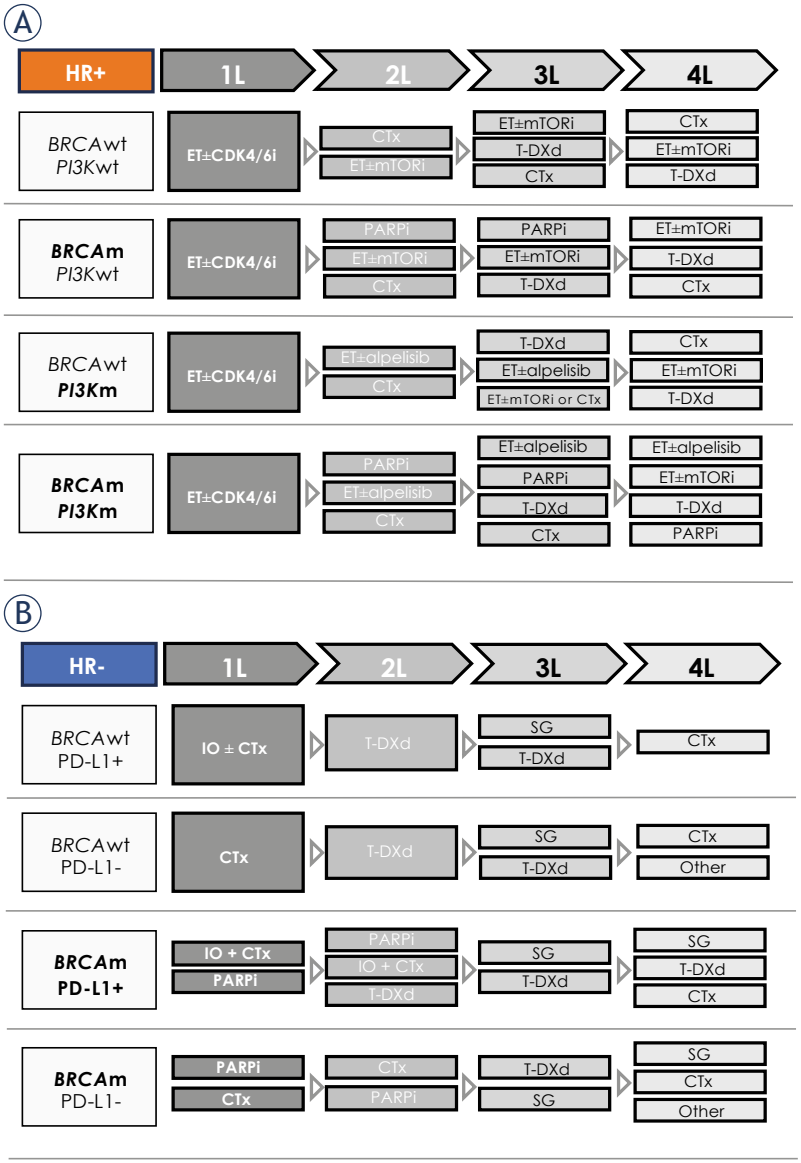


FIGURE 1. Algorithms for HER2-low mBC in light of evolving treatment paradigms, according to the HR status and other actionable targets: **(A)** HR+ and **(B)** HR-. The ideal scenario considers availability of all treatments in all lines and unrestricted treatment access. 1L/2L/3L/4L = first/second/third/fourth line; BRCAm = BReast CAncer gene mutations; BRCAwt = BReast CAncer gene wild type; CDK4/6i = cyclin-dependent kinase 4/6 inhibitor; CTx = chemotherapy; ET = endocrine therapy; HER2 = human epidermal growth factor receptor 2; HR = hormone receptor; IO = immunotherapy; mBC = metastatic breast cancer; mTORi = mammalian target of rapamycin inhibitor; PARPi = poly(adenosine diphosphate ribose) polymerase inhibitor; PD-L1 = programmed death-ligand 1; PI3Km = phosphatidylinositol 3-kinases mutations; PIK3wt = phosphatidylinositol 3-kinases wild type; SG = sacituzumab govitecan; T-DXd = trastuzumab deruxtecan. Treatment in 2L, 3L, 4L, and further lines is based on: previous therapy received; duration of response to previous treatment; patient's preferences, condition, and comorbidities; toxicities of previous therapies; presumed benefit of further lines of therapy; and treatment availability. Per current approved label, trastuzumab deruxtecan as monotherapy is indicated for the treatment of adult patients with unresectable or metastatic HER2-low breast cancer who have received prior chemotherapy in the metastatic setting or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy. Per current approved label, sacituzumab govitecan as monotherapy is indicated for the treatment of adult patients with unresectable or metastatic triple-negative BC who have received two or more prior systemic therapies, including at least one of them for advanced disease.

being limited. For example, only cyclin-dependant kinase 4 and 6 (CDK4/6) inhibitors as innovative medicines are being reimbursed in 1L and 2L for HR+ mBC. By contrast, alpelisib, poly(adenosine diphosphate ribose) polymerase (PARP) inhibitors, and the ADCs sacituzumab govitecan and T-DXd can be approved in some specific circumstances, but only in later lines when other therapy options are exhausted. In consequence, the meaningful clinical applicability of the drug is significantly decreased, because rates of treatment success in later lines are rather small.

In all four CEE countries, the ESMO guidelines and, in some countries, local guidelines with applicable updates are followed.[62-64] Whereas treatment decisions in 1L are aligned across countries, experts agreed that decisions in 2L/3L are individualized based on patient and tumor characteristics and treatment outcomes, although these decisions are highly dependent on reimbursement conditions. While innovative treatments are usually approved in Europe through a centralized procedure via the European Medicines Agency[65], the high costs of new anticancer drugs restrict their use until reimbursement. Previous reports from CEE have shown significant delays from marketing authorization to reimbursement of novel oncology medicines and reduced numbers of available drugs in this region, which undoubtedly leads to worsening patient outcomes.[21,24,66] For example, perhaps due to diverse reimbursement models and policies and lack of sustained investment in the oncology field, trastuzumab, one of the essential medicines for treatment of HER2-positive BC, did not receive full reimbursement in Eastern Europe and, with the exception of Slovenia and Croatia, was insufficiently procured to allow treatment access to all patients in need for several years.[22,23,28]

We have identified the following challenges applicable, to various extents, in all participating countries: significant delays in reimbursement decisions; limitative, restrictive reimbursement conditions that impact sequencing and/or treatment rechallenges; limited access to clinical trials with novel cancer medicines; and early access/bridging programs until treatment reimbursement. In addition, optimal sequencing remains to be determined, as levels of evidence are variable for different treatments.

Unmet needs

Unmet needs of equal importance for adequate access to treatment of HER2-low mBC were availability and/or full reimbursement of treatments

in all lines, extension of reimbursement criteria to all lines of treatment for medicines with marketing authorization granted, and reduced times from product marketing authorization to reimbursement for novel, innovative anticancer drugs. Avoiding repeating the situation of trastuzumab reimbursement and ensuring active involvement of all stakeholders in cancer care are prerequisites for preventing disparities in treatment of HER2-low BC patients between CEE countries.

Action plan

Key actions for optimizing access to HER2-low BC treatments are summarized below:

(1) While changing the reimbursement models at country level is beyond the scope of this initiative, the experts propose a treatment algorithm for HER2-low mBC aligned to the current evidence, guidelines, and clinical practice, according to HR status and other actionable targets, provided there is no limited access to treatments, including availability in all lines, and patients are not in visceral crisis (Figure 1).

(2) To prevent delays or lack of any patient access to treatments proven to prolong survival, a clear process mapping of the HER2-low mBC patient journey is strongly advised. Permanent communication with local decision authorities at every level and on multiple channels should be initiated by the medical community and supported by up-to-date and sound evidence of treatment benefits. For example, lobbying for alignment to ESMO-Magnitude of Clinical Benefit Scale (MCBS) for fast approval and reimbursement in the CEE area for drugs with scores of 4 and 5 would provide authorities with additional documentation and a reproducible methodology to assess the magnitude of the benefits ensured by novel anticancer drugs.[67] In addition, involving patient organizations to advocate change policies to improve access to medicines and cancer outcomes is needed. Although the actual role played by patient representatives across each country is less known and expected to vary, their inclusion into the open dialogue with the authorities should be encouraged and supported by clinicians.

Conclusions

This paper presents the opinions of oncology and pathology experts from four CEE countries on the optimal management of HER2-low mBC. Existing barriers to rapid diagnosis were identified, and treatment choices were proposed for real-world settings. Gaps and differences in the local epidemiology data on HER-2 low BC, certification of pathology laboratories and quality control, and availability of anticancer drugs for HER2-negative mBC across the CEE countries were identified. Preventing delays in HER2-low mBC patient access to diagnosis and timely and as-per guidelines therapies is crucial to improve outcomes.

Pathology reports should no longer report binary results as HER2-positive or -negative but include (ideally) the category of HER2-low and detail the positive score through the number of "+" because this is now becoming critical for treatment decisions. Clinicians should have pro-active discussions with policymakers and stakeholders, including patients and their representatives, in order to enable advances in HER2-low mBC diagnosis and treatment to truly optimize patient outcomes in the CEE region.

Acknowledgments

This initiative received no external funding, but medical writing support and the article processing charge were funded by AstraZeneca.

The authors would like to thank Ivana Magdić Blažević and Tina Jerič from AstraZeneca for project management and providing support in organizing the Expert Meeting. Medical writing support in drafting the manuscript and revising to address author feedback was provided by Ana Maria Iordan, MD, MSc of MedInteractiv (Bucharest, Romania) and was funded by AstraZeneca in accordance with Good Publication Practice (GPP) guidelines (https://www.ismpp.org/gpp-2022).

References

1. Schwartzberg L, Kim ES, Liu D, Schrag D. Precision Oncology: who, how, what, when, and when not? *Am Soc Clin Oncol Educ Book* 2017; **37:** 160-9. doi: 10.1200/EDBK_174176
2. Schettini F, Prat A. Dissecting the biological heterogeneity of HER2-positive breast cancer. *Breast* 2021; **59:** 339-50. doi: 10.1016/j.breast.2021.07.019
3. Yang C, Brezden-Masley C, Joy AA, Sehdev S, Modi S, Simmon C, et al. Targeting HER2-low in metastatic breast cancer: an evolving treatment paradigm. *Ther Adv Med Oncol* 2023; **15:** 1-19. doi: 10.1177/17588359231175440
4. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Saurer AG, et al. Breast cancer statistics, 2019. *CA Cancer J Clin* 2019; **69:** 438-51. doi: 10.3322/caac.21583
5. Tarantino P, Viale G, Press MF, Hu X, Penault-Llorca F, Bardiat A, et al. ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer *Ann Oncol* 2023; **34:** 645-59. doi: 10.1016/j.annonc.2023.05.008

6. Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, et al. HER2-Low breast cancer: pathological and clinical landscape. *J Clin Oncol* 2020; **38:** 1951-62. doi: 10.1200/JCO.19.02488

7. Wolff AC, Somerfield MR, Dowsett M, Hammond MEH, Hayes DF, McShane LM, et al. Human epidermal growth factor receptor 2 testing in breast cancer: ASCO–College of American Pathologists Guideline Update. *J Clin Oncol* 2023; **41:** 3867-72. doi: 10.1200/jco.22.02864

8. Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V, et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol* 2019; **20:** 1124-35. doi: 10.1016/S1470-2045(19)30328-6

9. Wang J, Liu Y, Zhang Q, Feng J, Chen X, Han Y, et al. RC48-ADC, a HER2-targeting antibody-drug conjugate, in patients with HER2-positive and HER2-low expressing advanced or metastatic breast cancer: a pooled analysis of two studies. [abstract]. *J Clin Oncol* 2021; **39(15 Suppl):** 1022. doi: 10.1200/JCO.2021.39.15_suppl.1022

10. Xu B, Wang J, Fang J, Chen X, Han Y, Li Q, et al. Abstract PD4-06: early clinical development of RC48-ADC in patients with HER2 positive metastatic breast cancer. [abstract]. San Antonio Breast Cancer Symposium; December 10-14, 2019; San Antonio, Texas, 2019 Dec 10-14. *Cancer Res* 2020; **80(4 Suppl):** PD4-06. doi: 10.1158/1538-7445.SABCS19-PD4-06

11. Corti C, Giugliano F, Nicolò E, Ascione L, Curigliano G. Antibody-drug conjugates for the treatment of breast cancer. *Cancers* 2021; **13:** 1-23. doi: 10.3390/cancers13122898

12. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-Low advanced breast cancer. *N Engl J Med* 2022; **387:** 9-20. doi: 10.1056/nejmoa2203690

13. Holthuis EI, Vondeling GT, Kuiper JG, Dezentje V, Rosenlund M, Overbeek JA, et al. Real-world data of HER2-low metastatic breast cancer: a population based cohort study. *Breast* 2022; **66:** 278-84. doi: 10.1016/j.breast.2022.11.003

14. Venetis K, Crimini E, Sajjadi E, Corti C, Guerino-Rocco E, Viale G, et al. HER2 Low, ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. *Front Mol Biosci* 2022; **9:** 1-12. doi: 10.3389/fmolb.2022.834651

15. Schlam I, Tolaney SM, Tarantino P. How I treat HER2-low advanced breast cancer. *Breast* 2023; **67:** 116-23. doi: 10.1016/j.breast.2023.01.005

16. Schettini F, Chic N, Brasó-Maristany F, Pare L, Pascual T, Conte B, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *NPJ Breast Cancer* 2021; **7:** 1. doi: 10.1038/s41523-020-00208-2

17. Nicolò E, Boscolo Bielo L, Curigliano G, Tarantino P. The HER2-low revolution in breast oncology: steps forward and emerging challenges. *Ther Adv Med Oncol* 2023; **15:** 1-16. doi: 10.1177/17588359231152842

18. Rassy E, Rached L, Pistilli B. Antibody drug conjugates targeting HER2: clinical development in metastatic breast cancer. *Breast* 2022; **66:** 217-26. doi: 10.1016/j.breast.2022.10.016

19. Ferraro E, Drago JZ, Modi S. Implementing antibody-drug conjugates (ADCs) in HER2-positive breast cancer: state of the art and future directions. *Breast Cancer Res* 2021; **23:** 84. doi: 10.1186/s13058-021-01459-y

20. Popović M, Silovski T, Križić M, Dedić Plavetić N. HER2 Low breast cancer: a new subtype or a trojan for cytotoxic drug delivery? *Int J Mol Sci* 2023; **24:** 8206. doi: 10.3390/ijms24098206

21. Newton M, Scott K, Troein P. EFPIA Patients W.A.I.T. indicator 2021 survey. [internet]. IQVIA; 2022. [cited 2023 Dec 15]. Available at: https://www.efpia.eu/media/676539/efpia-patient-wait-indicator_update-july-2022_final.pdf

22. Ades F, Senterre C, Zardavas D, De Azambuja E, Popescu R, Piccart M. Are life-saving anticancer drugs reaching all patients? Patterns and discrepancies of trastuzumab use in the European Union and the USA. *PLoS One* 2017; **12:** 1-11. doi: 10.1371/journal.pone.0172351

23. Trapani D, Curigliano G, Eniu A. Breast cancer: reimbursement policies and adoption of new therapeutic agents by national health systems. *Breast Care* 2019; **14:** 373-81. doi: 10.1159/000502637

24. Hofmarcher T, Szilagyiova P, Gustafsson A, Dolezal T, Rutkowski P, Baxter C, et al. Access to novel cancer medicines in four countries in Central and Eastern Europe in relation to clinical benefit. *ESMO Open* 2023; **8:** 101593. doi: 10.1016/j.esmoop.2023.101593

25. International Agency for Research on Cancer, World Health Organization. Breast cancer. Population Fact Sheets. [internet]. 2021. [cited 2023 Dec 16] Available at: https://gco.iarc.fr/today/fact-sheets-populations

26. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast* 2022; **66:** 15-23. doi: 10.1016/j.breast.2022.08.010

27. Vrdoljak E, Bodoky G, Jassem J, Popescu RA, Mardiak J, Pirker P, et al. Cancer control in central and eastern europe: current situation and recommendations for improvement. *Oncologist* 2016; **21:** 1183-90. doi: 10.1634/theoncologist.2016-0137

28. Vrdoljak E, Bodoky G, Jassem J, Popescu R, Pirker R, Čufer T, et al. Expenditures on oncology drugs and cancer mortality-to-incidence ratio in central and Eastern Europe. *Oncologist* 2019; **24:** e30-7. doi: 10.1634/theoncologist.2018-0093

29. Azadnajafabad S, Saeedi Moghaddam S, Mohammadi E, Delazar S, Rashedi S, Baradaran HR, et al. Patterns of better breast cancer care in countries with higher human development index and healthcare expenditure: insights from GLOBOCAN 2020. *Front Public Heal* 2023; **11:** 1137286. doi: 10.3389/fpubh.2023.1137286

30. Koczkodaj P, Sulkowska U, Gotlib J, Mańczuk M. Breast cancer mortality trends in Europe among women in perimenopausal and postmenopausal age (45+). *Arch Med Sci* 2020; **16:** 146-56. doi: 10.5114/aoms.2019.85198

31. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšič, et al. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet* 2018; **391:** 1023-75. doi: 10.1016/S0140-6736(17)33326-3

32. Zadnik V, Zagar T, Lokar K, Tomsic S, Konjevic AD, Zakotnik B. Trends in population-based cancer survival in Slovenia. *Radiol Oncol* 2021; **55:** 42-9. doi: 10.2478/raon-2021-0003

33. Vrdoljak E, Gligorov J, Wierinck L, Conte PF, Dr Greve J, Meunier F, et al. Addressing disparities and challenges in underserved patient populations with metastatic breast cancer in Europe. *Breast* 2021; **55:** 79-90. doi: 10.1016/j.breast.2020.12.005

34. ECIS - European cancer information system. Cancer Factsheets in EU-27 countries - 2020. Estimates of cancer incidence and mortality in 2020. Joint research centre. 2020. Available at: https://ecis.jrc.ec.europa.eu/

35. Gampenrieder SP, Rinnerthaler G, Tinchon C, Petzer A, Balic M, Heibl S, et al. Landscape of HER2-low metastatic breast cancer (MBC): results from the Austrian AGMT_MBC-Registry. *Breast Cancer Res* 2021; **23:** 1-9. doi: 10.1186/s13058-021-01492-x

36. Drev P, Blatnik O, Blazina J, Contreras J, Gašljević G, et al. [Determinations on 15,184 consecutive samples from the Oncology Institute in the period 2006 to 2021]. [Slovenian]. [internet]. 2021. p. 169-70. Available at: https://dirros.openscience.si/Dokument.php?id=21665&lang=slv

37. Auprih M, Gazic B, Drev P, Borstnar S. The frequency of HER2-low breast cancer among patients diagnosed at the Institute of Oncology Ljubljana from 2011 to 2021. Fourth Regional Congress of Medical Oncology - REKONIO 2023. Ljubljana, 2023 Sep 7-9. In: *Regional Congress of Medical Oncology REKONIO*; 2023. p. 63-4. Ljubljana: Slovenian Medical Association-Section for Medical Oncology, Oncology Institute of Ljubljana, REKOG. Available at: https://rekogconference.com

38. Bergeron A, Bertaut A, Beltjens F, Charon-Barra C, Amet A, Jankowski C, et al. Anticipating changes in the HER2 status of breast tumours with disease progression – towards better treatment decisions in the new era of HER2-low breast cancers. *Br J Cancer* 2023; **129:** 1-13. doi: 10.1038/s41416-023-02287-x

39. Dieci MV, Miglietta F. HER2: a never ending story. *Lancet Oncol* 2021; **22:** 1051-52. doi: 10.1016/S1470-2045(21)00349-1

40. Polidorio N, Veeravalli SS, Montagna G, Le T, Morrow M. Do HER2 low tumors have a distinct clinicopathologic phenotype? [abstract]. *J Clin Oncol* 2023; **41(16 Suppl):** 570. doi: 10.1200/jco.2023.41.16_suppl.570

41. Rugo HS, Wolf DM, Yau C, Petricoin E, Pohlmann PR, Pusztai L, et al. Correlation of HER2 low status in I-SPY2 with molecular subtype, response, and survival. [abstract]. *J Clin Oncol* 2023; **41(16 Suppl):** 514. doi: 10.1200/jco.2023.41.16_suppl.514

42. Djurmez O, Calamac M, Stanic N, Dimitrijevic M, Vukosavljevic J, Serovic K, et al,.Pathological complete response after neoadjuvantchemotherapy in patients with HER2 low and HER2 0 early breast cancer (eBC) experience from Insititute for Oncology and Radiology of Serbia (IORS). [abstract]. 18th St.Gallen International Breast Cancer Conference. 2023 Mar 15-18, Vienna, Austria. *Breast* 2023; **68(Suppl 1):** S64. Available at: https://breast-ibcc-2023.elsevierdigitaledition.com/63/#zoom=true

43. Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. *Br J Cancer* 2021; **125:** 164-75. doi: 10.1038/s41416-021-01328-7

44. Marchiò C, Annaratone L, Marques A, Casorzo L, Berrino E, Sapino A. Evolving concepts in HER2 evaluation in breast cancer: heterogeneity, HER2-low carcinomas and beyond. *Semin Cancer Biol* 2021; **72:** 123-35. doi: 10.1016/j.semcancer.2020.02.016

45. Hou Y, Nitta H, Li Z. HER2 intratumoral heterogeneity in breast cancer, an evolving concept. *Cancers* 2023; **15:** 1-12. doi: 10.3390/cancers15102664

46. Bar Y, Dedeoglu AS, Fell GG, Moffett NJ, Bovraz B, Ly A, et al. Dynamic HER2-low status among patients with triple negative breast cancer (TNBC): the impact of repeat biopsies. [abstract]. *J Clin Oncol* 2023; **41(16 Suppl):** 1005. doi: 10.1200/JCO.2023.41.16_suppl.1005

47. Miglietta F, Griguolo G, Bottosso M, Giarratano T, Mele ML, Fassan M, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer* 2021; **7:** 137. doi: 10.1038/s41523-021-00343-4

48. Mosele MF, Lusque A, Dieras V, Ducoulombier A, Pistilli B, Bachelot T, et al. LBA1 Unraveling the mechanism of action and resistance to trastuzumab deruxtecan (T-DXd): biomarker analyses from patients from DAISY trial. [abstract]. *Ann Oncol* 2022; **33(Suppl 3):** S123. doi: 10.1016/j.annonc.2022.03.277

49. Diéras V, Deluche E, Lusque A, Pistilli B, Bachelot T, Pierga JY, et al. Trastuzumab deruxtecan (T-DXd) for advanced breast cancer patients (ABC), regardless HER2 status: a phase II study with biomarkers analysis (DAISY). [abstract]. SABCS 2021 San Antonio Breast Cancer Symposium, 2022 Feb 15. *Cancer Res* 2022; **82(4 Suppl):** PD8-02. doi: 10.1158/1538-7445.SABCS21-PD8-02

50. Mazumder A, Shiao S, Haricharan S. HER2 activation and endocrine treatment resistance in HER2-negative breast cancer. *Endocrinol* 2021; **162:** 1-18. doi: 10.1210/endocr/bqab153

51. Pegram M, Jackisch C, Johnston SRD. Estrogen/HER2 receptor crosstalk in breast cancer: combination therapies to improve outcomes for patients with hormone receptor-positive/HER2-positive breast cancer. *NPJ Breast Cancer* 2023; **9:** 45. doi: 10.1038/s41523-023-00533-2

52. Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. *Nat Rev Drug Discov* 2023; **22:** 101-26. doi: 10.1038/s41573-022-00579-0

53. Wolff AC, Elizabeth Hale Hammond M, Allison KH, Harvey BE, Mangu PB, Bartlett JM, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology / College of American Pathologists Clinical Practice Guideline focused update. *J Clin Oncol* 2018; **36:** 2105-22. doi: 10.1200/JCO.2018.77.8738

54. Sajjadi E, Guerini-Rocco E, De Camilli E, Pala O, Mazzarol G, Venetis K, et al. Pathological identification of HER2-low breast cancer: tips, tricks, and troubleshooting for the optimal test. *Front Mol Biosci* 2023; **10:** 1-6. doi: 10.3389/fmolb.2023.1176309

55. Schnitt SJ, Tarantino P, Collins LC. The American Society of Clinical Oncology – College of American Pathologists Guideline update for human epidermal growth factor receptor 2 testing in breast cancer: how low can HER2 go? *Arch Pathol Lab Med* 2023; 147: 991-2. doi: 10.5858/arpa.2023-0187-ed

56. Ahn S, Woo JW, Lee K, Park SY. HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. *J Pathol Transl Med* 2020; **54:** 34-44. doi: 10.4132/jptm.2019.11.03

57. Fernandez AI, Liu M, Bellizzi A, Brock J, Fadale O, Hanley K, et al. Examination of low ERBB2 protein expression in breast cancer tissue. *JAMA Oncol* 2022; **8:** 1-4. doi: 10.1001/jamaoncol.2021.7239

58. Zaakouk M, Quinn C, Provenzano E, Boyd C, Callagy G, Elsheikh S, et al. Concordance of HER2-low scoring in breast carcinoma among expert pathologists in the United Kingdom and the republic of Ireland – on behalf of the UK National Coordinating Committee for Breast Pathology. *Breast* 2023; **70:** 82-91. doi: 10.1016/j.breast.2023.06.005

59. Ivkovic-Kapic T, Knezevic-Usaj S, Moldvaji E, Jovanic I, Milovanovic Z, Milantijevic M, et al. Interlaboratory concordance in HER2 testing: results of a Serbian ring-study. *J BUON* 2019; **24:** 1045-53. PMID: 31424659

60. Jonjić N, Mustać E, Tomić S, Jakić Razzumović J, Sarcević B, Blazicević, et al. Interlaboratory concordance in HER-2 positive breast cancer. *Acta Clin Croat* 2015; **54:** 479-84. PMID: 27017723

61. Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, et al. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst* 2002; **94:** 855-7. doi: 10.1093/jnci/94.11.855

62. Gennari A, André F, Barrios CH, Cortes J, de Azambuja E, DeMichele A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer *Ann Oncol* 2021; **32:** 1475-95. doi: 10.1016/j.annonc.2021.09.019

63. Curigliano G, Castelo-Branco, L Gennari A, Harbeck N, Criscitiello C, Trapani D on behalf of the CPG author group. ESMO Metastatic Breast Cancer Living Guideline, v1.1 May 2023. [internet]. [cited 2023 Dec 17]. Available at: https://www.esmo.org/living-guidelines/esmo-metastatic-breast-cancer-living-guideline

64. Lovasić IB, Koretić MB, Podolski P, Dedić Plavetić N, Silovski T, Pleština S, et al. [Clinical guidelines for diagnosis, treatment and monitoring of patients with invasive breast cancer – Croatian Oncology Society (BC-3 COS)]. [Croatian]. *Liječ Vjesn* 2022; **144:** 295-305. doi: 10.26800/LV-144-9-10-2

65. European Medicines Agency. From laboratory to patient - the journey of a medicine assessed by EMA. [internet]. *Eur Med Agency* 2019. [cited 2023 Dec 18]. Available at: https://www.ema.europa.eu/en/documents/other/laboratory-patient-journey-centrally-authorised-medicine_en.pdf

66. Cufer T, Ciuleanu TE, Berzinec P, Galffy G, Jakopovic M, Jassem J, et al. Access to novel drugs for non-small cell lung cancer in Central and Southeastern Europe: a Central European Cooperative Oncology Group analysis. *Oncologist* 2020; **25:** e598-601. doi: 10.1634/theoncologist.2019-0523

67. ESMO. ESMO-MCBS Scorecards. Scorecards for solid tumors. [internet]. [cited 2023 Dec 19]. Available at: https://www.esmo.org/guidelines/esmo-mcbs/esmo-mcbs-for-solid-tumours/esmo-mcbs-scorecards

research article

Unravelling the lung cancer diagnostic pathway: identifying gaps and opportunities for improvement

Mateja Marc Malovrh[1,2], Katja Adamic[1]

[1] University Clinic for Respiratory and Allergic Diseases Golnik, Golnik, Slovenia
[2] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Radiol Oncol 2024; 58(2): 268-278.

Received 18 December 2023
Accepted 20 January 2024

Correspondence to: Assist. Prof. Mateja Marc Malovrh, M.D., Ph.D., University Clinic of Respiratory and Allergic diseases Golnik, Slovenia.
E-mail: mateja.marc@klinika-golnik.si

Disclosure: No potential conflicts of interest were disclosed.

Background. A fast and well-organized complex diagnostic process is important for better success in the treatment of lung cancer patients. The aim of our study was to reveal the gaps and inefficiencies in the diagnostic process and to suggest improvement strategies in a single tertiary centre in Slovenia.

Patients and methods. We employed a comprehensive approach to carefully dissect all the steps in the diagnostic journey for individuals suspected of having lung cancer. We gathered and analysed information from employees and patients involved in the process by dedicated questionnaires. Further, we analysed the patients' data and calculated the diagnostic intervals for patients in two different periods.

Results. The major concerns among employees were stress and excessive administrative work. The important result of the visual journey and staff reports was the design of electronic diagnostic clinical pathway (eDCP), which could substantially increase safety and efficacy by diminishing the administrative burden of the employees. The patients were generally highly satisfied with diagnostic journey, but reported too long waiting times. By analysing two time periods, we revealed that diagnostic intervals exceeded the recommended timelines and got importantly shorter after two interventions - strengthening the diagnostic team and specially by purchase of additional PET-CT machine (the average time from general practitioner (GP) referral to the multidisciplinary treatment board (MDTB) decision was 50.8 [± 3.0] prior and 37.1 [± 2.3] days after the interventions).

Conclusions. The study illuminated opportunities for refining the diagnostic journey for lung cancer patients, underscoring the importance of both administrative and capacity-related enhancements.

Key words: lung cancer; diagnostic pathway; improvement

Introduction

Lung cancer remains a leading cause of cancer-related morbidity and mortality worldwide. Many patients are diagnosed at advanced stages of the disease.[1,2] Despite advances in treatment modalities, early detection, and our understanding of the molecular aspects of oncology, we still face challenges in improving patient care and outcomes.[3,4] Specifically, issues within the diagnostic process can lead to delayed diagnosis, treatment, and ultimately worse outcomes for lung cancer patients.[5,6] Previous studies have shown that the complexities of healthcare systems and disjointed care impact lung cancer patients.[7] Factors such as limited access to specialized services, especially for those in rural or remote areas, along with the need for coordinated care among various healthcare providers, result in delays and inefficiencies during diagnosis.[8] Although the implementation of

standardized care pathways has been proven to improve patient outcomes and satisfaction, their application varies across cancer types, settings, and populations.[9,10] Our study aimed to address these issues and enhance lung cancer care. We planned to analyse gaps in the diagnostic process for individuals suspected of having lung cancer at University Clinic of Respiratory and Allergic Diseases Golnik. Research from different regions and healthcare settings gave us the starting idea for the research.[11,12] Our work aims to comprehend the obstacles to timely and effective lung cancer diagnosis and suggest improvement strategies. By thoroughly assessing the diagnostic journey, our study delved into the vital aspects of lung cancer care paths. We identified factors that aid or hinder their implementation and assessed their impact on patient outcomes. Ultimately, our findings will be able to guide the creation of customized lung cancer care pathways that cater to our population's and healthcare system's specific needs. The objective was to elevate the quality and efficiency of care for individuals with suspected lung cancer.

Patients and methods

To conduct this study, we employed a comprehensive approach to analyse the diagnostic journey for individuals suspected of having lung cancer. We undertook the following steps to ensure a thorough understanding of the process:

Visual representation of the patient journey

We created a visual representation of the patient journey with input from an interdisciplinary team. The team included interventional pulmonology specialists (involved in triage, outpatient exams, (day)hospital work, and invasive procedures such as bronchoscopies and pleural punctures), radiologists for CT consultations and transthoracic biopsy guidance, pathologists, nurses in outpatient and inpatient settings, and an administrator and coordinator responsible for administrative tasks like issuing discharge letters and forwarding delayed reports from imaging or pathology investigations (especially PET CT or MRI performed in other institutions) to physicians. The patient journey illustration showed the stages and steps in the diagnostic process and interactions among healthcare providers. The key organizational characteristics of a clinic that diagnoses one-third of Slovenian patients with lung cancer are listed below:

a. Patients with suspected lung cancer were managed in a specialized multidisciplinary tertiary center offering a range of necessary examinations, excluding PET-CT and MRI.
b. Many examinations were conducted on an outpatient basis, making it ideal for identifying and preparing patients for invasive diagnostics, which were performed in hospitalized patients.
c. A proficient triage system was established, involving a coordinator, interventional pulmonologist, and radiologist. The coordinator managed referrals, provided patient information, and organized outpatient exams or hospital admissions. The interventional pulmonologist and radiologist determined the need for further invasive diagnostics.
d. Ideally, patients had outpatient exams before invasive diagnostics. Patients with prior outpatient exams were scheduled for invasive procedures (e.g., bronchoscopy, CT or US-guided transthoracic biopsy, US-guided lymph node aspiration, thoracentesis) on admission day, often as day-hospital cases.
e. Directly admitted patients underwent invasive diagnostics the following day.
f. The diagnostic process concluded by presenting patient data to the multidisciplinary treatment board (MDTB) for decision-making, communicating treatment choices to the patient, and scheduling lung cancer specialist follow-up.

Patient data collection and analysis

After obtaining their written consent, we analysed data from patients referred to the clinic with suspected lung cancer from January 1, 2023, to March 31, 2023. Information was gathered from the hospital's triage list, recording key events (date of referral, date of first / second appointment), triage decision, and methods of management (first visit at outpatient department, direct hospital admission, redirection to other facilities). Additional data on patients referred in January 2023 were extracted from the hospital information data system, including age, sex, final diagnosis, hospitalization duration, invasive procedures performed and MDTB treatment decision. Diagnostic intervals for hospitalized individuals with suspected lung cancer referred in January and June 2023 were computed from an excel spreadsheet and the Hospital Information system. All individual data were anonymized and in accordance with the General

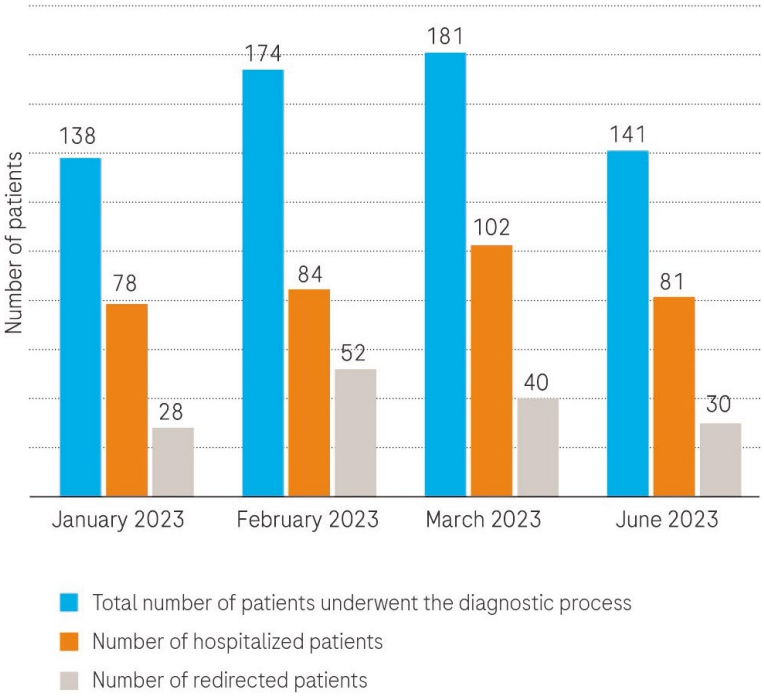


FIGURE 1. Triage for patients with lung infiltrates from January 1 to the end of March 2023 and in June 2023 (total number of referred patients, number of hospitalized patients, and number of redirected patients per month).

Data Protection Regulation. Ethical approvals were obtained from the Medical Ethics Committee of the Republic of Slovenia Nr. 0120-317/2016/2.

Hospital staff survey

An online survey was administered to hospital staff to gather further insights and perspectives on the diagnostic patient journey and identify potential areas for improvement (Supplementary Table 1).

Patient questionnaires

Four questionnaires (Q1–4) were designed especially for this survey to capture patient perspectives at different diagnostic journey stages:

g. Q1 - referral by the general practitioner (GP) to acceptance at the outpatient clinic (Supplementary Table 2).;
h. Q2 - after the outpatient clinic visit (Supplementary Table 3).;
i. Q3 - following hospitalization with an invasive diagnostic procedure (Supplementary Table 4).;
j. Q4 - after receiving a diagnosis (Supplementary Table 5).

After obtaining their written consent, the patients completed the questionnaires anonymously via the iPad they received at the hospital visits (Q1-Q3). The answers for Q4 questionnaire were obtained by phone call one week after the final diagnosis by medical students.

Validation workshops

The accuracy and comprehensiveness of the patient journey map was validated through two workshops involving interdisciplinary team members who had previously contributed insights. These workshops facilitated discussions and feedback to refine the patient journey representation.

Results

Analysis of patients' data from triage and hospitalized patients with lung cancer

The analysis of the three-month period (from January 1, 2023, to March 31, 2023) revealed that a total of 493 patients underwent the diagnostic process for lung infiltrates. On average, this accounted for 164 patients per month (ranging between 138 and 181). Among the referred patients, 120 individuals (24.3%) were redirected to alternative facilities following the initial triage, which involved chest X-rays, CT scans and medical documentation assessment. This redirection occurred due to the absence of suspicion for malignant disease. Of all the patients referred, 264 (53.8%) required hospitalization for invasive diagnostics. In June 2023, out of the 141 referred patients, 30 individuals (27%) were redirected to other facilities after the initial triage. Furthermore, 82 patients (58.2%) needed hospitalization for further diagnostic procedures (Figure 1).

Hospitalized patients from triage in January 2023

A detailed analysis was conducted on a subgroup of 75 patients who were referred for diagnostic evaluation in January 2023 and necessitated hospitalization. Within this subset, the final diagnoses encompassed various categories, with 39 patients (representing 52% of the hospitalized individuals and 28% of the referred patients) receiving a diagnosis of lung cancer, 9 cases involving lung metastases originating from other primary cancers, 16 patients with benign lesions, and 11 cases involving pleural diseases. Mean age of 39 patients with

lung cancer was 70.0 years (± 1.3 Standard Error of the Mean [SEM]), 15.4% were older than 80 years, 7.7% younger than 60 years. Out of this group, 23 were male (59%). On average, their hospital stay spanned 2.6 (± 0.3) nights, with five individuals managed as day-hospital cases. Within this subgroup, 18 patients were categorized as stage I (46.2%), 5 as stage II (12.8%), 7 as stage III (17.9%), and 9 as stage IV (23.1%), based on disease TNM staging. The mean number of invasive procedures conducted per patient was 1.4 (± 0.09). Specifically, 19 patients underwent solely bronchoscopy, 12 received a combination of bronchoscopy and transthoracic needle aspiration (TTNA), two patients underwent only TTNA, three patients underwent ultrasound-guided peripheral lymph node puncture, and one patient underwent thoracentesis. Additionally, circulating DNA in plasma (ctDNA) analysis was performed in 2 patients with poor performance status. The MDTB recommendations varied among the patients, with 17 individuals advised to undergo surgery, 7 recommended for radical radiotherapy, 5 recommended for radical radiotherapy with concomitant systemic treatment, 5 prescribed systemic therapy, and five patients deemed suitable for best supportive care.

Diagnostic intervals for patients with lung cancer

We calculated the time it took to diagnose lung cancer patients referred to our clinic in January 2023 and in June 2023. We selected these two time periods because they coincided with two significant changes that impacted the diagnostic process. First, a new PET CT machine was acquired (the fourth in Slovenia), and second, organizational changes were implemented within the clinic. These organizational changes included strengthening the day-hospital operations and involving other wards in the reception of lung cancer patients from the waiting list, resulting in a 50% increase in our hospital's capacity to manage lung cancer patients.

Here are the key findings from our analysis:

For the 39 hospitalized lung cancer patients referred in January 2023:

- The average time from GP referral to the first examination in the clinic was 17.9 (± 0.9) days.
- The average time from GP referral to the final diagnosis (MDTB treatment decision) was 50.8 (± 3.0) days.
- 25% of patients received their first clinic appointment in under two weeks, and 20% received their final diagnosis within the recommended 31 days.
- The mean waiting time for a PET CT scan was 28.3 (± 3.4) days.

For the 38 hospitalized lung cancer patients referred in June 2023:

- The average time from GP referral to the first examination in the clinic was 13.6 (± 0.9) days.
- The average time from GP referral to the final diagnosis was 38.1 (± 2.3) days.
- 60% of patients received their first clinic appointment within two weeks, and 31.5% received their final diagnosis within the recommended 31 days.
- The mean waiting time for a PET CT scan was 19.5 (± 2.8) days.

Hospital staff survey results

The results from a survey (Supplementary Table 1) conducted among 13 responsive team members, comprising four pulmonology specialists, one coordinator, one administrator, and seven nurses, have provided valuable insights into the work conditions within our hospital.

Here are the key findings:

- Work overload: A significant 90% of respondents expressed that they felt overloaded with their workload.
- Multiple workplace demands: Many staff members also faced challenges related to concurrent work across various hospital departments on the same day (ward, emergency department, bronchoscopy, outpatient pulmonology department, student tutoring). This multitasking affected their ability to dedicate sufficient time to patient discussions, explanations of the diagnostic procedures, final diagnoses, and treatment plans, particularly in outpatient settings and during brief hospital encounters.
- Job satisfaction: The majority of participants reported satisfaction with their workplace, appreciating the responsible, non-monotonous, and meaningful nature of their roles.
- Stress levels: All respondents reported experiencing at least moderate levels of stress, with 25% indicating severe stress at work.

Challenges identified:

- Patient care: The majority pulmonology specialists highlighted the challenges of manag-

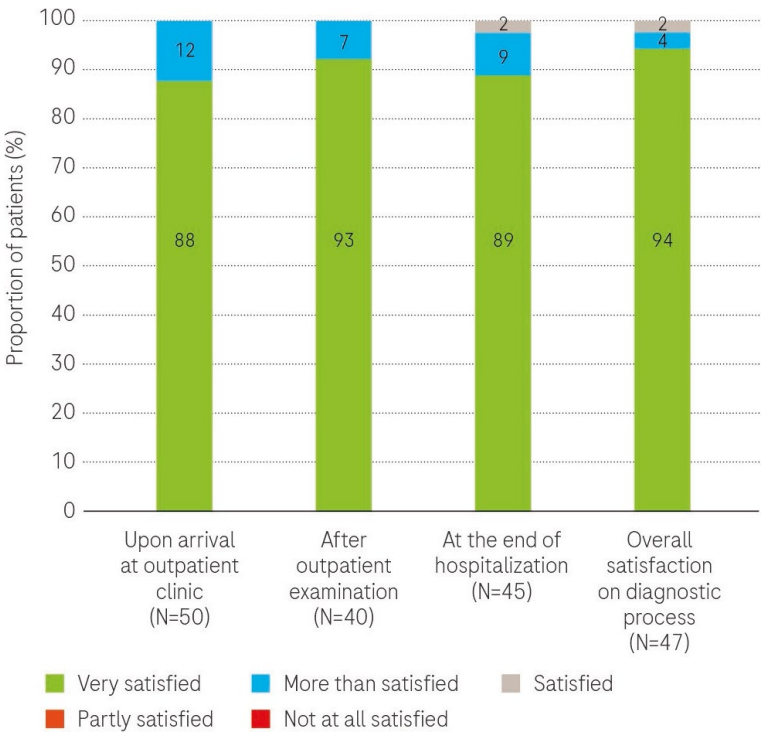


FIGURE 2. Patient satisfaction across diagnostic process stages. The proportion of patients who were asked: How satisfied were you during the diagnostic process? and answered with 1 (not satisfied) to 5 (very satisfied).

ing numerous patients at various stages of the diagnostic process. They expressed dissatisfaction with the time-consuming system for tracking the newly arrived results of already discharged patients. The existing information system lacked alerts regarding patients not presented to the MDTB or the completion of their diagnostic path. To mitigate delays and potential loss of patient documentation, an Excel table was introduced in the past to track patients' progress in the diagnostic pathway, including test results from pathology or radiology departments and presentation dates to the MDTB council (only for cases where such evaluation is needed). However, this process was found to be time-consuming and prone to inconsistencies and errors.

- Process duration: The extended duration of the entire diagnostic process was a concern.
- Administrative burden: Redundant administrative tasks were cited as a significant issue.
- Time pressure: The awareness that time constraints could impact patient outcomes added to their stress.
- These survey results shed light on the need for targeted interventions to alleviate workload pressures, streamline administrative tasks, and enhance the quality of patient care and communication within our hospital.

Patient questionnaire results

We collected responses from patient questionnaires (Supplementary Tables 2–5) at various stages of their patient journey. Here is a breakdown of when and how many of these questionnaires were administered:

- Questionnaire 1 (Q1, Supplementary Table 2): Administered to 52 patients upon their arrival at the outpatient clinic in September 2022.
- Questionnaire 2 (Q2, Supplementary Table 3): Administered to the same patients after they completed their outpatient examination.
- Questionnaire 3 (Q3, Supplementary Table 4): Collected from 47 patients at the end of their hospitalization, spanning two time periods, in September-October 2022 and May-June 2023.
- Questionnaire 4 (Q4, Supplementary Table 5): Gathered from the same patients as Q3 approximately one week after their presentation to MDTB council, during the mentioned time periods.

Patient response rates vary across questions, and the number of respondents for each question is indicated next to the corresponding figure (see the N numbers listed with each Figure).

The majority of patients expressed high levels of satisfaction with all aspects of their experience throughout the diagnostic process for lung cancer, encompassing the period from their initial visit to the outpatient clinic to their discharge and the subsequent waiting period for the MDTB decision, as well as the receipt of the MDTB treatment decision information (as illustrated in Figure 2).

These results underscore the positive feedback received from patients regarding both the organizational and professional aspects of their diagnostic journey.

In our evaluation of the patient experience, we found the following key points:

- Outpatient medical check-ups: Medical check-ups in the outpatient department were quick, with all patients completing them in under 30 minutes. A substantial 63% of patients finished their check-ups in under 15 minutes. All patients were satisfied with the information provided by the medical staff, with the exception of one patient who missed PET-CT information.

- Hospital admissions: Among hospitalized patients, the breakdown was as follows:
  - 40% were admitted after check-up in the outpatient department.
  - 42% were triaged for direct admission.
  - 18% with acute symptoms bypassed triage and were admitted through the emergency unit.

For 80% of hospitalized patients, a bed was available in less than 1 hour, while for the remaining 20%, it took up to 2 hours.

- Information and communication:
  - 89% of patients felt they received sufficient information from healthcare professionals
  - 20% expressed a desire for more time to converse with the doctor.
  - Over 90% believed they could understand information about their illness and knew who to contact for additional questions.
- MDTB decision communication: The information about the MDTB decision was effectively conveyed to the majority of patients, through either phone calls or in-person conversations. Patients comprehended the information and were aware of the subsequent steps. Relatives were also adequately informed.
- Stress levels: 51% of patients reported no stress during the diagnostic process. The remaining patients experienced stress due to various reasons, including: facing the diagnosis, prolonged waiting times for a final diagnosis, insufficient information, other factors such as sharing a room with three patients, Covid-19 infection, communication issues with staff, feelings of helplessness, social concerns, and fear. In Figure 3, we illustrate the proportions of patients reporting stress due to different reasons during their diagnostic journey.
- Patient-reported waiting times: we have provided detailed data on patient-reported waiting times for major events in their diagnostic journey in Figure 4A and 4B.
- Key findings from the data include:
  - Over 50% of patients reported waiting for more than 2 weeks for their initial appointment at the clinic, whether it was for an outpatient examination or hospital admission.
  - Similarly, for the period from GP referral to the receipt of a final diagnosis, more than 50% of patients reported waiting times that exceeded the recommended four-week period.

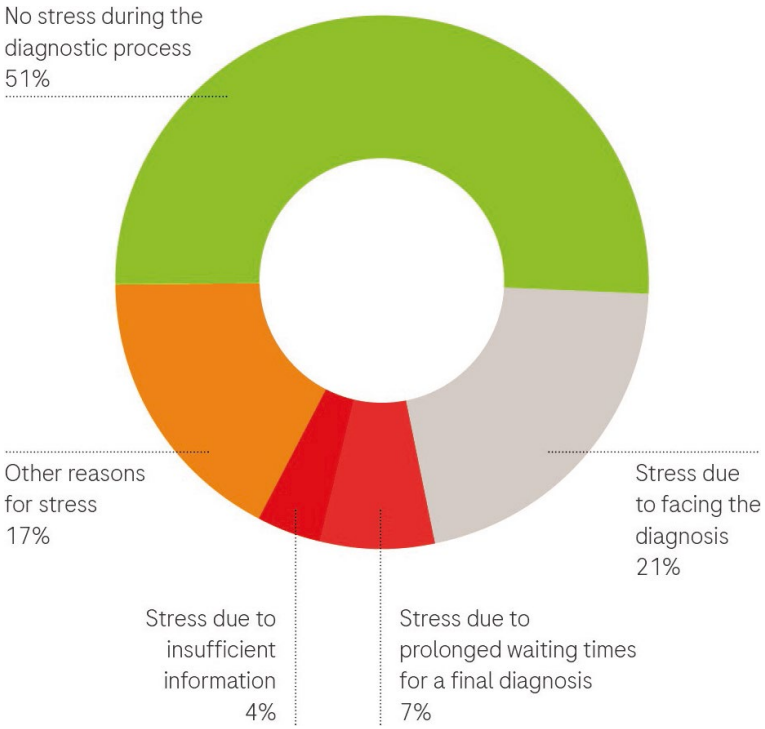


FIGURE 3. Sources of stress among patients during the diagnostic process (N = 47). Other reasons were sharing a room with three patients, Covid-19 infection, communication issues with staff, feelings of helplessness, social concerns, and fear.

Validation, data analysis and opportunities for improvement

Upon conducting a comprehensive assessment that included visual mapping of the patient journey and analysis of both hospital staff and patient surveys, several challenges and potential enhancements have become known. These improvements have the potential to elevate satisfaction levels and reduce stress for all involved parties.

Organizational challenges

- Patient tracking: Currently, patient tracking is managed simultaneously through Excel spreadsheets, which places an additional administrative burden on physicians and increases the likelihood of errors.
- Redundant administrative work: We have identified redundant administrative tasks, including the maintenance of patient records in both the Hospital Information System and separate files.
- Transcription of dictated notes: Administrative resources are dedicated to transcribing doctors'

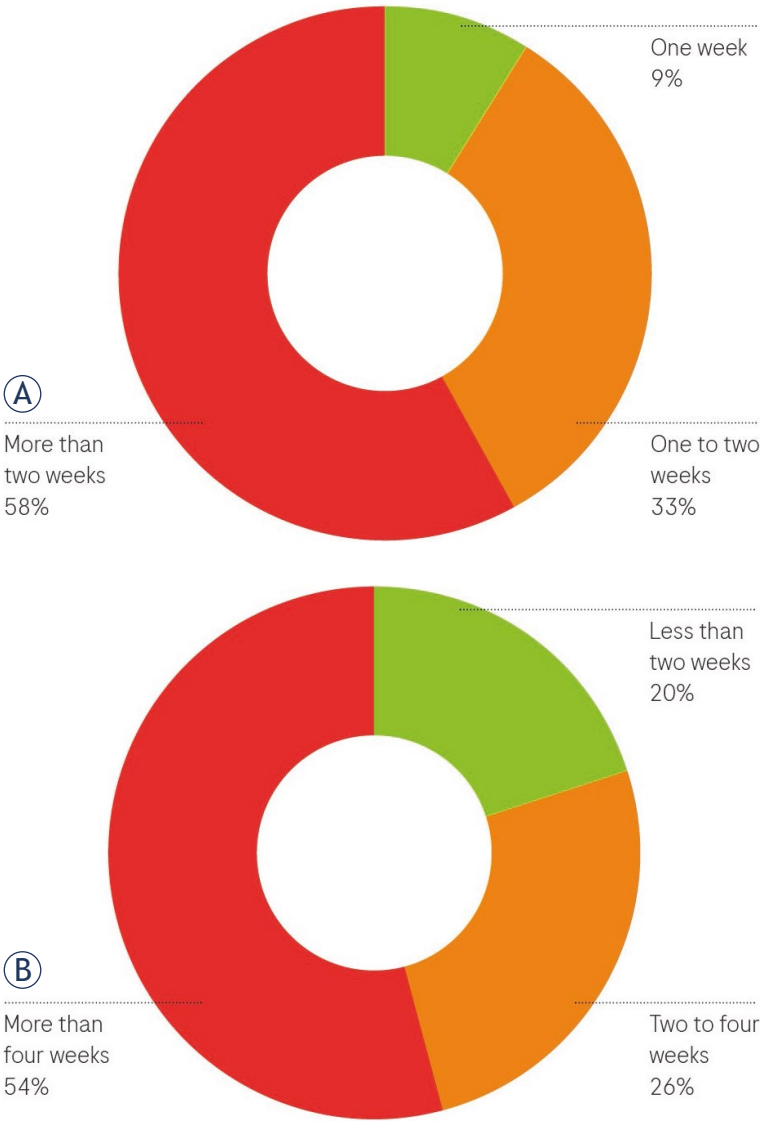


FIGURE 4. Patient reported waiting times from the GP referral to an initial appointment at the Golnik University Clinic (N = 52) **(A)**; and for receiving a final diagnosis **(B)**.

dictated notes, resulting in inefficient use of work force.

- MDTB data organization: The organization of patient data required for the MDTB is sub-optimal, necessitating additional administrative work for documentation preparation, although most data are accessible in the information system.

In response to these challenges, we designed an electronic diagnostic clinical pathway (e-DCP), which could be incorporated in the existing clinical informational system. The e-DCP's structure would encompass predefined options for various investigations, ensuring precise tracking of each patient's progress within the diagnostic journey. Additional administrative efficiency gains could be realized by automating the generation of a concise summary of patient essential information extracted from the Electronic Health Record (EHR) for the MDTB proceedings. Notably, the system is designed to promptly flag any gaps or missing information and alert the physician when the patient is prepared for MDTB presentation.

Patient-centric challenges

- Lack of systematic patient feedback: Currently, patient feedback is not systematically collected, potentially overlooking valuable insights from the patient perspective.
- Communication channels: Patient communication with the hospital is limited to postal mail, telephone, or email. Exploring additional digital communication channels could enhance the patient experience.
- Patient awareness: Patients often lack explicit information about their current position within the patient journey and are unaware of the expected next steps.

Based on our analysis, data from two different periods, and patient reports, it is evident that in the majority of cases, the final diagnosis exceeds the recommended 31-day timeframe. This is primarily attributed to extended waiting times from referral to the first appointment and the challenge of limited PET-CT machines, resulting in lengthy waiting periods. Addressing these organizational and patient-centric challenges, as well as streamlining the diagnostic process, will be essential to improve the overall experience for patients and hospital staff alike.

Discussion

This study takes a comprehensive approach to enhance our understanding of the diagnostic trajectory for patients with lung cancer, focusing on patient and medical staff experiences at a tertiary care center and pre-treatment temporal intervals. To our knowledge, this is the first in-depth survey of patient and medical staff experiences during the diagnostic path of lung cancer in tertiary centers.

Previous study by Rankin NM and colleagues provided insights into how patients with suspected lung cancer and their general practitioners (GPs) experience the diagnostic journey. Their findings highlighted that the lack of defined diagnostic

pathways to respiratory specialist assessment and hospital clinics was a clear source of frustration for both patients and GPs. They recommended the implementation of national lung cancer pathways, which have shown to improve outcomes for lung cancer patients and may help address GP frustrations and health system barriers.[13] However, our case differs from Rankin's study, as our lung cancer diagnostic path is well defined, offering a simple and uniform process of referring patients with suspected lung cancer to a tertiary diagnostic center for all GPs and pulmonologists, regardless of the region. Additionally, our tertiary multidisciplinary diagnostic center operates with a well-coordinated and structured diagnostic pathway. These factors likely contribute significantly to the high rates of patient satisfaction and surprisingly low reported stress levels in our setting. Despite the well-structured and coordinated diagnostic pathway for lung cancer, our investigation revealed critical areas in need of improvement. These potential enhancements primarily pertain to either hospital staff or patients.

The findings from an online survey among hospital staff have highlighted two significant areas of concern: stress and excessive administrative workload. Stress primarily results from the limited time available to manage patient care. Additionally, extended timeframes until the final diagnosis and deficiencies in administrative patient tracking contribute to stress and frustration. To address these issues, the most frequently proposed improvements include: (1) Streamlining administrative tasks to reduce workload; (2) Implementing a system for regular and automated notification of test results after a patient's discharge from the hospital; and (3) Enhancing access to specific medical services, such as hospital admissions for invasive diagnostics, PET-CT scans and MRIs. These measures have been suggested as means to alleviate stress and enhance the overall efficiency of patient care.

Significant strides in administrative patient management can be achieved by seamlessly integrating an electronic Diagnostic Clinical Pathway (e-DCP) into the existing Hospital Information System. The e-DCP concept envisions predefined options and a comprehensive patient tracking system that spans all diagnostic stages, from initial triage to the final diagnosis and presentation to MDTB for treatment decisions. We have carefully designed this e-DCP, and its implementation is currently pending. This automation would significantly reduce the present workload associated with documentation preparation. The ultimate goal of e-DCP implementation is to entirely eliminate the need for paper records and the current Excel spreadsheet-based patient tracking system.

Furthermore, once the patient data summary is systematically organized, it should be automatically integrated into both the Clinical and National Lung Cancer Registry, further streamlining the process and enhancing data accuracy.

Further, in the current scenario, considerable administrative resources are dedicated to transcribing physicians' dictated notes. This inefficiency could be significantly streamlined by embracing appropriate Speech Recognition technology, designed to directly transcribe doctors' notes into the Patient's Electronic Health Record (EHR). Ideally, this application would convert unstructured text into a standardized format, enhancing overall efficiency and accuracy.

A significant drawback in the process was the extended timeframe from the initial referral to the final diagnosis, a factor that also contributed significantly to stress among both patients and medical staff. The influence of diagnostic process speed on patient outcomes and survival has been extensively explored in the literature.[12,14-18] These studies have yielded mixed results due to the high heterogeneity of patients and variations in diagnostic pathways, which often prioritize faster evaluation for more severe cases. Nonetheless, it is widely acknowledged that delayed confirmation of cancer diagnosis elevates patient anxiety and distress.[19] In response to these concerns, numerous European countries and the USA have published organizational guidelines featuring recommended diagnostic and treatment intervals. Notable organizations like the British Thoracic Society (BTS), the National Institute for Health and Care Excellence (NICE), Swedish and Danish Lung Cancer Groups (SLCG, DLCG), the American College of Chest Physicians (ACCP), and the Institute of Medicine (IOM) have all contributed to establishing these crucial benchmarks.

The delays in diagnostic timelines represent a well-recognized and pervasive issue within the healthcare systems. Numerous medical centres have reported that diagnostic and treatment intervals frequently exceed the recommended timeframes for a significant portion of their patients. Addressing these challenges necessitates comprehensive improvements in the care pathways for lung cancer patients across various dimensions. Previous studies have consistently identified several common factors contributing to diagnostic

delays, including prolonged waiting times for diagnostic procedures, multiple attempts required to establish a diagnosis, limited access to high-yield investigations, delays in staging procedures, and protracted turnaround times for results.[20-23] Effective strategies to mitigate these delays have included the establishment of rapid access clinics designed to streamline the coordination of diagnostic procedures, the implementation of structured cancer diagnostic pathways, and the initiation of quality improvement projects that have successfully reduced redundant investigations and unnecessary inpatient admissions.[14,24-27] An intriguing study conducted in Texas employed an electronic medical record trigger system to prospectively identify patients at risk of experiencing delays in their diagnostic evaluations.[28]

In our specific case, the prolonged waiting time for the initial clinic examination emerged as the primary culprit behind diagnostic delays, primarily due to the constraints posed by limited hospital capacities for lung cancer patients. Furthermore, the extended waiting times for PET-CT scans significantly contributed to the overall time required for arriving at a final diagnosis. Considering that the majority of our patients presented with non-metastatic disease and approximately two-thirds of them necessitated a PET-CT scan before treatment decision could be made, it becomes less surprising that the time to reach a final diagnosis often exceeded the recommended 31-day threshold. An analysis encompassing 39 and 38 patients referred during two distinct periods, January and June 2023, substantiated the critical role of the factors mentioned above. A minor clinic reorganization undertaken in the spring resulted in increased capacities for managing lung cancer patients, leading to an average reduction of 4 days in the time interval from GP referral to the initial clinic examination. Moreover, this reorganization enabled 60% of patients to undergo their first assessment at the clinic within the recommended 14 days. Additionally, the notable decrease in the average waiting time for PET-CT scans in June, which was nearly 9 days, could be attributed to the acquisition of a new PET-CT machine. Collectively, these two strategic changes significantly shortened the overall time required to reach a final diagnosis by an average of 12.7 days.

Previous retrospective study from our clinic reported that 61 patients with lung cancer were diagnosed out of 159 patients who were examined in 2008 in specialized out-patient clinic for lung lesions in 12 months. The authors did not check the time from GP referral to the first visit, but reported the median time from the onset of symptoms to the first visit in outpatient clinic, which was 67 days. The median time from the first visit at clinic to the diagnosis was 10 days and from diagnosis to the beginning of treatment of 12 days. The important difference between the two analysed groups was a proportion of patients in whom PET CT was mandatory prior to treatment decision – 77% of patients in current study were of stage I-III and needed PET CT in comparison with less than half in 2008.[29]

Patients expressed high levels of satisfaction throughout every stage of the diagnostic pathway, spanning from the initial GP referral to the receipt of a final diagnosis. Surprisingly, more than half of the patients reported minimal stress during the diagnostic period. Those who did experience stress typically attributed it to concerns related to their diagnosis, personal or environmental factors. Notably, less than 10% felt stressed due to extended waiting times, and less than 5% cited a lack of information as a stress-inducing factor. These findings are particularly noteworthy in light of a previous study that highlighted the frustration experienced by patients and GPs when faced with undefined diagnostic pathways leading to respiratory specialist assessments and hospital clinics.[13] It appears that the presence of a uniformly organized and well-coordinated pathway played a pivotal role in both the high levels of patient satisfaction and the low reported stress levels. Within this structured pathway, patients received holistic management and guidance from a single center, which facilitated appointment scheduling for all necessary investigations, managed test results, and maintained regular communication with patients. To further enhance the patient experience and alleviate anxiety, the implementation of automated notifications, such as emails or SMS messages, to keep patients informed about their progress along the diagnostic journey is recommended. Such a system could also reduce the volume of incoming patient inquiries regarding results and appointment status.

Although medical staff have expressed limitations in their available time for patients and caregivers interactions, patients generally reported receiving sufficient information about their medical management from the healthcare team. Nevertheless, we believe that the creation and distribution of an informational brochure or an electronic application (e-application) containing comprehensive details about the patient's journey,

including individual stages and diagnostic procedures, could further alleviate patient anxieties. Such printed resource may particularly benefit individuals who prefer offline, easily accessible information, reinforcing an inclusive approach to patient education. Moreover, the integration of Patient Reported Outcomes Measures (PROMs) and Patient Reported Experience Measures (PREMs) should be incorporated into routine practice. These measures offer a holistic assessment of care quality from the patient's perspective, fostering a patient-centric healthcare culture and nurturing a feedback-based continuous improvement approach.[30]

While our study provides valuable insights, it is important to recognize its limitations. We conducted our research at a single center within the country, limiting the generalizability of our findings to a national level. Additionally, our study was limited in scope due to a relatively short timeframe. To ensure consistent treatment for patients with suspected lung cancer nationwide and to gain a comprehensive understanding of the issue, further research at a national level is needed. This broader investigation would address the specific needs and challenges faced by patients in various regions, ultimately enhancing the quality of care and outcomes for individuals with suspected lung cancer on a national scale.

Conclusions

Our study, employing a comprehensive methodology, not only gathered insights from healthcare professionals involved in the diagnostic pathway but also incorporated valuable perspectives from patients themselves. This multifaceted approach provided a deep understanding of the diagnostic patient journey and served as a foundation for developing customized strategies for improvement. While it is evident that the patient journey from GP referral to MDTB treatment decisions is well-structured, coordinated, and garners high levels of patient satisfaction, our survey has uncovered critical areas requiring enhancement. Foremost among these is the development of an Electronic Diagnostic Clinical Pathway (eDCP), a pivotal initiative that can significantly enhance the process by alleviating unnecessary administrative burdens on staff. Moreover, it can provide a secure and reliable checklist and analytical system for regular process evaluations, ensuring ongoing improvements. On a systemic level, it is imperative to further bolster clinic capacities dedicated to patients with lung cancer. In particular, national investments in additional PET-CT machines, accompanied by the necessary medical personnel, are urgently needed to expedite the diagnostic process. In sum, this study illuminated opportunities for refining the diagnostic journey for lung cancer patients, underscoring the importance of both administrative and capacity-related enhancements.

Acknowledgments

The authors would like to express their sincere gratitude to Ivona Perić, Janez Bernik, and Mateja Žužek Perc from Roche Pharmaceutical Company d.o.o. for their initiative and methodological guidance throughout the project. We are thankful for their technical support in delivering online questionnaires to medical professionals and patients, analyzing results, as well as their assistance with writing. We also extend our appreciation to our coworkers at UC Golnik for their valuable contributions. Furthermore, we want to acknowledge the patients and caregivers for their participation and feedback in the survey.

References

11. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* 2021; **71:** 7-33. doi: 10.3322/caac.21654

12. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; **144:** 1941-53. doi: 10.1002/ijc.31937

13. Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023; **34:** 339-57. doi: 10.1016/j.annonc.2022.12.009

14. Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, et al. Non-oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023; **34:** 358-76. doi: 10.1016/j.annonc.2022.12.013

15. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65:** 87-108. doi: 10.3322/caac.21262

16. Murray PV, O'Brien ME, Sayer R, Cooke N, Knowles G, Miller AC, et al. The pathway study: results of a pilot feasibility study in patients suspected of having lung carcinoma investigated in a conventional chest clinic setting compared to a centralised two-stop pathway. *Lung Cancer* 2003; **42:** 283-90. doi: 10.1016/s0169-5002(03)00358-1

17. Copeland J, Neal E, Phillips W, Hofferberth S, Lathan C, Donington J, et al. Restructuring lung cancer care to accelerate diagnosis and treatment in patients vulnerable to healthcare disparities using an innovative care model. *MethodsX* 2023; **11:** 102338. doi: 10.1016/j.mex.2023.102338

18. Dunn J, Garvey G, Valery PC, Ball D, Fong KM, Vinod S, et al. Barriers to lung cancer care: health professionals' perspectives. *Support Care Cancer* 2017; **25:** 497-504. doi: 10.1007/s00520-016-3428-3

19. Kutubudin F, Robinson R, Deus P, Hughes K, Wight AG. Impact of national optimal lung cancer pathway – can we meet the 28 day standard by 2020? [abstract]. *Thorax* 2018; **73(Suppl 4):** A140. doi: 10.1136/thorax-2018-212555.229

20. Jaly A, Conroy S, Mohsin N. Implementing the National Optimal Lung Cancer Pathway; STHK experience. European Congress of Radiology - ECR 2020; Poster: C-12308. [Internet]. Available at: https://dx.doi.org/10.26044/ecr2020/C-12308. doi: 10.26044/ecr2020/C-12308

21. Fung-Kee-Fung M, Maziak DE, Pantarotto JR, Smylie J, Taylor L, Timlin T, et al. Regional process redesign of lung cancer care: a learning health system pilot project. *Curr Oncol* 2018; **25:** 59-66. doi: 10.3747/co.25.3719

22. Jacobsen MM, Silverstein SC, Quinn M, Waterston LB, Thomas CA, Benneyan JC, et al. Timeliness of access to lung cancer diagnosis and treatment: a scoping literature review. *Lung Cancer* 2017; **112:** 156-64. doi: 10.1016/j.lungcan.2017.08.011

23. Rankin NM, York S, Stone E, Barnes D, McGregor D, Lai M, et al. Pathways to lung cancer diagnosis: a qualitative study of patients and general practitioners about diagnostic and pretreatment intervals. *Ann Am Thorac Soc* 2017; **14:** 742-53. doi: 10.1513/AnnalsATS.201610-817OC

24. Ost DE, Yeung SCJ, Tanoue LT, Gould MK. Clinical and organizational factors in the initial evaluation of patients with lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* 2013; **143(5 Suppl):** e121S-41S. doi: 10.1378/chest.12-2352

25. Kanashiki M, Satoh H, Ishikawa H, Yamashita YT, Ohtsuka M, Sekizawa K. Time from finding abnormality on mass-screening to final diagnosis of lung cancer. *Oncol Rep* 2003; **10:** 649-52. doi: 10.3892/or.10.3.649

26. Kashiwabara K, Koshi S, Itonaga K, Nakahara O, Tanaka M, Toyonaga M. Outcome in patients with lung cancer found on lung cancer mass screening roentgenograms, but who did not subsequently consult a doctor. *Lung Cancer* 2003; **40:** 67-72. doi: 10.1016/s0169-5002(02)00505-6

27. Salomaa ER, Sallinen S, Hiekkanen H, Liippo K. Delays in the diagnosis and treatment of lung cancer. *Chest* 2005; **128:** 2282-8. doi: 10.1378/chest.128.4.2282

28. Saint-Jacques N, Rayson D, Al-Fayea T, Virik K, Morzycki W, Younis T. Waiting times in early-stage non-small cell lung cancer (NSCLC). *J Thorac Oncol* 2008; **3:** 865-70. doi: 10.1097/JTO.0b013e318180210c

29. Risberg T, Sorbye SW, Norum J, Wist EA. Diagnostic delay causes more psychological distress in female than in male cancer patients. *Anticancer Res* 1996; **16:** 995-9. PMID: 8687166.

30. Malalasekera A, Nahm S, Blinman PL, Kao SC, Dhillon HM, Vardy JL. How long is too long? A scoping review of health system delays in lung cancer. *Eur Respir Rev* 2018; **27:** 180045. doi: 10.1183/16000617.0045-2018

31. Neal RD, Robbe IJ, Lewis M, Williamson I, Hanson J. The complexity and difficulty of diagnosing lung cancer: findings from a national primary-care study in Wales. *Prim Health Care Res Dev* 2015; **16:** 436-49. doi: 10.1017/S1463423614000516

32. Al Achkar M, Zigman Suchsland M, Walter FM, Neal RD, Goulart BHL, Thompson MJ. Experiences along the diagnostic pathway for patients with advanced lung cancer in the USA: a qualitative study. *BMJ Open* 2021; **11:** e045056. doi: 10.1136/bmjopen-2020-045056

33. White V, Bergin RJ, Thomas RJ, Whitfield K, Weller D. The pathway to diagnosis and treatment for surgically managed lung cancer patients. *Fam Pract* 2020; **37:** 234-41. doi: 10.1093/fampra/cmz064.z

34. Dunican E, Uzbeck M, Clince J, Toner S, Royston D, Logan MP, et al. Outcomes of patients presenting to a dedicated rapid access lung cancer clinic. *Ir Med J* 2011; **104:** 265-8. PMID: 22132593

35. Aasebo U, Strom HH, Postmyr M. The Lean method as a clinical pathway facilitator in patients with lung cancer. *Clin Respir J* 2012; **6:**169-74. doi: 10.1111/j.1752-699X.2011.00271.x

36. Hueto Pérez De Heredia J, Cebollero Rivas P, Cascante Rodrigo JA, Andrade Vela I, Pascal Martínez I, Boldú Mitjans J, et al. Evaluation of the use of a rapid diagnostic consultation of lung cancer. Delay time of diagnosis and therapy. *Arch Bronconeumol* 2012; **48:** 267-73. doi: 10.1016/j.arbr.2012.06.003

37. Lo DS, Zeldin RA, Skrastins R, Fraser IM, Newman H, Monavvari A, et al. Time to treat: a system redesign focusing on decreasing the time from suspicion of lung cancer to diagnosis. *J Thorac Oncol* 2007; **2:** 1001-6. doi: 10.1097/JTO.0b013e318158d4b6

38. Murphy DR, Wu L, Thomas EJ, Forjuoh SN, Meyer AN, Singh H. Electronic trigger-based intervention to *reduce delays in diagnostic evaluation for cancer: a cluster randomized controlled trial. J Clin Oncol* 2015; **33:** 3560-7. doi: 10.1200/JCO.2015.61.1301

39. Triller N, Bereš V, Rozman A. [Delays in the diagnosis and treatment of lung cancer: can the period between the onset of symptoms and the diagnosis and treatment be shortened?] [Slovenian]. [Internet]. *Zdrav Vestn* 2010; **79:** 618-22. [cited 2020 Jan 10]. Available from: https://vestnik.szd.si/index.php/ZdravVest/article/view/287

40. Aapro M, Bossi P, Dasari A, Fallowfield L, Gascon P, Geller M, et al. Digital health for optimal supportive care in oncology: benefits, limits, and future perspectives. *Support Care Cancer* 2020; **28:** 4589-12. doi: 10.1007/s00520-020-05539-1

research article

Influence of different intraoperative fluid management on postoperative outcome after abdominal tumours resection

Matej Jenko[1,2], Katarina Mencin[1,2], Vesna Novak-Jankovic[1,2], Alenka Spindler-Vesel[1,2]

[1] Department of Anesthesiology and Surgical Intensive Care, University Medical Centre Ljubljana, Slovenia
[2] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Radiol Oncol 2024; 58(2): 279-288.

Received 31 August 2023
Accepted 10 December 2023

Correspondence to: Assist. Matej Jenko, M.D., Department of Anesthesiology and Surgical Intencive Care, University Medical Centre Ljubljana, Slovenia. E-mail: matej.jenko@kclj.si

Disclosure: No potential conflicts of interest were disclosed.

Background. Intraoperative fluid management is a crucial aspect of cancer surgery, including colorectal surgery and pancreatoduodenectomy. The study tests if intraoperative multimodal monitoring reduces postoperative morbidity and duration of hospitalisation in patients undergoing major abdominal surgery treated by the same anaesthetic protocols with epidural analgesia.

Patients and methods. A prospective study was conducted in 2 parallel groups. High-risk surgical patients undergoing major abdominal surgery were randomly selected in the control group (CG), where standard monitoring was applied (44 patients), and the protocol group (PG), where cerebral oxygenation and extended hemodynamic monitoring were used with the protocol for intraoperative interventions (44 patients).

Results. There were no differences in the median length of hospital stay, CG 9 days (interquartile range [IQR] 8 days), PG 9 (5.5), p = 0.851. There was no difference in postoperative renal of cardiac impairment. Procalcitonin was significantly higher (highest postoperative value in the first 3 days) in CG, 0.75 mcg/L (IQR 3.19 mcg/L), than in PG, 0.3 mcg/L (0.88 mcg/L), p = 0.001. PG patients received a larger volume of intraoperative fluid; median intraoperative fluid balance +1300 ml (IQR 1063 ml) than CG; +375 ml (IQR 438 ml), p < 0.001.

Conclusions. There were significant differences in intraoperative fluid management and vasopressor use. The median postoperative value of procalcitonin was significantly higher in CG, suggesting differences in immune response to tissue trauma in different intraoperative fluid status, but there was no difference in postoperative morbidity or hospital stay.

Key words: postoperative complications; intraoperative monitoring; multimodal monitoring; hemodynamic monitoring; cerebral tissue oxygenation; abdominal surgery

Introduction

Intraoperative fluid management is a crucial aspect of cancer surgery, as it may significantly impact patient outcomes and postoperative complications. The optimal approach to fluid therapy during cancer surgery remains a topic of debate and ongoing research. Several studies have investigated the effects of different fluid management strategies on morbidity, mortality, and postoperative complications in various types of cancer surgeries. Restrictive fluid management has been shown to be superior to standard fluid management in preventing postoperative complications in abdominal surgery. Additionally, goal-directed fluid therapy targeting hemodynamic variables such as cardiac output and stroke volume has been found to decrease fluid balance and reduce inflammatory reactions after lung cancer surgery.[1]

It is important to note that the management of fluid balance in cancer surgery is complex and depends on various factors such as the type of surgery, patient characteristics, and underlying conditions. The use of enhanced recovery after surgery (ERAS) protocols, which include specific guidelines for perioperative fluid management, has been recommended in oncology surgeries.[2] Outcome of treatment has often been influenced by several variables.[3]

Continuous intraoperative measurement of blood flow and related variables was studied several times to show the benefit for patients. New monitors and treatment protocols with predefined treatment limits (goal-directed optimization of hemodynamic parameters) suggested an improvement in long-term patient outcome and a reduction in morbidity and mortality of more than 50% in some studies.[4-7] They aim to optimise microcirculation and improve oxygen delivery by correcting specific hemodynamic parameters.[8] The benefit of personalised and targeted oxygen delivery algorithms that incorporate both fluid resuscitation and vasoactive drugs applied to high-risk surgical patients was shown.[9] However, flow monitoring alone when added to conventional monitoring has much less effect on improving outcomes and reducing mortality than anticipated.[10,11] When using this strategy there was no decrease in mortality and the length of stay decreased on average by only one day.[10] Also a composite outcome of complications or mortality at 30 days is not reduced by this strategy.[11] In addition to hemodynamic variables, other important parameters, such as regional cerebral oxygenation (rSO2), measured by near-infrared spectroscopy (NIRS), should be continuously monitored to improve outcomes. Especially in the elderly, the reduction of regional cerebral oxygenation can lead to a poor outcome.[12-14] Monitors that assess the degree of cortical suppression (e.g. BIS, Aspect Medical Systems, Cambridge, USA) facilitate anaesthetic titration and have been shown to reduce anaesthetic exposure.[15,16]

In most studies, all new methods have been studied separately, and there is a lack of studies showing the effect of joint (multimodal) monitoring on mortality and occurrence of complications. All data collected indicate that the combined use of new methods (monitoring blood flow with assessment of fluid status, depth of anaesthesia and tissue oxygenation) with adherence to an appropriate protocol could dramatically improve perioperative management and outcome of high-risk surgical patients.[17,18] The important cofactor that may interfere with the results of the studies is the different anaesthesia techniques used in the patients included in the studies (presence or absence of an epidural catheter, different anaesthetic techniques used).[19-21]

The present study tests the hypothesis that intraoperative multimodal monitoring with hemodynamic optimisation and maintenance of optimal cerebral oxygenation reduces the rate of postoperative complications. Furthermore, multimodal monitoring can reduce the duration of hospitalisation in patients undergoing major abdominal surgery. To minimise bias, all patients in both groups have received the same intraoperative anaesthetic technique with epidural postoperative analgesia and all patients underwent similar gastrointestinal surgical procedures.

Patients and methods

A prospective randomised trial with 2 parallel groups was conducted at the University Medical Centre (UMC) Ljubljana in years 2015–2018. Patients from the Clinical Department of Abdominal Surgery were included in the study. Adult patients who underwent one of the following major abdominal cancer surgeries were included: stomach surgery, pancreatic surgery, and large intestinal resections. Only high-risk surgical patients, defined as American Society of Anaesthesia (ASA) class 2 or 3 with P-Possum predicted mortality >4% fulfilled criteria for inclusion.[22] ASA physical status classification system class 2 are patients with mild systemic disease, while class 3 patients are patients with a severe systemic disease that is not life-threatening.[23] P-possum is Physiological and Operative Severity Score for the enumeration of Mortality and morbidity. With the result, we are able to predict perioperative mortality.[24] Exclusion criteria were underage, pregnant women, laparoscopic surgery, and palliative procedures.

The study was approved by the Slovenian National Medical Ethics Committee. It was registered with ClinicalTrials.gov, Surgical Outcome and Multimodal Monitoring (SOMM) Identifier: NCT02293473. The article has previously unpublished data from the study.

Power analysis was performed using simulation of results with the Mann-Whitney U test. For a 2-day difference in stay length, with power 0.8 and significance level 0.05, 16 patients in each group are needed. To show the difference in LOS

at one day, 40 patients in each group are needed. The calculations are based on a small pilot study with 12 patients in each group. The expected Cohen value -d is 0.660 for a difference of 1 day in length of stay. We have slightly increased the number of patients recruited due to expected loss during follow-up.

All patients scheduled for abdominal cancer surgery were visited by a member of our team a day prior to surgery to obtain informed consent and to answer any questions. Before anaesthesia, patients were randomly assigned into two groups using covariate adaptive randomisation. The covariates considered were age, weight, and the ASA status of the patients. The groups were protocol group (PG) and control group (CG). The randomisation was carried out by a member of our study team. Two anaesthesiologists (who had not participated in randomisation) conducted the intraoperative management. They performed an intraoperative protocol determined by randomisation. The personnel who conducted postoperative management and postoperative data collection were unaware of how intraoperative management was conducted or the group of patients. The data collected and the patient group were linked after the data collection process was completed.

Anaesthesia management

Before the procedure, thoracic epidural catheter was inserted in the left lateral position (Th 7–8 or Th 9–10 for rectal surgery) and the tested with 3 ml of 2% lidocaine was performed. After monitoring and placement of the intravenous line, the infusion of dexmedetomidine was started (0.5 mcg/kg/hour). The continuous infusion ended after skin suture at the end of the procedure.

Then a standard induction to general anaesthesia (propofol, sufentanyl, rocuronium) was performed. Anaesthesia was maintained by iv infusion of propofol. The depth of anaesthesia in both groups was adjusted to keep the bispectral index (BIS) 40–55.[25] Analgesia was provided by 15 ml of 0.25% epidurally levobupivacaine, with a 15 mcg sufentanyl supplement. 1–2 hours after epidural bolus of local anaesthetic, patient-controlled epidural analgesia (PCEA) was started with constant infusion rate and additional patient-controlled boluses for postoperative analgesia (PCEA (0.125% levobupivacaine 200 ml, morphine 4 mg, clonidine 0.075 mg; infusion rate 5 ml/h, bolus 5 ml, lock time 30 minutes). Relaxation was provided with rocuronium and monitored with the train-of-four monitor (TOF). Sugammadex was provided to reverse neuromuscular block at the end of operation.

The haemoglobin level was measured every two hours or at the events with blood loss over 500ml. It was kept above 80 g/L. A fall in haemoglobin was coped with blood transfusion. Oesophageal measured body temperature was kept in the range between 36 and 37 °C.

Postoperatively, the patients were transferred to postoperative recovery and then to Abdominal Surgery high dependency unit (HDU).

Protocol group

Monitors that calculate stroke volume (SV) and cardiac output (CO) from a standard radial arterial line (LiDCO Rapid, LiDCO Cardiac Sensor Systems, Cambridge, UK) were applied. The near-infrared spectroscopy (NIRS) monitor (INVOS, Medtronic, USA) was used in the protocol group. As a non-invasive technology that continuously monitors regional tissue oxygenation, it was used unilaterally to monitor cerebral oxygenation in the left hemisphere. A baseline prior to induction was recorded. Baseline values of the nominal stroke index (SI), cardiac index (CI), BIS, mean arterial pressure (MAP), and regional oxygen saturation (rSO2) were recorded.

The patients have received 2 ml/kg/h of balanced fluids + replacement for fluid loss (with a ratio of 3 units of balanced crystalloids per every unit of blood loss, until the Hb 80. Then the blood transfusion was started. In the event of immediate blood loss of more than 500 ml, colloids were administered with the ratio to blood loss 1:1. The exact number of fluids given, dependent on monitored hemodynamic variables.

Phenylephrine was administered when the SVV was below 13% of variation, CI was in normal range and there was hypotension. CI, MAP, and SI were maintained within 80% of baseline values. In the event of a 20% fall in regional cerebral oxygenation (rSO2) or values rSO2% below 60 in the absence of a fall in CI or blood loss, we adjusted ventilation so that PaCO2 was kept in the high normal range (5–5,5kPa).

Control group

The same anesthetic regime was used in PG; there was no hemodynamic monitor. Measurement of cerebral oxygenation was also absent. The patients received 2 ml/kg/h of balanced fluids and additional fluid for replacement for fluid loss as in proto-

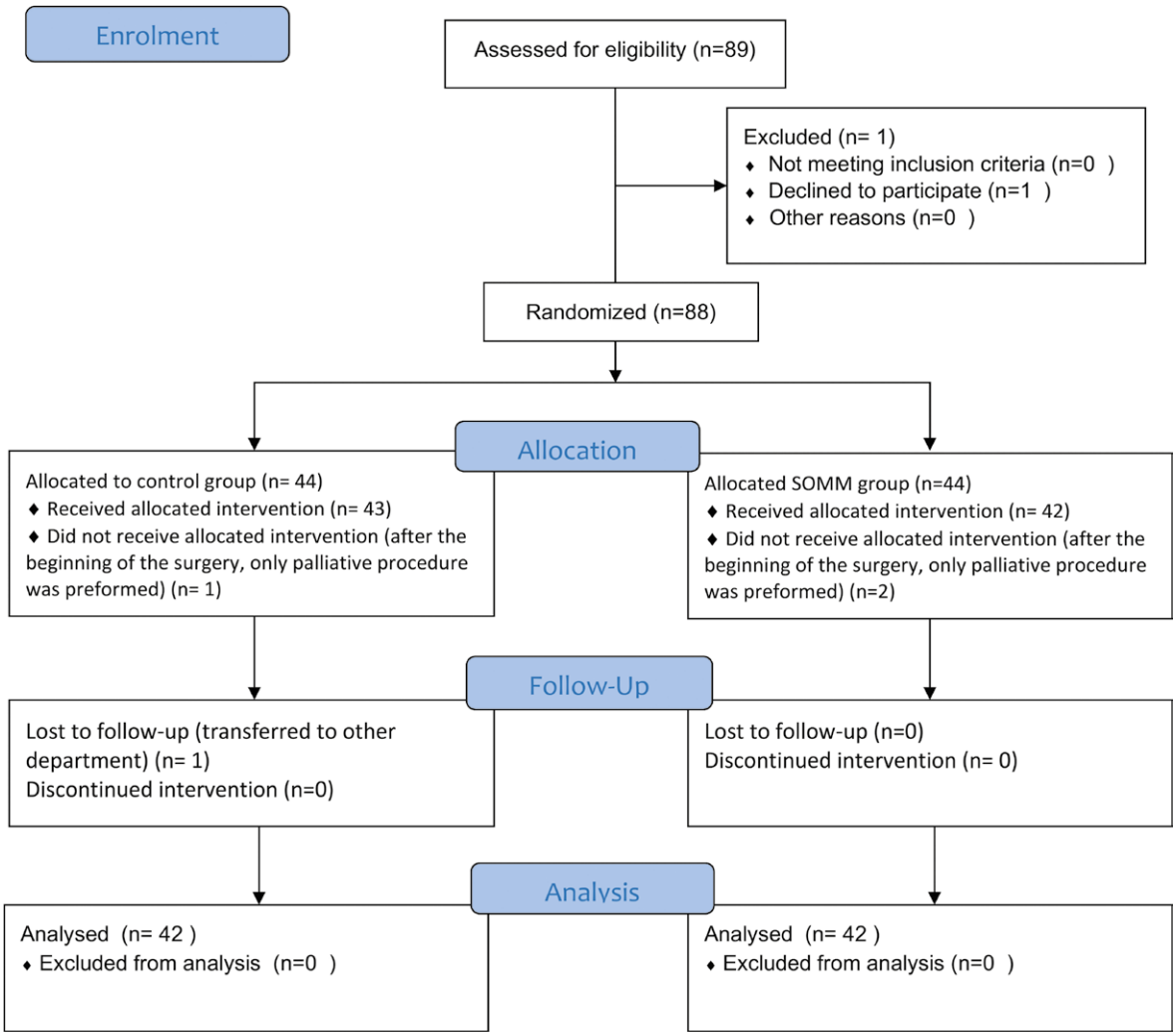


FIGURE 1. Consort diagram of the study.

col group. If there were no clinical signs of hypovolemia, phenylephrine was used in treatment of hypotension.

Data collection

Postoperatively we collected the following data: length of stay, length of stay in HDU, re-admission to HDU or intensive care unit (ICU), quality of wound healing, reoperations, 30-day mortality. We have observed complications (sepsis, pneumonia, acute respiratory infection, pleural effusion, myocardial infarction, pulmonary embolism, stroke, infection).

Hospital discharge criteria

To reduce unintended variations, strict discharge criteria were implemented. A hemodynamically stable patient without active infection, proper wound healing, and who has completed the first phase of rehabilitation to assisted mobility (or mobility comparable to preoperative) was discharged. If due to administrative reasons formal discharge was not possible, we considered him discharged if all the criteria were met.

Mental status testing

Preoperative mini mental state test (MMSE) examination was conducted.[26] The aim was to measure possible postoperative cognitive decline. Postoperatively, the same testing was conducted after patient was admitted to the ward from high dependency unit.

TABLE 1. Intraoperative fluid management

	Control group Median (interquartile range) ml	Protocol group Median (interquartile range) ml	P-value (Mann – Whitney U test)
Intraoperative blood loss	300 (425)	500 (500)	0.182
RBC transfusion	0 (0)	0 (0)	0.185
FFP transfusion	0 (0)	0 (0)	1
Platelet transfusion	0 (0)	0 (0)	0.317
Intraoperative fluid balance	+375 (438)	+1300 (1063)	0.000[1]
Intraoperative urinary output	205 (100)	300 (200)	0.078

1 = shows statistically significant difference; FFP = fresh frozen plasma; RBC = red blood cell

Statistical analysis

The results were analysed using R: A Language and Environment for Statistical Computing. The results of intraoperative management, the results of postoperative creatinine, the demographics of the patients, and the length of stay are presented as the median and interquartile range. Groups were compared using the Mann-Whitney U test, the level of significance of 0.05 was considered statistically significant.

Intraoperative observations, postoperative complications, and ASA classification are presented as the absolute number of patients with a certain intervention/observation. Groups are compared using the Chi-square test or Fisher's exact test, where appropriate. A level of significance of 0.05 is considered statistically significant.

When comparing postoperative complications, several comparisons are made on the same sample. The level of significance was adjusted accordingly to the Bonferroni correction, and a p-value of 0.001 is considered statistically significant.

Results

We randomly selected 88 patients, 44 in each group. Regarding intraoperative management and postoperative complications, 84 patients were analysed, 4 were excluded after randomisation because the intraoperative protocol was not strictly followed. Consort diagram of the study is shown in Figure 1.

The average age of the included patients was 65 ± 12 years in CG and 66 ± 8 years in PG (P = 0.265, Mann-Whitney U test). The average weight was 64 ± 10 kg in CG and 66 ± 12 kg in PG (p = 0.177, Mann-Whitney U test). 18 patients with ASA 2 status were in CG and 16 in the protocol (p = 0.154, Chi-square test). 24 ASA3 patients were included in CG and 26 in PG (p = 0.117, Chi-square test). The median physiological P-Possum in CG was 21 (interquartile range [IQR] 7) and 20 (IQR 8) in PG (p = 0.322, Mann-Whitney U test). The median operative P-possum was 13 in CG (IQR 5) and 13 (IQR 7) in PG. (p = 0.260, Mann-Whitney U test).

TABLE 2. Intraoperative observations and interventions

	Control group (Number of patients out of 42 with a certain observation/ intervention)	Protocol group (Number of patients out of 42 with a certain observation/ intervention)	P-value
Bolus of phenylephrine during procedure	31	38	0.043[1,2]
Vasoactive support with norepinephrine	1	2	0.500[3]
Mean arterial pressure less than 70mmHg at any time during the procedure	31	36	0.226[2]
Mean arterial pressure less than 50mmHg at any time during the procedure	7	8	0.353[2]

[1] statistically significant difference; [2] Pearson Chi-square; [3] Fisher's exact test

TABLE 3. Comparison of length of stay

	Control group Median (Interquartile range) days	Protocol group Median (Interquartile range) days	P-value (Mann – Whitney U test)
Longest stay in the hospital	9 (8)	9 (5.5)	0.851
Duration of stay in the HDU	4 (3)	3 (1.3)	0.122

HDU = high-depense unit

The time of perioperative fasting was 13 ± 2 hours, similar in both groups. The median duration of the surgery (from surgical incision to last suture) is 123 minutes in PG (interquartile range, IQR 35 min), and 120 minutes (IQR 47min) in CG (Mann-Whitney U test, p = 0.157). There was no difference in intraoperative propofol consumption between PG (1.32 g) and CG (1.30 g), (p = 0.860, Mann-Whitney U test.

Table 1 shows intraoperative fluid management in both groups and Table 2 intraoperative interventions with respect to hemodynamic variables.

In PG, we have observed the results of the NIRS monitor. In 7 cases (out of 42), there was a decrease of more than 20% of the preoperative value during the procedure. The absolute value was never below 45%.

TABLE 4. List of postoperative complications in the first three days after the procedure in both groups

Postoperative complication/intervention	Control group (Number of patients out of 42 with a certain observation/ intervention)	Protocol group (Number of patients out of 42 with a certain observation/ intervention)	Value P*
Readmission to the HDU	5	1	0.136[1]
Admission to the ICU	2	0	0.247[2]
Revision surgery	6	0	0.026[1]
The patient has died before discharge	1	0	0.500[2]
Complications related to the operative procedure (dehiscence, inflammation) first day after the procedure	0	2	0.494[2]
Complications related to the operative procedure (dehiscence, inflammation) third day after the procedure	3	2	1[2]
RBC transfusion needed on the first day after the procedure	2	0	0.513[2]
RBC transfusion required the second or third day after the procedure.	1	2	0.500[2]
Acute kidney disease	3	4	0,500[2]
Troponin leak	0	3	0,241[2]
Median level of C-reactive protein (difference between highest postoperative level in 3 days and preoperative level) Laboratory reference range (0−5 mcg/L)	125 (118)	115 (122)	0.106[3]
Median level of procalcitonin (highest postoperative value in the first 3 days) Laboratory reference range (0−0.50 mcg/L)	0.75 (3.19)	0.3 (0.88)	0.001[3]

Due to multiple comparisons, the significance of the p-value was adjusted accordingly to the Bonferroni correction (significant p value for the variables in the table was < 0.001);

[1] Pearson's Chi-square; [2] Fisher's exact test; [3] independent samples Mann-Whitney U test

HDU = high-depence unit; ICU = intensive care unit; RBC = red blood cell

None of the intraoperative interventions influenced hospital or high-response unit (HDU) stay as shown in Table 3.

One person (in CG) died during hospitalisation. Several postoperative complications were observed, the distribution among groups was the same as shown in Table 4.

The results of the Mini mental state examination are shown in Figure 1. There were no differences between groups, neither was the postoperative result significantly different.

Discussion

Findings of our study do not support the benefit of goal directed fluid therapy and cerebral oxygenation monitoring during surgery. There was no decrease the incidence of postoperative complications or duration of hospital stay. However, as opposed to some other studies, our groups were homogenous in terms of surgical procedure anaesthetic and pain management. If those factors are optimised, the contribution of multimodal monitoring seems to be lower than anticipated. Nevertheless, there were some important differences in fluid and vasopressor management among the groups.

Changes in intraoperative management

The use of multimodal monitoring resulted in differences in intraoperative management. The amount of fluid infused was higher in PG and vasoactive drugs were used more often. That suggests a trend towards more dynamic microcirculation. The most noticeable change in the postoperative period (related to differences in operative management) is a significant difference in the level of procalcitonin. Detailed discussion of those conclusions is provided below.

Fluid optimisation strategy

The results of the number of fluids given during the surgical procedure present an unexpectedly high fluid load in our PG. This group has received almost twice the number of fluids given in the CG. Intraoperative blood loss is comparable, and PG has a large positive intraoperative fluid balance. Thacker *et al.*, reports the relation between higher fluid load and longer stay.[27] However, the length of stay was similar in both groups in our study. The choice of fluid (colloides or crystalloides) does not seem to have impact on overall morbidity.[28]

One study compared goal-directed therapy (GDT) with standard fluid therapy in cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC). The study found that the use of a fluid therapy protocol combined with GDT was associated with a significant reduction in morbidity, length of hospital stay, and mortality compared to standard fluid therapy.[29] Similarly, Yu *et al*. conducted a controlled before-and-after study to evaluate the benefits of intraoperative goal-directed fluid therapy in major gynaecologic oncology surgery. The study found that the implementation of goal-directed fluid management was associated with a reduced risk of postoperative morbidities, particularly surgical site infections.[30] Another study analysed the impact of intraoperative fluid balance during pancreatoduodenectomy on the development of postoperative pancreatic fistula (POPF). The study found that fluid balance was significantly associated with the development of POPF, highlighting the importance of appropriate fluid management in pancreatic surgery.[31]

In addition, questions are raised about what the optimal goals of hemodynamic parameters (healthy population-derived normal values, preoperative values, maximal values) should be. Studies have shown that optimising cardiac output and oxygen delivery to higher values intraoperatively (supranormal) did not affect postoperative complications rate, intensive care unit stay, or hospital stay length.[32-34] The question of fluid concentration has also been raised. The liberal approach can lead to oedema of the intestines and other tissues that may be responsible for poor tissue healing and other complications.[35] In abdominal surgery protocol-based fluid restriction reduced the incidence of perioperative complications such as cardiopulmonary events and altered intestinal motility while improving wound and anastomotic healing and reducing hospital stay compared to liberal fluid management.[9,10] One of the trials has shown a 52% lower rate of major postoperative complications in the restrictive group than in the conventional group.[11]

Our study presents opposite results where the optimised group received a larger number of fluids. Our protocol has clearly defined steps when to add inotropes or fluid. One reason for the fluid load would be vasodilation due to epidural analgesia (all patients in our study have epidural analgesia), although vasoconstrictor (phenylephrine) in boluses was predicted to counteract the effect.[36] When comparing studies, 60 – 80% of the included patients have epidural analgesia.[21,37] Lopes *et al.*,

reports a significant decrease in ICU and hospital stay in the intervention group with an even greater difference in the number of infused crystalloids and colloids. They report a total volume of fluids infused 7 ml/kg /h in CG (roughly the same as in our study) and 21 ml/kg/h in the intervention group (12,5 in our study).[38] The choice of fluids and their timing also differs greatly.[20] The optimised group has received a larger volume of fluids, especially, although not exclusively, colloids.[39] Rare studies report other factors that greatly affect patient fluid status at the beginning, for example how long prior to the procedure are fasted, are fast track protocols implemented, etc. When trying to explain why sometimes one fluid regime (for example, restrictive) improves the outcome for the most, but not for all, we must realise that instead of restrictive or liberal there is only patient-directed fluid regime. Every patient should receive as much fluid as needed and at an appropriate time.[40]

Differences in the use of vasopressors

Significantly more patients with PG require vasopressor support with phenylephrine. Some articles suggest that anaesthesia after induction also causes venodilatation and not only arteriolar vasodilation (and consequently the decrease in MAP due to a change in volume out of the arterial tree into the dilated venous compartment).[41-43] Phenylephrine infusion before induction minimises this effect, but this is hardly the complete explanation of the difference. To keep hemodynamic parameters as close to starting values as possible, the anaesthetist in PG probably reacted earlier than in CG. In CG, if MAP was kept to some extent (due to reflex mechanisms) there was no information about hemodynamic changes that would require intervention (fall in cardiac output and stroke index). The number of MAP falls below 70 mmHg and 50 mmHg is similar in both groups, but that does not mean that the duration of hypotension is the same.

Monitoring depth of anaesthesia

BIS was used in both groups. This is in accordance with hospital policy, as total intravenous infusion was used to prevent intraoperative awareness.[44] In the context of multimodal monitoring, we omit an important variable that, without doubt, influences the outcome. Probably not only cognitive decline, but mortality and morbidity in general are related to too deep anaesthesia, a common occurrence without monitoring, especially in elderly people.[25,45-47]

The role of cerebral oximetry

Our study does not confirm the benefit of using a cerebral oximetry monitor during major abdominal surgery, at least it does not influence the results as presented in this study. Cerebral oxygenation monitoring cannot be considered as a monitor of overall tissue oxygenation.[48] The incidence of renal impairment can be considered one of the measures of adequate oxygenation.

Postoperative complications and length of stay

Neither the length of stay in the HDU nor the hospital stay decrease in PG. There are some postoperative complications such as the need for revision surgery, indication for antibiotic treatment third day after the procedure, or readmission to HDU that occur largely in the CG. Only comparison of individual complication does not show a statistically significant difference, but if we sum up all three, there is an obvious and statistically significant difference. Some other studies report more convincing but similar results.[49]

C-reactive protein after surgery was elevated in both groups. There were no significant differences between the groups. A significant difference in the highest postoperative levels (in first 3 days) of procalcitonin was noted. Despite this, no clinically or microbiologically evident bacterial infection was confirmed. The level of procalcitonin increases in response to a pro-inflammatory stimulus, especially of bacterial origin.[50] The median value in CG is above the reference range. Since the surgical management in both groups was similar, different fluid status might explain stronger inflammatory response to tissue trauma in one group. It is important to note that procalcitonin is not a specific marker for bacterial infections and can be influenced by various factors, including noninfectious inflammatory reactions and tissue trauma.[51,52] Therefore, the role of procalcitonin in noninfectious tissue trauma is still not well-defined, and further research is needed to determine its diagnostic utility in this context.[53]

Troponin leak was observed in 3 patients with PG. The increase is only marginally above the laboratory threshold value for positive. Acute myocardial infarction was ruled out in those patients with a high degree of confidence. Anyway, this can be related to a higher fluid load in the PG.

Mini Mental State Examination Testing

No major short-term differences in cognitive function are seen. Cognitive changes, related to the anaesthesia are much more subtile.[54-58]

Strengths and limitations of the study

The patients involved in the study are very homogeneous in terms of surgical procedures, perioperative surgical management, and comorbidities. Demographic characteristics of both groups were similar. The type of anaesthesia was the same (total intravenous anesthesia [TIVA] with propofol and epidural analgesia) in all the patients observed. Compared to other prospective studies, the number of patients included is comparable.[59]

Multimodal monitoring would probably provide more benefit, if used throughout the entire HDU stay not only during the surgical procedure.

Conclusions

In the present study, the joint use of hemodynamic monitoring and cerebral monitoring does not significantly decrease the length of stay in HDU or hospital stay in cancer patients after abdominal tumor resection. There is a difference in the volume of fluids infused, that is larger in the protocol group. There is also significantly higher use of vasopressors in the protocol group. The median postoperative value of procalcitonin was significantly higher in control group, suggesting differences in immune response to tissue trauma in different intraoperative fluid status.

There were no significant differences in the number of other postoperative complications observed in the postoperative period. The use of expensive additional monitoring may not provide benefit when used in general abdominal cancer surgery.

References

1. Kubo Y, Tanaka K, Yamasaki M, Yamashita K, Makino T, Saito T, et al. The impact of perioperative fluid balance on postoperative complications after esophagectomy for esophageal cancer. *J Clin Med* 2022; **11:** 3219. doi: 10.3390/jcm11113219
2. Noblett SE, Snowden CP, Shenton BK, Horgan AF. Randomized clinical trial assessing the effect of Doppler-optimized fluid management on outcome after elective colorectal resection. *Br J Surg* 2006; **93:** 1069-76. doi: 10.1002/bjs.5454
3. Potrc S, Ivanecz A, Pivec V, Marolt U, Rudolf S, Iljevec B, et al. Impact factors for perioperative morbidity and mortality and repercussion of perioperative morbidity and long-term survival in pancreatic head resection. *Radiol Oncol* 2018; **52:** 54-64. doi: 10.1515/raon-2017-0036
4. Sandham JD, Hull RD, Brant RF, Knox L, Pineo GF, Doig CJ, et al. A randomized, controlled trial of the use of pulmonary-artery catheters in high-risk surgical patients. *N Engl J Med* 2003; **348:** 5-14. doi: 10.1056/NEJMoa021108
5. Walsh SR, Tang T, Bass S, Gaunt ME. Doppler-guided intra-operative fluid management during major abdominal surgery: systematic review and meta-analysis. *Int J Clin Pract* 2008; **62:** 466-70. doi: 10.1111/j.1742-1241.2007.01516.x
6. Gurgel ST, Do Nascimento P. Maintaining tissue perfusion in high-risk surgical patients: a systematic review of randomized clinical trials. *Anesth Analg* 2011; **112:** 1384-91. doi: 10.1213/ANE.0b013e3182055384
7. Navarro LHC, Bloomstone JA, Auler JOC, Cannesson M,Della Rocca G,Tong J Gan TJ, et al. Perioperative fluid therapy: a statement from the International fluid optimization group. *Perioper Med* 2015; **4:** 3. doi: 10.1186/s13741-015-0014-z
8. Saugel B, Flick M, Bendjelid K, Critchley LAH, Vistisen ST, Scheeren TWL. Journal of clinical monitoring and computing end of year summary 2018: hemodynamic monitoring and management. *J Clin Monit Comput* 2019; **33:** 211-22. doi: 10.1007/s10877-019-00297-w
9. Giglio MT, Marucci M, Testini M, Brienza N. Goal-directed haemodynamic therapy and gastrointestinal complications in major surgery: a meta-analysis of randomized controlled trials. *Br J Anaesth* 2009; **103:** 637-46. doi: 10.1093/bja/aep279
10. Grocott MPW, Dushianthan A, Hamilton MA, Mythen MG, Harrison D, Rowan K. Perioperative increase in global blood flow to explicit defined goals and outcomes after surgery: a Cochrane systematic review. *Br J Anaesth* 2013; **111:** 535-48. doi: 10.1093/bja/aet155
11. Pearse RM, Harrison DA, MacDonald N, Gillies MA, Blunt M, Ackland G, et al. Effect of a perioperative, cardiac output–guided hemodynamic therapy algorithm on outcomes following major gastrointestinal surgery. *JAMA* 2014; **311:** 2181. doi: 10.1001/jama.2014.5305
12. Murkin JM, Arango M. Near-infrared spectroscopy as an index of brain and tissue oxygenation. *Br J Anaesth* 2009; **103:** 3-13. doi: 10.1093/bja/aep299
13. Slater JP, Guarino T, Stack J, Vinod K, Bustami RT, Brown JM, et al. Cerebral oxygen desaturation predicts cognitive decline and longer hospital stay after cardiac surgery. *Ann Thorac Surg* 2009; **87:** 36-45. doi: 10.1016/j.athoracsur.2008.08.070
14. Bisgaard J, Gilssa T, Ronholm E, Toft P. Optimising stroke volume and oxygen delivery in abdominal aortic surgery: a randomised controlled trial. *Acta Anaesthesiol Scand* 2013; **57:** 178-88. doi: 10.1111/j.1399-6576.2012.02756.x
15. Chan MTV, Cheng BCP, Lee TMC, Gin T. BIS-guided anesthesia decreases postoperative delirium and cognitive decline. *J Neurosurg Anesthesiol* 2013; **25:** 33-42. doi: 10.1097/ANA.0b013e3182712fba
16. Ballard C, Jones E, Gauge N, Aarsland D, Nilsen OB, Saxby BK, et al. Optimised anaesthesia to reduce post operative cognitive decline (POCD) in older patients undergoing elective surgery, a randomised controlled trial. *PLoS One* 2012; **7:** 1-9. doi: 10.1371/journal.pone.0037410
17. Green D, Paklet L. Latest developments in peri-operative monitoring of the high-risk major surgery patient. *Int J Surg* 2010; **8:** 90-9. doi: 10.1016/j.ijsu.2009.12.004
18. Fernandes A, Rodrigues J, Antunes L. Development of a preoperative risk score on admission in surgical intermediate care unit in gastrointestinal cancer surgery. *Perioper Med* 2020; **9:** 1-9. doi: 10.1186/s13741-020-00151-7
19. Kaufmann T, Clement RP, Scheeren TWL, Saugel B, Keus F, van der Horst ICC. Perioperative goal-directed therapy: a systematic review without meta-analysis. *Acta Anaesthesiol Scand* 2018; **62:** 1340-55. doi: 10.1111/aas.13212
20. Harten J, Crozier JEM, McCreath B, Hay A, McMillan DC, McArdle CS, et al. Effect of intraoperative fluid optimisation on renal function in patients undergoing emergency abdominal surgery: a randomised controlled pilot study (ISRCTN 11799696). *Int J Surg* 2008; **6:** 197-204. doi: 10.1016/j.ijsu.2008.03.002

21. Benes J, Chytra I, Altmann P, Hluchy M, Kasal E, Svitak R, et al. Intraoperative fluid optimization using stroke volume variation in high risk surgical patients: results of prospective randomized study. *Crit Care* 2010; **14:** R118. doi: 10.1186/cc9070

22. Prytherch DR, Whiteley MS, Higgins B, Weaver PC, Prout WG, Powell SJ. POSSUM and Portsmouth POSSUM for predicting mortality. *Br J Surg* 1998; **85:** 1217-20. doi: 10.1046/j.1365-2168.1998.00840.x

23. Bose S, Talmor D. Who is a high-risk surgical patient? *Curr Opin Crit Care* 2018; **24:** 547-53. doi: 10.1097/MCC.0000000000000556

24. Scott S, Lund JN, Gold S, Elliott R, Vater M, Chakrabarty M, et al. An evaluation of POSSUM and P-POSSUM scoring in predicting post-operative mortality in a level 1 critical care setting. *BMC Anesthesiol* 2014; **14:** 1-7. doi: 10.1186/1471-2253-14-104

25. Bidd H, Tan A, Green D. Using bispectral index and cerebral oximetry to guide hemodynamic therapy in high-risk surgical patients. *Perioper Med* 2013; **2:** 11. doi: 10.1186/2047-0525-2-11

26. Patten SB, Fick GH. Clinical interpretation of the mini-mental state. *Gen Hosp Psychiatry* 1993; **15:** 254-9. doi: 10.1016/0163-8343(93)90040-U

27. Thacker JKM, Mountford WK, Ernst FR, Krukas MR, Mythen MG. Perioperative fluid utilization variability and association with outcomes. *Ann Surg* 2016; **263:** 502-10. doi: 10.1097/SLA.0000000000001402

28. Markovic-Bozic J, Visocnik B, Music P, Potocnik I, Vesel AS. Crystalloids vs. colloids for fluid optimization in patients undergoing brain tumour surgery. *Radiol Oncol* 2022; **56:** 50814. doi:10.2478/raon-2022-0035

29. Colantonio L, Claroni C, Fabrizi L, Marcelli ME, Sofra M, Giannarelli D. A randomized trial of goal directed vs standard fluid therapy in cytoreductive surgery with hyperthermic intraperitoneal chemotherapy. *J Gastrointest Surg* 2015; **19:** 722-9. doi: 10.1007/s11605-015-2743-1

30. Yu J, Che L, Zhu A, Xu L, Huang Y. Goal-directed intraoperative fluid therapy benefits patients undergoing major gynecologic oncology surgery: a controlled before-and-after study. *Front Oncol* 2022; **12:** 1-8. doi: 10.3389/fonc.2022.833273

31. Zhang L, Zhang Y, Shen L. Effects of intraoperative fluid balance during pancreatoduodenectomy on postoperative pancreatic fistula: an observational cohort study. *BMC Surg* 2023; **23:** 1-9. doi: 10.1186/s12893-023-01978-9

32. Shoemaker WC, Appel PL, Kram HB, Waxman K, Lee TS. Prospective trial of supranormal values of survivors as therapeutic goals in high-risk surgical patients. *Chest* 1988; **94:** 1176-86. doi: 10.1378/chest.94.6.1176

33. Velmahos GC, Demetriades D, Shoemaker WC, Chan LS, Tatevossian R, Wo CC, et al. Endpoints of resuscitation of critically injured patients: normal or supranormal? A prospective randomized trial. *Ann Surg* 2000; **232:** 409-18. doi: 10.1007/BF03019819

34. Kim HJ, Kim EJ, Lee HJ, Min JY, Kim TW, Choi EC, et al. Effect of goal-directed haemodynamic therapy in free flap reconstruction for head and neck cancer. *Acta Anaesthesiol Scand* 2018; **62:** 903-14. doi: 10.1111/aas.13100

35. Licker M, Hagerman A, Bedat B, Ellenberger C, Triponez F, Schorer R, et al. Restricted, optimized or liberal fluid strategy in thoracic surgery: a narrative review. *Saudi J Anaesth* 2021; **15:** 324. doi: 10.4103/sja.sja_1155_20

36. Jozwiak M, Rex S, Bendjelid K. Boosting systemic pressure with phenylephrine: arterial or venous modulation? *J Clin Monit Comput* 2018; **32:** 967-8. doi: 10.1007/s10877-018-0177-5

37. Zheng H, Guo H, Ye J, Chen L, Ma H. Goal-directed fluid therapy in gastrointestinal surgery in older coronary heart disease patients: Randomized trial. *World J Surg* 2013; **37:** 2820-9. doi: 10.1007/s00268-013-2203-6

38. Lopes MR, Oliveira MA, Pereira V, Lemos I, Auler J, Michard F. Goal-directed fluid management based on pulse pressure variation monitoring during high-risk surgery: a pilot randomized controlled trial. *Crit Care* 2007; **11:** R100. doi: 10.1186/cc6117

39. Benes J, Giglio M, Brienza N, Michard F. The effects of goal-directed fluid therapy based on dynamic parameters on post-surgical outcome: a meta-analysis of randomized controlled trials. *Crit Care* 2014; **18:** 584. doi: 10.1186/s13054-014-0584-z

40. Kirov MY, Kuzkov V V, Molnar Z. Perioperative haemodynamic therapy. *Curr Opin Crit Care* 2010; **16:** 384-92. doi: 10.1097/MCC.0b013e32833ab81e

41. Green DW. Cardiac output decrease and propofol: what is the mechanism? *Br J Anaesth* 2015; **114:** 163-4 doi: 10.1093/bja/aeu424

42. Wolff CB, Green DW. Clarification of the circulatory patho-physiology of anaesthesia - Implications for high-risk surgical patients. *Int J Surg* 2014; **12:** 1348-56. doi: 10.1016/j.ijsu.2014.10.034

43. Moller Petrun A, Kamenik M. Bispectral index-guided induction of general anaesthesia in patients undergoing major abdominal surgery using propofol or etomidate: a double-blind, randomized, clinical trial. *Br J Anaesth* 2013; **110:** 388-96. doi: 10.1093/bja/aes416

44. Avidan MS, Zhang L, Burnside BA, Finkel KJ, Searleman AC, Selvidge JA, et al. Anesthesia awareness and the bispectral index. *N Engl J Med* 2008; **358:** 1097-108. doi: 10.1056/NEJMoa0707361

45. Zhou C, Zhu Y, Liu Z, Ruan L. Effect of dexmedetomidine on postoperative cognitive dysfunction in elderly patients after general anaesthesia: a meta-analysis. *J Int Med Res* 2016; **44:** 1182-90. doi: 10.1177/0300060516671623

46. Hanning CD. Postoperative cognitive dysfunction. *Br J Anaesth* 2005; **95:** 82-7. doi: 10.1093/bja/aei062

47. Jildenstål PK, Hallén JL, Rawal N, Berggren L. Does depth of anesthesia influence postoperative cognitive dysfunction or inflammatory response following major ent surgery? *J Anesth Clin Res* 2012; **3:** 6. doi: 10.4172/2155-6148.1000220

48. Klijn E, van Velzen MHN, Lima AP, Bakker J, van Bommel J, Groeneveld ABJ. Tissue perfusion and oxygenation to monitor fluid responsiveness in critically ill, septic patients after initial resuscitation: a prospective observational study. *J Clin Monit Comput* 2015; **29:** 707-12. doi: 10.1007/s10877-014-9653-8

49. Salzwedel C, Puig J, Carstens A, Bein B, Molnar Z, Kiss K, et al. Perioperative goal-directed hemodynamic therapy based on radial arterial pulse pressure variation and continuous cardiac index trending reduces postoperative complications after major abdominal surgery: a multi-center, prospective, randomized study. *Crit Care* 2013; **17:** R191. doi: 10.1186/cc12885

50. Hamade B, Huang DT. Procalcitonin: where are we now? *Crit Care Clin* 2020; **36:** 23-40. doi: 10.1016/j.ccc.2019.08.003

51. Park JH, Wee JH, Choi SP, Oh JH, Cheol S. Assessment of serum biomarkers and coagulation/fibrinolysis markers for prediction of neurological outcomes of out of cardiac arrest patients treated with therapeutic hypothermia. *Clin Exp Emerg Med* 2019; **6:** 9-18. doi: 10.15441/ceem.17.273

52. Ribaric Filekovic S, Turel M, Knafelj R. Prophylactic versus clinically-driven antibiotics in comatose survivors of out-of-hospital cardiac arrest – a randomized pilot study. *Resuscitation* 2017; **111:** 103-9. doi: 10.1016/j.resuscitation.2016.11.025

53. Alons IME, Verheul RJ, Kuipers I, Jellema K, Wermer MJ, Algra A, et al. Procalcitonin in cerebrospinal fluid in meningitis: a prospective diagnostic study. *Brain Behav* 2016; **6:** 1-7. doi: 10.1002/brb3.545

54. Avidan MS, Evers AS. Review of clinical evidence for persistent cognitive decline or incident dementia attributable to surgery or general anesthesia. *J Alzheimer's Dis* 2011; **24:** 201-16. doi: 10.3233/JAD-2011-101680

55. Shoair O, Grasso II M, Lahaye L, Daniel R, Biddle C, Slattum P. Incidence and risk factors for postoperative cognitive dysfunction in older adults undergoing major noncardiac surgery: a prospective study. *J Anaesthesiol Clin Pharmacol* 2015; **31:** 30. doi: 10.4103/0970-9185.150530

56. Monk TG, Weldon BC, Garvan CW, Dede DE, van der Aa MT, Heilman KM, et al. Predictors of cognitive dysfunction after major noncardiac surgery. *Anesthesiology* 2008; **108:** 18-30. doi: 10.1097/01.anes.0000296071.19434.1e

57. Avidan MS, Searleman AC, Storandt M, Barnett K, Vannucci A, Saager L, et al. Long-term cognitive decline in older subjects was not attributable to noncardiac surgery or major illness. *Anesthesiology* 2009; **111:** 964-70. doi: 10.1097/ALN.0b013e3181bc9719

58. Marasco SF, Sharwood LN, Abramson MJ. No improvement in neurocognitive outcomes after off-pump versus on-pump coronary revascularisation: a meta-analysis. *Eur J Cardio-thoracic Surg* 2008; **33:** 961-70. doi: 10.1016/j.ejcts.2008.03.022

59. Challand C, Struthers R, Sneyd JR, Erasmus PD, Mellor N, Hosie KB, et al. Randomized controlled trial of intraoperative goal-directed fluid therapy in aerobically fit and unfit patients having major colorectal surgery. *Br J Anaesth* 2012; **108:** 53-62. doi: 10.1093/bja/aer273

research article

Dosimetry and efficiency comparison of knowledge-based and manual planning using volumetric modulated arc therapy for craniospinal irradiation

Wei-Ta Tsai[1,2], Hui-Ling Hsieh[2], Shih-Kai Hung[2,3], Chi-Fu Zeng[2], Ming-Fen Lee[2], Po-Hao Lin[2], Chia-Yi Lin[2], Wei-Chih Li[4], Wen-Yen Chiou[2,3], Tung-Hsin Wu[1]

[1] Department of Biomedical Imaging and Radiological Sciences, National Yang Ming Chiao Tung University, Taipei, Taiwan
[2] Department of Radiation Oncology, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Chiayi, Taiwan
[3] School of Medicine, Tzu Chi University, Hualien, Taiwan
[4] Departments of Radiation Oncology, Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taichung, Taiwan

Radiol Oncol 2024; 58(2): 289-299.

Received 4 November 2023
Accepted 3 January 2024

Correspondence to: Tung-Hsin Wu, Ph.D., Department of Biomedical Imaging and Radiological Sciences, National Yang Ming Chiao Tung University, No. 155, Sec. 2, Linong St., Beitou Dist., Taipei City 112304, Taiwan, E-mail: tung@ym.edu.tw; Tel: (886) 02-28201095 and Wen-Yen Chiou, MD, Ph.D., Department of Radiation Oncology, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, No. 2, Ming Sheng Road, Dalin Town, Chiayi, 622401, Taiwan, E-mail: cwyncku@gmail.com; Tel: (886) 05-2648000 extension 5695.

Disclosure: No potential conflicts of interest were disclosed.

Background. Craniospinal irradiation (CSI) poses a challenge to treatment planning due to the large target, field junction, and multiple organs at risk (OARs) involved. The aim of this study was to evaluate the performance of knowledge-based planning (KBP) in CSI by comparing original manual plans (MP), KBP RapidPlan initial plans (RP_I), and KBP RapidPlan final plans (RP_F), which received further re-optimization to meet the dose constraints.

Patients and methods. Dose distributions in the target were evaluated in terms of coverage, mean dose, conformity index (CI), and homogeneity index (HI). The dosimetric results of OARs, planning time, and monitor unit (MU) were evaluated.

Results. All MP and RP_F plans met the plan goals, and 89.36% of RP_I plans met the plan goals. The Wilcoxon tests showed comparable target coverage, CI, and HI for the MP and RP_F groups; however, worst plan quality was demonstrated in the RP_I plans than in MP and RP_F. For the OARs, RP_F and RP_I groups had better dosimetric results than the MP group ($P < 0.05$ for optic nerves, eyes, parotid glands, and heart). The planning time was significantly reduced by the KBP from an average of 677.80 min in MP to 227.66 min ($P < 0.05$) and 307.76 min ($P < 0.05$) in RP_I, and RP_F, respectively. MU was not significantly different between these three groups.

Conclusions. The KBP can significantly reduce planning time in CSI. Manual re-optimization after the initial KBP is recommended to enhance the plan quality.

Key words: knowledge-based planning; RapidPlan; craniospinal irradiation; volumetric modulated arc therapy

Introduction

Prophylactic or therapeutic craniospinal irradiation (CSI) is an option for managing certain primary brain tumors, such as medulloblastoma, or hematologic malignancies.[1] Since the maximum field of the linear accelerator is 40 cm by 40 cm, the conventional three-dimensional conformal radiation therapy (3D-CRT) techniques for CSI use two opposed lateral craniocervical fields adjoined by two adjacent posterior spinal fields. In conventional CSI techniques, the fields are matched between

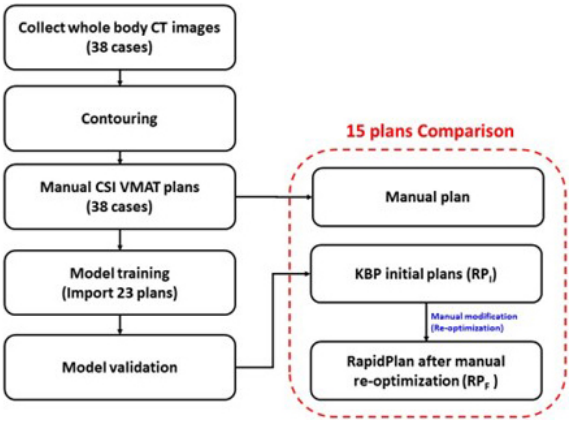


FIGURE 1. Flowchart of the study design.

CSI = craniospinal irradiation; CT = computed tomography; KBP = knowledge-based planning; VMAT = volumetric modulated arc therapy

the lateral and posterior fields, creating over- or underdosage within the spinal cord. To address this issue, 3D-CRT with the moving junction technique[2,3], which involves changing different junction locations daily during the treatment course, is an option to blur the dose ununiform effect.

The moving junction technique in 3D-CRT requires the use of multiple treatment plans, which increases the complexity of treatment planning and daily treatment. Moreover, the CSI moving junction technique can only reduce the dose ununiform effect but cannot obtain dose homogeneity as a common treatment. With the development of commercial treatment planning system (TPS), volumetric modulated arc therapy (VMAT) with multi-isocenter optimization[4] was introduced. VMAT with 360-degree beams can achieve higher conformity and better dispersion of normal organs compared to conventional 3D-CRT.[5,6] The VMAT technique with large field overlaps for low-dose gradient junction could tolerate greater positional shifts while maintaining homogeneous dose.[7,8] However, planning CSI using the high-precision VMAT technique is challenging and time-consuming for medical physicists due to the long treatment field from the brain to the lumbosacral region, which significantly exceeds the treatment field size of a linear accelerator and involves more than ten organs at risk. Because CSI treatment is relatively rare and only patients with possible malignancy tumor cells seeding in the craniospinal canal receive this treatment, medical physicists in many institutions are unfamiliar with this technique. The rarity of the expertise and complex planning processes make this process resource-intensive.

Knowledge-based planning (KBP) is based on a model of estimating dose-volume histograms (DVHs), which is configured by a library of historical treatment plans with the aim of improving planning efficiency.[9] In previous studies, KBP has been adopted to treat patients with several cancer types, such as head and neck cancers and pelvic malignancies.[10-13] KBP showed improved planning efficiency with well-reserved plan quality in those cancer sites. However, compared to those cancer sites, CSI would require more treatment isocenters and patients moving with junction feathering. Moreover, more organs at risk (OARs) needed to be considered in CSI than other treatment sites. Reviewing the literature, previous CSI studies have not compared the plan quality and cost-effectiveness of the general manual plan method and the KBP with and without re-optimization.

This study aimed to compare the plan quality and efficiency of the original manual plans (MP), KBP initial plans (RP_I) (RapidPlan™, Varian Medical Systems, Palo Alto, USA), and KBP final plans, which received further re-optimization (RP_F) for CSI.

Patients and methods

Ethics statement

The Institutional Review Board of the Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation approved this study (approval number, B10804011-1) and waived the requirement for written informed consent from the patients involved because only anonymized images were retrospectively analyzed, and this study did not affect the actual treatments these patients received before.

Patients

This study retrospectively collected computed tomography (CT) image sets of 38 anonymized adults assessed between 2014 and 2019. All the image sets met the requirement of immobilization, supine position, and scan from head to pelvis. The slice thickness and matrix size were 3–5 mm and 512 × 512 voxels, respectively (Figure 1).

Target and OAR delineation

The clinical target volume (CTV) includes the whole brain and spinal cord, typically extended to the lumbar spine L3 level. Assembled CTV was

separated into CTV-brain, CTV-spine-superior, and CTV-spine-inferior for the multiple field optimization (Figure 2). The PTV-brain was constructed by symmetrically extending the CTV-brain by 3 mm and by adding 5 mm margin to the spine area. The maximum and minimum lengths of the CTV were 77.83 cm and 65.40 cm, while those of the PTV were 78.80 cm and 66.38 cm. The mean lengths of the CTV and PTV were 71.15 ± 4.28 cm and 72.23 ± 4.16 cm, respectively. The mean CTV and PTV were 1413.40 ± 162.18 cm^3 and 1823.93 ± 192.14 cm^3, respectively. For planning evaluation purposes, the PTV-brain, PTV-spine-superior, and PTV-spine-inferior were combined as PTV.

Dose prescription

The dose prescription was 36 Gy in 18 daily fractions. All plans were normalized so that 95% of the PTV received 100% of the prescribed dose.

Treatment planning

The 38 CT image sets of anonymized adults were imported to Eclipse TPS version 13.6 (Varian Medical Systems, Palo Alto, CA, USA). Overall, six medical physicists were participated in this study. Plans for each patient were reviewed and approved by the same physician. A TrueBeam linear accelerator (Varian Medical Systems, Palo Alto, CA, USA) equipped with a 120-leaf multileaf collimator was selected. All plans were set as 6 megavoltage for the VMAT technique. Analytical Anisotropic Algorithm dose calculation algorithm, 2.5 mm dose calculation grid, and jaw tracking were used. The mean lateral field size for the brain field is 14.76 ± 0.08 cm, while the average lateral field size for the spine field is 12.42 ± 2.52 cm. These dimensions are adjusted to encompass the entire target within a reasonable rotation range. Jaw tracking technique is used to minimize the impact of transmission leakage dose to normal organ. The collimator rotation angle is set within a range of ± 35 degrees for the head and ± 12 degrees for the spine, according to the physicist's discretion at the time.

The whole target length was more than 100 cm, whereas the maximum single-field size of a linear accelerator at the isocenter is 40 × 40 cm. Therefore, multiple fields and three isocenters were required. The PTV-brain used two full arcs, with the isocenter positioned at the center of the brain. For the PTV-spine, two or four partial arcs were used on the PTV-spine-superior, and PTV-spine-inferior to avoid the 60–120-degree and 240–300-degree direction for arm sparing. For the sake of clinical convenience, the three isocenters were aligned along the same X-axis (left-right). The spine isocenter shared the same X and Y coordinates, differing only along the Z-axis (craniocaudal) (Figure 2).

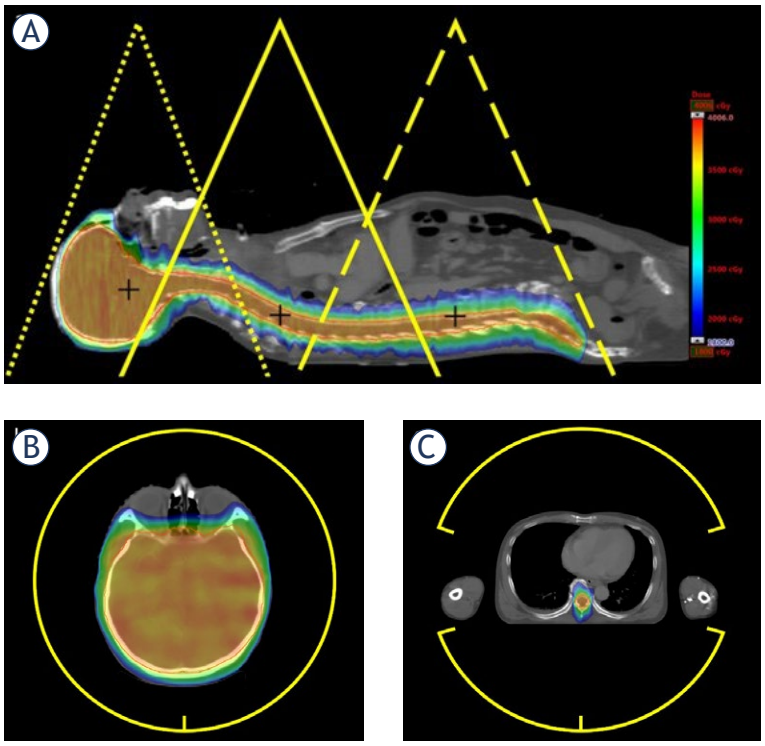


FIGURE 2. Example of the target and field setup. **(A)** The arrangement of the brain field (dotted lines), spine-superior field (solid lines), spine-inferior field (dashed lines), and their isocenters. Each field overlaps at least 5 cm for the low-dose gradient junction. **(B)** Full arc was used on the brain field. **(C)** Partial arc was used in the spinal fields for arm sparing.

A total of 38 MPs were generated for the 38 patients, with 23 MPs used to train the RapidPlan (RP) model, and 15 MPs used for validation and comparison (Figure 1). Using RP, 15 RP initial plans (RP_I) were generated without manual modification, on which we performed further manual re-optimization to generate 15 RP final plans (RP_F). Finally, we compared the following three plan groups: MP, RP_I, and RP_F.

Knowledge-based planning

The RapidPlan is a commercial KBP program integrated within the Eclipse TPS. The KBP program references a library of previously clinically accepted treatment plans. It analyzes the geometric and dosimetric features, such as structure sets, field geometry, dose matrices and plan prescriptions of those plans to train a statistical model. This mod-

el is then used to predict an achievable range of DVHs and generate dose-volume objectives for a new plan.

RapidPlan algorithm

The RapidPlan algorithm comprises two main components: model configuration and DVH estimation. The model configuration component is responsible for setting up new DVH estimation models, which are subsequently utilized in the DVH estimation component to generate estimates for an individual plan. The model configuration component encompasses two distinct phases: data extraction and model training. On the other hand, the DVH estimation component encompasses the phases of estimation generation and objective generation.

The minimum requirement of data extraction and model training was 20 plans with their targets and OARs. Among the 20 randomly selected plans for model training, the right lens of three plans were too small to evaluate. Therefore, we added three more plans to meet the training requirement.

The model training phase within the DVH estimation algorithm is dedicated to the creation of DVH estimation models. The estimation generation phase calculates for each supported structure the same metrics that were calculated during the data extraction of the DVH estimation model, except for the DVH. Once the estimation generation phase has derived the upper and lower bound DVHs, the optimization objectives placement phase translates them into optimization objectives.

Plan quality, planning time, and monitor unit comparison

There were 27 dosimetric goals of irradiated fields and OARs were evaluated for the three groups among 15 patients. One patient had previously undergone thyroidectomy, and his thyroid dose could not be evaluated. This resulted in a total of 404 items being calculated for model evaluation. Dosimetric characteristics, such as V_{95}, V_{100}, V_{107}, D_{mean}, D_{max}, and D_2 of CTV, and PTV, were evaluated. In addition, conformity index (CI) and homogeneity index (HI) of the targets and dose gradient ($R_{x\%}$) were compared.[14] The Radiation Therapy Oncology Group (RTOG) criteria define CI values to be between 1.0 and 2.0 in accordance with the protocol, 2.0 to 2.5 and 0.9 to 1.0 as a minor deviation, and > 2.5 and < 0.9 as a major deviation from the protocol. The CI was defined as a ratio between the volume covered by the reference isodose (36 Gy) and the target volume, as in Equation [1].

$$CI_{RTOG} = \frac{V_{RI}}{TV} \quad [1]$$

where V_{RI} = Reference isodose volume and TV = target volume.

The HI is the ratio between maximum isodose and reference isodose. The formula of HI was shown as Equation [2]. The ideal value is 1, which increases as the plan becomes less homogeneous.

$$HI_{RTOG} = \frac{I_{max}}{RI} \quad [2]$$

Where I_{max} = maximum isodose in the target and RI = reference isodose.

The dose gradient ($R_{x\%}$) formula is given below:

$$R_{x\%} = \frac{V_{x\%}}{TV} \quad [3]$$

where $V_{x\%}$ = percentage of isodose volume, and TV = target volume.

The pre-optimization, optimization, and re-optimization planning times were compared. The pre-optimization time included OARs contouring and field setup, and the re-optimization time was the time of further optimization and calculation until the plan was satisfied. Average monitor units (MUs) were also evaluated.

Statistical analysis

The Wilcoxon test was used to compare the differences between the three groups. The differences in the dose coverage, mean dose of the targets, and OARs were compared with a 95% confidence interval. All tests were two-sided. A p value of < 0.05 was considered statistically significant. SPSS statistical package (version 17; SPSS Inc., Chicago, IL) was used for all statistical analysis.

Results

Target coverage and OAR sparing

Table 1 shows the dosimetric results of targets. For the V_{100}, V_{107}, D_{max}, and D_2 of the CTV, both MP and RP_F groups were significantly better than RP_I ($P < 0.01$). MP and RP_F in most subjects were not significantly different, except for V_{95}. For PTV, the V_{100} was normalized to 95% prescribed dose for all three groups, MP, RP_I, and RP_F. MP and RP_F groups had significantly better V_{107}, D_{max}, D_2, and HI than did the RP_I group ($P < 0.01$). The MP group had a worse CI than the other groups. In addition, among 13 compared parameters (Table 1), the RP_I had worse results in 84.62% (11/13) parameters

TABLE 1. Dosimetric comparison between manual plans, RapidPlan initial, and RapidPlan final

Parameters	Goals	Results			*P value*		
		MP	RP_I	RP_F	MP *vs.* RP_I	MP *vs.* RP_F	RP_I *vs.* RP_F
CTV							
V_{95} [%]	> 99	99.99 ± 0.03	99.98 ± 0.03	99.97 ± 0.03	0.36	0.03*	0.09
V_{100} [%]	> 99	99.20 ± 0.17	98.37 ± 0.33	99.37 ± 0.23	< 0.01**	0.07	< 0.01**
V_{107} [%]	Minimize	0.62 ± 0.59	2.94 ± 4.33	0.46 ± 0.66	< 0.01**	0.16	< 0.01**
D_{mean} [Gy]	36	37.23 ± 0.18	37.31 ± 0.21	37.22 ± 0.24	0.07	0.87	0.13
D_{max} [Gy]	Minimize	39.38 ± 0.40	40.38 ± 0.57	39.42 ± 0.41	< 0.01**	0.78	< 0.01**
D_2 [%]	< 107	106.12 ± 0.73	106.95 ± 0.96	105.72 ± 0.84	< 0.01**	0.19	< 0.01**
PTV							
V_{95} [%]	> 98	99.68 ± 0.15	99.55 ± 0.23	99.24 ± 0.32	0.03*	< 0.01**	< 0.01**
V_{100} [%]	= 95	95.00 ± 0.00	95.00 ± 0.00	95.00 ± 0.00	-	-	-
V_{107} [%]	Minimize	0.62 ± 0.57	3.01 ± 4.12	0.44 ± 0.61	< 0.01**	0.17	< 0.01**
D_{mean} [Gy]	36	37.10 ± 0.16	37.22 ± 0.18	37.08 ± 0.20	0.05	0.73	0.01*
D_{max} [%]	< 112	109.99 ± 1.17	112.89 ± 1.78	110.17 ± 1.14	< 0.01**	0.57	< 0.01**
D_2 [%]	< 107	106.09 ± 0.73	107.00 ± 0.93	105.71 ± 0.80	< 0.01**	0.21	< 0.01**
CI	1	0.98 ± 0.01	1.01 ± 0.01	1.00 ± 0.01	< 0.01**	< 0.01**	0.01*
HI	1	1.10 ± 0.01	1.13 ± 0.02	1.10 ± 0.01	< 0.01**	0.57	< 0.01**

CI = conformity index; CTV = clinical target volume; Dx = minimum dose received by the hottest x% volume; HI = homogeneity index; MP = manual plan; PTV = planning target volume; RP_I = RapidPlan initial; RP_F = RapidPlan final; Vx = volume receiving at least x dose; * = $P < 0.05$; ** = $P < 0.01$

compared to the MP and RP_F groups, which had the best results in 30.77% (4/13) and 61.53% (8/13) parameters, respectively. The value of HI was the same in the MP and RP_F groups.

Furthermore, there were 14 OARs and 20 evaluation parameters for these OARs (Table 2). RP_F and RP_I had better dosimetric results than MP for the D_{mean} of optic nerves, parotid glands, heart, and esophagus, and D_{max} of eyes (all $P < 0.05$). The RP_F group was significantly better than the RP_I group in 11 parameters ($P \leq 0.01$); no parameter in the RP_F group was worse than any parameter in the RP_I group. RP_F had comparable results to the MP group in the other OARs including, brain, brain stem, chiasma, lens, thyroid, lungs, liver, and kidneys. In conclusion, when comparing the three groups, except the heart $V_{40,}$ which was 0% in all these three groups, the MP and RP_I groups obtained the worst results in 63.16% (12/19) and 36.84% (7/19) OAR parameters, respectively. On the contrary, the RP_F group had 73.68% (14/19) OAR parameters that were superior or equal to the other two groups.

Overall, the RP_F group achieved superior or equal best results in 71.88% (23/32) of the 32 evaluation parameters of the targets (13) and OARs (19), which excluding the PTV $V_{100\%}$ and heart V_{40Gy}, because the volumes were the same in all three groups.

In this study, we evaluated the quality of the treatment plans for three groups of 15 patients each. We used 27 parameters to evaluate each plan, for a total of 404 parameters, due to one patient who did not have a thyroid gland. We did not include the parameters CTV $V_{107\%}$, CTV D_{mean}, CTV D_{max}, PTV $V_{107\%}$, PTV D_{mean}, CI, and HI in the evaluation because they did not have specific goal values. The plan quality pass rate of the MP and RP_F groups was 100% (404/404) according to the plan goals of targets and OARs. The RP_I group pass rate was 89.36% (361/404). When evaluating the failures of the RP_I group, although no patient in the RP_I group passed the CTV V_{100} goal of 99%, the minimum and median values of RP_I CTV V_{100} were 97.83% and 98.44%, respectively, and both the CTV V_{95} and the PTV V_{95} of RP_I group reached the goals.

TABLE 2. Dosimetric goals and results for organs at risk

OAR parameters	Goals	Results			*P value*		
		MP	RP_I	RP_F	MP *vs.* RP_I	MP *vs.* RP_F	RP_I *vs.* RP_F
Brain							
D_{max} [Gy]	< 60	39.34 ± 0.39	40.24 ± 0.61	39.28 ± 0.37	< 0.01**	0.46	< 0.01**
Brain stem							
D_{max} [Gy]	< 54	38.48 ± 0.41	39.03 ± 0.46	38.51 ± 0.28	< 0.01**	0.91	< 0.01**
Chiasm							
D_{mean} [Gy]	< 50	37.15 ± 0.35	37.10 ± 0.32	36.95 ± 0.32	0.69	0.07	0.06
D_{max} [Gy]	< 55	38.13 ± 0.40	38.73 ± 0.55	38.20 ± 0.26	0.01*	0.46	< 0.01**
Optic nerves							
D_{mean} [Gy]	< 50	27.61 ± 3.40	22.90 ± 2.39	22.42 ± 2.29	< 0.01**	< 0.01**	0.05
D_{max} [Gy]	< 55	37.13 ± 0.69	36.36 ± 1.72	36.15 ± 1.39	0.13	0.02*	0.13
Eyes							
D_{max} [Gy]	< 50	25.55 ± 3.57	22.52 ± 3.83	21.60 ± 3.86	0.02*	0.01*	0.01*
Lens							
D_{max} [Gy]	< 10	8.40 ± 0.68	8.87 ± 1.00	8.10 ± 0.55	0.11	0.21	< 0.01**
Parotid glands							
D_{mean} [Gy]	< 25	7.38 ± 2.52	5.16 ± 0.39	4.95 ± 0.39	< 0.01**	< 0.01**	< 0.01**
Spinal cord							
D_{max} [Gy]	< 50	38.93 ± 0.51	39.81 ± 0.67	39.04 ± 0.56	< 0.01**	< 0.01**	< 0.01**
Thyroid							
D_{max} [Gy]	< 45	17.23 ± 4.04	16.68 ± 2.13	16.38 ± 2.04	0.59	0.36	0.07
Lungs							
D_{mean} [Gy]	< 13	4.63 ± 0.30	4.95 ± 0.43	4.63 ± 0.24	0.03*	0.73	< 0.01**
V_{20Gy} [%]	< 22	0.06 ± 0.11	0.04 ± 0.08	0.03 ± 0.04	0.64	0.44	0.33
V_{5Gy} [%]	< 42	36.48 ± 2.88	42.77 ± 5.62	37.11 ± 2.87	0.01*	0.69	< 0.01**
Heart							
D_{mean} [Gy]	< 10	6.76 ± 1.47	5.53 ± 0.82	5.70 ± 0.93	0.01*	0.02*	0.33
V_{40Gy} [%]	< 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-	-
V_{18Gy} [%]	< 5	0.04 ± 0.10	0.01 ± 0.03	0.01 ± 0.02	0.31	0.23	0.41
Esophagus							
D_{mean} [Gy]	< 34	14.34 ± 1.62	13.23 ± 1.63	13.30 ± 1.74	0.01*	< 0.01**	0.96
Liver							
D_{mean} [Gy]	< 30	4.82 ± 0.94	4.57 ± 0.73	4.45 ± 0.70	0.61	0.33	< 0.01**
Kidneys							
D_{mean} [Gy]	< 18	2.81 ± 1.17	2.47 ± 0.46	2.38 ± 0.43	0.96	0.73	< 0.01**

OAR = organ at risk; MP = manual plan; RP_I = RapidPlan initial; RP_F = RapidPlan final; Vx = volume receiving at least x dose; * = $P < 0.05$; ** = $P < 0.01$

TABLE 3. The mean dose of the OARs outside the targets contours

Organ	Mean dose			*P value*		
	MP	RP_I	RP_F	MP *vs.* RP_I	MP *vs.* RP_F	RP_I *vs.* RP_F
Optic nerves	**27.61 ± 3.40**	22.90 ± 2.39	22.42 ± 2.29	< 0.01**	< 0.01**	0.05
Eyes	**12.11 ± 1.99**	10.16 ± 0.55	9.83 ± 0.74	0.01*	0.01*	0.02*
Lens	7.09 ± 0.67	**7.21 ± 0.52**	6.78 ± 0.39	0.43	0.16	< 0.01**
Parotid glands	**7.38 ± 2.52**	5.16 ± 0.39	4.95 ± 0.39	< 0.01**	< 0.01**	< 0.01**
Thyroid	**10.41 ± 3.37**	9.00 ± 1.94	8.51 ± 2.07	0.06	0.04*	< 0.01**
Lungs	4.63 ± 0.30	**4.95 ± 0.43**	4.63 ± 0.24	0.03*	0.73	< 0.01**
Heart	**6.76 ± 1.47**	5.53 ± 0.82	5.70 ± 0.93	0.01*	0.02*	0.33
Liver	**4.82 ± 0.94**	4.57 ± 0.73	4.45 ± 0.70	0.61	0.33	< 0.01**
Kidneys	**2.81 ± 1.17**	2.47 ± 0.46	2.38 ± 0.43	0.96	0.73	< 0.01**

Bold type = the highest D_{mean} in the three groups; MP = manual plan; RP_I = RapidPlan initial; RP_F = RapidPlan final; Underline mark = the lowest D_{mean} in the three groups; * = $P < 0.05$; ** = $P < 0.01$

The pass rates of CTV D_2, PTV D_{max}, and PTV D_2, for the RP_I group, were 66.67% (10/15), 33.33% (5/15), and 66.67% (10/15), respectively. In addition, in the OAR, the lens D_{max} and lungs V_5 of the RP_I group did not meet the goals. The pass rate of the lens D_{max} was 93.33% (14/15) for the RP_I group. In one RP_I plan, the lens D_{max} was 10.98 Gy > 10 Gy. Lastly, the RP_I lungs V_5 pass rate was 53.33% (8/15).

Table 3 shows the mean dose of the 9 OARs. The highest OARs D_{mean} of the optic nerves, eyes, parotid glands, thyroid, heart, liver, and kidneys; and lens and lungs in these three groups were obtained in the MP group (78%, 7/9) and RP_I group (22%, 2/9), respectively. The lowest OARs D_{mean} were mostly in the RP_F group (89%, 8/9). Comparing RP_I and MP, RP_F and RP_I, and RP_F and MP groups, the RP_I group significantly reduced the doses of optic nerves, eyes, parotid glands, and heart than the MP group; the RP_F group further significantly reduced the doses of eyes, lenses, parotid glands, thyroid, lungs, liver, and kidneys than the RP_I group ($P \leq 0.05$); and RP_F significantly reduced the doses of optic nerves, eyes, parotid glands, thyroid, and heart, respectively than the MP group ($P < 0.05$).

In the low-dose region of normal tissue, we employed $R_{50\%}$, $R_{30\%}$, and $R_{10\%}$ as dose gradient indicators. The values for MP, RP_I, and RP_F at $R_{50\%}$ were 2.27 ± 0.13, 2.26 ± 0.16, and 2.26 ± 0.14, respectively. For $R_{30\%}$, the values were 3.96 ± 0.31, 3.95 ± 0.32, and 3.94 ± 0.37, respectively. The corresponding values for $R_{10\%}$ were 10.15 ± 1.93, 10.08 ± 1.69, and 10.00 ± 1.74. There were no statistically significant differences among the three groups (P > 0.05).

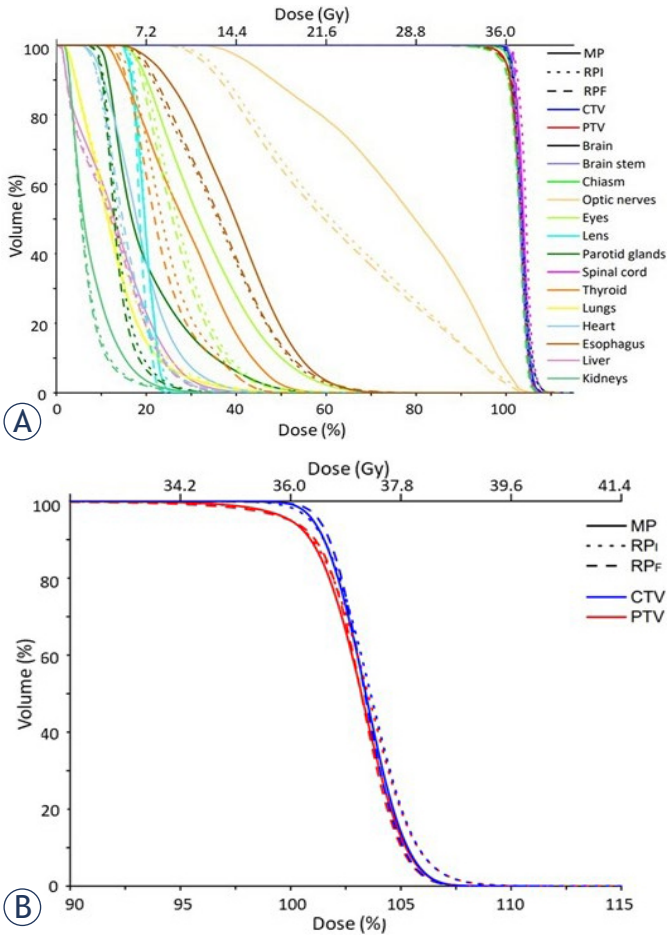


FIGURE 3. (A) Population-averaged dose-volume histogram (DVH) for all organs at risk and targets. **(B)** The population-averaged DVH for targets only.

CTV = clinical target volume; MP = manual optimization plan; PTV = planning target volumes; RPI = RapidPlan initial; RPF = final RapidPlan after manual re-optimization

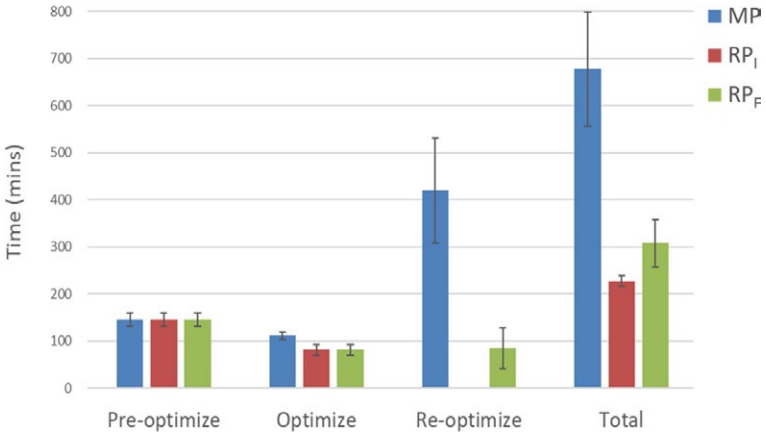


FIGURE 4. Comparison of the planning time for MP, RP_I, and RP_F. The error bar represents one standard deviation.

MP = manual optimization plan; RP_I = RapidPlan initial; RP_F = final RapidPlan after manual re-optimization

Figure 3A showed the population-averaged DVH of targets and OARs. In the DVH, the doses of optic nerves, eyes, lens, parotid glands, thyroid, liver, and kidneys in RP_F or RP_I were lower than those in MP. Furthermore, the DVH of RP_F OARs was better than those of RP_I OARs. Figure 3B shows the targets coverage of CTV and PTV. In the shoulder part of the DVH, with the 95% volume of targets, the MP and RP_I groups had the same targets coverage, while the RP_F group had a slightly better 95% volume dose coverage than the other two groups. The DVH tail part, the high dose in 5% volume, showed that the RP_I had the highest dose in the craniospinal area. The population-averaged DVH showed that the RP_F group had the best targets coverage, homogenous targets dose distribution, and OAR dose avoidance among these three groups.

Treatment planning time

The pre-optimization time was the same in all three groups (146 minutes, Figure 4). The optimization process took a significantly longer time in the MP group than in the RP_I and RP_F groups with 111.45, 81.68, and 81.68 minutes ($P < 0.05$), respectively. The re-optimization time in the MP was significantly longer than in the RP_F group (420.36 versus 85.13 minutes, $P < 0.05$). There was no re-optimization in the RP_I group. Overall, the entire planning time was longer in the MP group than in the RP_I (677.80 versus 227.66 minutes, $P < 0.05$) and RP_F (677.80 versus 307.76 minutes, $P < 0.05$) groups. The total planning time-saving rates (saved planning time) of RP_I and RP_F were 66.41% (450.14 minutes) and 54.59% (370.04 minutes), respectively, compared to the MP group.

MU comparison

The average MU values with one standard deviation of MP, RP_I, and RP_F groups were 935.24 ± 128.44, 1013.22 ± 114.92, and 1026.46 ± 149.43, respectively, with no significant difference between these three groups (all $P > 0.05$).

Discussion

Our research discovered that by utilizing 23 plans to develop the KBP model in combination with RP and re-optimization in CSI, we were able to significantly shorten the planning time by half and enhance plan quality.

Incorporating more patients in the model libraries for model training have a possibility to lead to fewer outliers and more consistent plan quality.[15-17] However, the application of the CSI technique in clinical practice is not common in most hospitals. In this study, because CSI treatment is relatively rare, we searched databases covering the previous 6 years and found only 38 CT image sets. The Varian accelerator company recommended a minimum of 20 to 25 treatment plans in training set for a specific target. According to the study by Jim P. Tol *et al.*[18], Increasing the number of plans used in model training was found to produce comparable results. Based on recommendations, previous experience, and the limited availability of clinical CSI cases, we used 23 plans to complete the model training and compared them with 15 manual plans.

The traditional CSI used patient prone position to reduce the OARs radiation dose via simple two lateral opposed and posterior-anterior (PA) fields. However, this technique can create dose ununiform in the field junction area. The commonly encountered pediatric CSI typically requires two fields and one junction to achieve coverage. This study aims to validate whether KBP can perform effectively in more complex scenarios, utilizing adult CSI as a test case. We used the VMAT technique to disperse the radiation dose in OARs and enhance the homogeneity of the targets dose. The VMAT technique delivers radiation from all angles, which causes it to be attenuated as it passes

through the couch. Our medical physicist compensated for this effect by calculating the attenuation of the couch.[19] Furthermore, cone beam computed tomography ensured an accurate treatment location. Therefore, in this study, all treatment plans were designed using the supine position, which could make patients more comfortable, relaxed, and stable during treatment.[3,20]

Although the plan parameter pass rate of RP_I was only 89.36%, the RP_I target coverage of minimum CTV V_{95} and PTV V_{95} values were ≥ 99.90% and ≥ 99.00%, respectively, which were both higher than 95%, the clinical common plan acceptable criteria.[21] Compared with the traditional 3D-CRT technique, by which the high dose area might receive approximately twice the prescribed dose at the field overlapping sites, the highest PTV D_{max} in RP_I was 115.57% which was much lower than the traditional 3D-CRT technique. For OARs, all 14 plans in RP_I achieved the goal (< 10 Gy) except for one plan with lens D_{max} 10.98 Gy, which did not reach the goal. Table 2 shows that the heart D_{mean} in RP_I was also the lowest of the three groups. Although, Uehara *et al.* reported that KBP was found clinically unacceptable after a single optimization without manual objective constraints in head and neck cancer.[22] Most studies in the other body sites, such as gynecological, prostate, and rectal cancers, support that the RP plan would be comparable to the manual plan.[23] In our study, the RP_I plans were clinically acceptable for CSI and approved by the physician.

The DVH distribution is one of the vital plan evaluation tools. The DVH of OARs (Figure 3) showed that most of the OARs in the MP group received higher doses than RP_I and RP_F, as shown by the D_{mean} and D_{max} in Table 2. In the target DVH (Figure 3B), the RP_F group had better 95% volume dose coverage and better performance at reducing high doses than the other two groups. According to our CI results, there was a minor deviation of the target in the MP group; however, RP_I or RP_F could have achieved the planning goal. Furthermore, HI values in this study show that MP and RP_F groups had better homogeneity than did RP_I. Previous studies on lung cancer or prostate cancer showed that KBP could reduce the OARs dose[23]; however, target coverage and dose homogeneity of KBP did not always have better results than the manual plan. Our study on CSI showed that RP improved the plan quality of OARs and that additional re-optimization after initial RP could improve the plan quality, as previous studies showed in other cancer sites.[24-27]

In terms of cardiac doses, all three plans (MP, RP_I, and RP_F) exhibited notably low V_{40Gy} and V_{18Gy} values, comfortably below the established cardiac dose constriants. It is pertinent to mention that the mean cardiac dose for RP_I was already lower than that for MP. Therefore, the primary focus during the optimization process was not predominantly on further reducing cardiac dose. In the case of RP_I, the lungs V_{5Gy} value(42.77 ± 5.62%) surpassed the target threshold of 42%. Subsequently, in the ensuing RP_F optimization, concerted efforts were undertaken to amplify the reduction of lungs V_{5Gy} values, resulting in a dose shift towards the heart. Nevertheless, from a statistical perspective, the P-value for the comparison between RP_I and RP_F exceeded 0.05.

In our study, RP_I and RP_F reduced planning time compared to MP by 66.41% (450.14 minutes) and 54.59% (370.04 minutes), respectively. The result showed that KBP for CSI might save more planning time in complex plans with many OARs than in general cancer sites. Previously, Wells *et al.*[28] reported that KBP could reduce planning time by approximately 30 minutes per breast cancer patient. Visak *et al.*[29] reported that all the RP plans required less than 30 minutes of planning time for lung cancer. Masi *et al.*[30] showed that the time required for the production of the KBP plan was 6–15 minutes, compared to manual planning requiring 30–150 minutes for a commercial TPS and 15–60 minutes after 8 months of commercial TPS usage in prostate cancer. Furthermore, Chatterjee *et al.*[31] showed that the KBP planning time for the multiform brain glioblastoma was typically 13 minutes for VMAT, compared to the typical 4 hours for the manual planning method. Amaloo *et al.*[32] showed that the total planning time was reduced from 120 minutes to 20 minutes in prostate cancer patients. In a study of nasopharyngeal cancer, Chang *et al.*[33] concluded that the total RP planning time is only about one-fifth that of MP. Similarly, our KBP study for CSI, a very long treatment size from the brain to the lumbosacral area, could effectively reduce the planning time while improving the plan quality, as shown in previous KBP studies for other cancer sites.

Conclusions

This study used 23 plans to train the KBP CSI model and investigated the difference between MP and RP for the same patients and found that RP plans after re-optimization could halve the planning

time and improve plan quality. According to our study result, medical physicists at low CSI patient volume hospitals could efficiently produce CSI plans by the KBP method.

Acknowledgements

We would like to thank Ms. Feng-Chun Hsu for statistical support, Dr. Liang-Cheng Chen for his advice for clinical aspects of this project. This study was supported by research grants from the Dalin Tzu Chi Hospital (grant number: DTCRD109-I-18). The funders had no role in the study design, data collection and analysis, decision to publish, or the preparation of the manuscript.

References

1. Seidel C, Heider S, Hau P, Glasow A, Dietzsch S, Kortmann RD. Radiotherapy in medulloblastoma-evolution of treatment, current concepts and future perspectives. *Cancers* 2021; **13:** 5945. doi: 10.3390/cancers13235945
2. Kiltie AE, Povall JM, Taylor RE. The need for the moving junction in craniospinal irradiation. *Br J Radiol* 2000; **73:** 650-4. doi: 10.1259/bjr.73.870.10911789
3. Mani KR, Sapru S, Maria Das KJ, Basu A. A supine cranio-spinal irradiation technique using moving field junctions. *Pol J Med Phys Eng* 2016; **22:** 79-83. doi: 10.1515/pjmpe-2016-0014
4. Mancosu P, Cozzi L, Muren LP. Total marrow irradiation for hematopoietic malignancies using volumetric modulated arc therapy: a review of treatment planning studies. *Phys Imaging Radiat Oncol* 2019; **11:** 47-53. doi: 10.1016/j.phro.2019.08.001
5. Seravalli E, Bosman M, Lassen-Ramshad Y, Vestergaard A, Oldenburger F, Visser J, et al. Dosimetric comparison of five different techniques for craniospinal irradiation across 15 European centers: analysis on behalf of the SIOP-E-BTG (radiotherapy working group). *Acta Oncol* 2018; **57:** 1240-9. doi: 10.1080/0284186X.2018.1465588
6. Prabhu RS, Dhakal R, Piantino M, Bahar N, Meaders KS, Fasola CE, et al. Volumetric modulated arc therapy (VMAT) craniospinal irradiation (CSI) for children and adults: a practical guide for implementation. *Pract Radiat Oncol* 2022; **12:** e101-e9. doi: 10.1016/j.prro.2021.11.005
7. Sarkar B, Pradhan A. Choice of appropriate beam model and gantry rotational angle for low-dose gradient-based craniospinal irradiation using volumetric-modulated arc therapy. *J Radiother Pract* 2016; **16:** 53-64. doi: 10.1017/s146039691600042x
8. Sarkar B, Munshi A, Manikandan A, Roy S, Ganesh T, Mohanti BK, et al. A low gradient junction technique of craniospinal irradiation using volumetric-modulated arc therapy and its advantages over the conventional therapy. *Cancer Radiother* 2018; **22:** 62-72. doi: 10.1016/j.canrad.2017.07.047
9. Hussein M, Heijmen BJM, Verellen D, Nisbet A. Automation in intensity modulated radiotherapy treatment planning-a review of recent innovations. *Br J Radiol* 2018; **91:** 20180270. doi: 10.1259/bjr.20180270
10. Ma C, Huang F. Assessment of a knowledge-based RapidPlan model for patients with postoperative cervical cancer. *Prec Radiat Oncol* 2017; **1:** 102-7. doi: 10.1002/pro6.23
11. Fogliata A, Reggiori G, Stravato A, Lobefalo F, Franzese C, Franceschini D, et al. RapidPlan head and neck model: the objectives and possible clinical benefit. *Radiat Oncol* 2017; **12:** 73. doi: 10.1186/s13014-017-0808-x
12. Hu J, Liu B, Xie W, Zhu J, Yu X, Gu H, et al. Quantitative comparison of knowledge-based and manual intensity modulated radiation therapy planning for nasopharyngeal carcinoma. *Front Oncol* 2020; **10:** 551763. doi: 10.3389/fonc.2020.551763
13. Castriconi R, Fiorino C, Passoni P, Broggi S, Di Muzio NG, Cattaneo GM, et al. Knowledge-based automatic optimization of adaptive early-regression-guided VMAT for rectal cancer. *Phys Med* 2020; **70:** 58-64. doi: 10.1016/j.ejmp.2020.01.016
14. Shaw E, Kline R, Gillin M, Souhami L, Hirschfeld A, Dinapoli R, et al. Radiation Therapy Oncology Group: radiosurgery quality assurance guidelines. *Int J Radiat Oncol Biol Phys* 1993; **27:** 1231-9. doi: 10.1016/0360-3016(93)90548-a
15. Boutilier JJ, Craig T, Sharpe MB, Chan TC. Sample size requirements for knowledge-based treatment planning. *Med Phys* 2016; **43:** 1212-21. doi: 10.1118/1.4941363
16. Cagni E, Botti A, Wang Y, Iori M, Petit SF, Heijmen BJM. Pareto-optimal plans as ground truth for validation of a commercial system for knowledge-based DVH-prediction. *Phys Med* 2018; **55:** 98-106. doi: 10.1016/j.ejmp.2018.11.002
17. Wang M, Gu H, Hu J, Liang J, Xu S, Qi Z. Evaluation of a highly refined prediction model in knowledge-based volumetric modulated arc therapy planning for cervical cancer. *Radiat Oncol* 2021; **16:** 58. doi: 10.1186/s13014-021-01783-9
18. Tol JP, Delaney AR, Dahele M, Slotman BJ, Verbakel WF. Evaluation of a knowledge-based planning solution for head and neck cancer. *Int J Radiat Oncol Biol Phys* 2015; **91:** 612-20. doi: 10.1016/j.ijrobp.2014.11.014
19. Yu CY, Chou WT, Liao YJ, Lee JH, Liang JA, Hsu SM. Impact of radiation attenuation by a carbon fiber couch on patient dose verification. *Sci Rep* 2017; **7:** 43336. doi: 10.1038/srep43336
20. Sarkar B, Munshi A, Ganesh T, Manikandan A, Mohanti BK. Dosimetric comparison of short and full arc in spinal PTV in volumetric-modulated arc therapy-based craniospinal irradiation. *Med Dosim* 2020; **45:** 1-6. doi: 10.1016/j.meddos.2019.03.003
21. Dietzsch S, Braesigk A, Seidel C, Remmele J, Kitzing R, Schlender T, et al. Pretreatment central quality control for craniospinal irradiation in non-metastatic medulloblastoma: first experiences of the German radiotherapy quality control panel in the SIOP PNET5 MB trial. *Strahlenther Onkol* 2021; **197:** 674-82. doi: 10.1007/s00066-020-01707-8
22. Uehara T, Monzen H, Tamura M, Ishikawa K, Doi H, Nishimura Y. Dose-volume histogram analysis and clinical evaluation of knowledge-based plans with manual objective constraints for pharyngeal cancer. *J Radiat Res* 2020; **61:** 499-505. doi: 10.1093/jrr/rraa021
23. Ge Y, Wu QJ. Knowledge-based planning for intensity-modulated radiation therapy: a review of data-driven approaches. *Med Phys* 2019; **46:** 2760-75. doi: 10.1002/mp.13526
24. Wu H, Jiang F, Yue H, Zhang H, Wang K, Zhang Y. Applying a RapidPlan model trained on a technique and orientation to another: a feasibility and dosimetric evaluation. *Radiat Oncol* 2016; **11:** 108. doi: 10.1186/s13014-016-0684-9
25. Castriconi R, Fiorino C, Broggi S, Cozzarini C, Di Muzio N, Calandrino R, et al. Comprehensive Intra-Institution stepping validation of knowledge-based models for automatic plan optimization. *Phys Med* 2019; **57:** 231-7. doi: 10.1016/j.ejmp.2018.12.002
26. Kamima T, Ueda Y, Fukunaga JI, Shimizu Y, Tamura M, Ishikawa K, et al. Multi-institutional evaluation of knowledge-based planning performance of volumetric modulated arc therapy (VMAT) for head and neck cancer. *Phys Med* 2019; **64:** 174-81. doi: 10.1016/j.ejmp.2019.07.004
27. Fogliata A, Cozzi L, Reggiori G, Stravato A, Lobefalo F, Franzese C, et al. RapidPlan knowledge based planning: iterative learning process and model ability to steer planning strategies. *Radiat Oncol* 2019; **14:** 187. doi: 10.1186/s13014-019-1403-0
28. Wells DM, Walrath D, Craighead PS. Improvement in tangential breast planning efficiency using a knowledge-based expert system. *Med Dosim* 2000; **25:** 133-8. doi: 10.1016/s0958-3947(00)00039-x
29. Visak J, McGarry RC, Randall ME, Pokhrel D. Development and clinical validation of a robust knowledge-based planning model for stereotactic body radiotherapy treatment of centrally located lung tumors. *J Appl Clin Med Phys* 2021; **22:** 146-55. doi: 10.1002/acm2.13120
30. Masi K, Archer P, Jackson W, Sun Y, Schipper M, Hamstra D, et al. Knowledge-based treatment planning and its potential role in the transition between treatment planning systems. *Med Dosim* 2018; **43:** 251-7. doi: 10.1016/j.meddos.2017.10.001

31. Chatterjee A, Serban M, Abdulkarim B, Panet-Raymond V, Souhami L, Shenouda G, et al. Performance of knowledge-based radiation therapy planning for the glioblastoma disease site. *Int J Radiat Oncol Biol Phys* 2017; **99:** 1021-8. doi: 10.1016/j.ijrobp.2017.07.012

32. Amaloo C, Hayes L, Manning M, Liu H, Wiant D. Can automated treatment plans gain traction in the clinic? *J Appl Clin Med Phys* 2019; **20:** 29-35. doi: 10.1002/acm2.12674

33. Chang ATY, Hung AWM, Cheung FWK, Lee MCH, Chan OSH, Philips H, et al. Comparison of planning quality and efficiency between conventional and knowledge-based algorithms in nasopharyngeal cancer patients using intensity modulated radiation therapy. *Int J Radiat Oncol Biol Phys* 2016; **95:** 981-90. doi: 10.1016/j.ijrobp.2016.02.017

study protocol

Determination of copper and other trace elements in serum samples from patients with biliary tract cancers: prospective noninterventional nonrandomized clinical study protocol

Martina Rebersek[1,2], Nezka Hribernik[1,2], Katarina Markovic[3], Stefan Markovic[3], Katja Ursic Valentinuzzi[4,5], Maja Cemazar[4,6], Tea Zuliani[3,7], Radmila Milacic[3,7], Janez Scancar[3,7]

[1] Department of Medical Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[2] Medical Faculty, University of Ljubljana, Ljubljana, Slovenia
[3] Jožef Stefan Institute, Ljubljana, Slovenia
[4] Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[5] Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[6] Faculty of Health Sciences, University of Primorska, Izola, Slovenia
[7] Jožef Stefan International Postgraduate School, Ljubljana, Slovenia

Radiol Oncol 2024; 58(2): 300-310.

Received 18 February 2024
Accepted 9 March 2024

Correspondence to: Assist. Prof. Martina Reberšek, M.D., Ph.D., Department of Medical Oncology, Institute of Oncology Ljubljana, Zaloška 2, SI-1000 Ljubljana, Slovenia. E-mail: mrebersek@onko-i.si

Disclosure: No potential conflicts of interest were disclosed.

Background. Biliary tract cancers (BTCs) are usually diagnosed at an advanced stage, when the disease is incurable. Currently used tumor biomarkers have limited diagnostic value for BTCs, so there is an urgent need for sensitive and specific biomarkers for their earlier diagnosis. Deregulation of the homeostasis of trace elements is involved in the carcinogenesis of different cancers, including BTCs. The objective of the study is to determine/compare the total concentrations of copper (Cu), zinc (Zn) and iron (Fe) and the proportions of free Cu and Cu bound to ceruloplasmin (Cp) and the isotopic ratio of $^{65}Cu/^{63}Cu$ in serum samples from healthy volunteers and cancer patients using inductively coupled plasma-mass spectrometry-based methods (ICP-MS).

Patients and methods. In this prospective, noninterventional, nonrandomized study 20 patients and 20 healthy volunteers will be enrolled to identify serum Cu, Zn and Fe levels, Cu isotopic fractionation as a predictive biomarker of response to systemic therapy of BTCs, which will be evaluated by computed tomography. Newly developed analytical methods based on ICP-MS will be applied to metal-based biomarker research in oncology.

Conclusions. In the study the comparison of the total concentration of selected trace elements, the proportion of free Cu and Cu bound to Cp and the isotopic ratio of $^{65}Cu/^{63}Cu$ in serum samples from healthy volunteers and cancer patients will be conducted to provide the foundation for the development of a BTC cancer screening methodology and the data on their usability as a potential predictive biomarker for BTCs of response to systemic therapy.

Key words: trace elements; copper; predictive biomarkers of response; biliary tract cancer; systemic treatment

Introduction

Biliary tract cancers (BTCs) are a heterogeneous group of uncommon and rare epithelial tumors arising from biliary duct cells. Most of them are adenocarcinomas and represent < 1% of all human cancers or 3% of gastrointestinal cancers.[1,2] Based on anatomical location, BTCs are subdivided into

intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC) that comprises perihilar cholangiocarcinoma and distal cholangiocarcinoma, and gallbladder carcinoma. This anatomical classification parallels distinct biological and molecular features.[1-4]

Most patients with BTCs are aged 65 or older.[1-4] Mortality rates are approximately 1–2/100,000 for ICC and below 1/100,000 for ECC in most countries.[1] Increasing mortality from ICC rises was observed globally, due to risk factors and possibly, in part, due to better disease classification.[1] Mortality from ECC decreases, most likely because of better diagnostics following the increased use of laparoscopic cholecystectomy.[1] According to the Cancer Registry of Slovenia, there were 223 new microscopically confirmed cases of BTCs in 2020.[5] Cholangiocarcinoma accounts for 10%–15% of all primary intrahepatic tumors and is the second most common primary liver cancer after hepatocellular carcinoma.[1,3] The main etiological factors are chronic viral infections (hepatitis B virus and hepatitis C virus), cirrhosis or nonalcoholic fatty liver, obesity, alcohol consumption, tobacco, diabetes mellitus, chronic inflammation of the bile ducts and biliary stasis.[2,3]

Most BTC patients have advanced disease at presentation and relapse despite surgery.[3,4] Metastatic disease is still incurable, with 5% five-year overall survival (OS) without treatment. The first treatment is surgery of the primary tumor, detected at an early stage in selected patients, and is the only potentially curative treatment, if radically resected (R0) without residual disease.[3,4,6] Five-year survival ranges depend on the stage of the disease and are from 9% to 25%, 10% to 15% and 15% to 35% for ICC, ECC and gallbladder carcinoma, respectively.[3] Over the last 10 years, the prognosis of patients has changed significantly, mainly due to the availability of systemic treatment, both adjuvant and, in particular, systemic treatment of metastatic disease with targeted drugs.[3,4,7,8]

The combination of cisplatin and gemcitabine is an approved first-line treatment for unresectable or advanced BTC, with a 30% improvement in overall survival and progression-free survival compared to gemcitabine monotherapy and a statistically significant longer median overall survival for patients on combination therapy.[9] The phase III TOPAZ-1 clinical trial showed that prior treatment with immunotherapy and chemotherapy in advanced BTCs has a benefit on overall survival.[10] In the phase III TOPAZ-1 clinical trial, the addition of the anti-PD-L1 immunotherapy durvalumab, to gemcitabine and cisplatin significantly improved survival without additional toxicity compared to cisplatin and gemcitabine combination chemotherapy alone, with a higher objective response rate and longer recurrence-free survival. Thus, combination chemotherapy with cisplatin and gemcitabine in combination with durvalumab immunotherapy is recommended as standard first-line treatment for patients with advanced, metastatic BTC. In recently published results of the phase III KEYNOTE-966 clinical trial in the intention-to-treat population, treatment with anti-PD-1 immunotherapy pembrolizumab in combination with gemcitabine and cisplatin significantly improved the primary endpoint of OS.[4] At the first interim analysis, treatment with pembrolizumab in combination with gemcitabine and cisplatin did not result in a statistically significant benefit in progression-free survival (PFS). Similar results were obtained in the final analysis for PFS.

Given the current understanding of the biology and the molecular heterogeneity of subgroups of BTCs, it is recommended that extensive molecular genetic profiling be performed prior to the initiation of systemic treatment of advanced metastatic disease.[2-4] Molecular genetic profiling includes microsatellite high instability-high (MSI-H), isocitrate dehydrogenase (IDH1/2) mutations, B-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations, human epidermal growth factor receptor 2 (HER2) overexpression or amplification, positive tumors neurotrophic tyrosine kinase receptor (*NTRK*), fibroblast growth factor receptor (*FGRF*) and rearrangement during transfection (*RET*) fusions, as this may allow for personalized, patient-tailored treatment and thus a better prognosis.[2-4]

As patients with BTCs are asymptomatic in early stages without both specific clinical presentation and specific serum tumor biomarkers, it is difficult to distinguish BTCs from metastatic disease of other cancers.[2] Tumor markers can be diagnostic, for tumor screening and early detection, prognostic or predictive for response to treatment. However, widely accepted biomarkers for diagnosing and dynamically monitoring BTCs are still lacking. Currently applied tumor markers carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) have limited diagnostic value because of their low sensitivity and specificity for BTCs.[2-4] CA 19-9 tends to have higher specificity than CEA (92.7% vs. 79.2%, respectively); however, its sensitivity tends to be lower (50% vs. 79.4%, respectively).[2,3,4] Moreover, they are not specific for gallbladder cancer and can also be significantly

elevated in benign diseases of the liver or in other metastatic cancers. When markedly elevated, CA 19-9 is associated with poorer prognosis, and it can also be useful as a predictive biomarker for the tumor's dynamic changes and thus for response to systemic treatment.[2-4]

Trace elements

Essential trace elements are needed in minute amounts for normal physiology. Among them are iodine (I), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), selenium (Se), cobalt (Co) and molybdenum (Mo).[11] Alterations in levels and changes in the expression of proteins involved in metal metabolism have been demonstrated in a variety of cancers. First, the hyperproliferation of cancer cells renders them more reliant on iron than normal cells. Targeting iron metabolism in cancer cells is an emerging field of therapeutics.[12] When essential trace elements (Mn, Co, Zn, Cu, Se) in the serum, cell fraction, cerebrospinal fluid and tumor tissue samples of malignant brain cancer patients were analyzed, it was shown that elemental profiles in these samples were significantly altered in these cancer patients compared to the healthy individuals. Higher contents of trace elements (particularly Mn, Se, and lead (Pb)) could also be involved in the pathogenesis of brain tumors. Therefore, the urine-to-serum ratio of essential trace elements was proposed as an appropriate diagnostic biomarker in malignant brain tumors.[13]

Copper

Cu is an essential trace element with a precisely regulated amount in our bodies.[14] Cu is present in all tissues. It is stored primarily in the liver and then in the muscles, heart, kidneys and brain. In the blood, it is transported bound to the protein Cp.[14-16] It is a coenzyme of many enzymes (e.g., Cu/Zn superoxide dismutase, ceruloplasmin, cytochrome oxidase, tyrosinase, dopamine hydroxylase, lysine oxidase, catalase, selenium-dependent peroxidase, etc.) that are important for cellular respiration and defense against free radicals. It also affects glutathione function. Consequently, Cu deficiency impairs cellular respiration and the regulation of reactive oxygen species. Deregulation of oxidative stress, due to excessive production of reactive oxygen species, impairs cellular DNA repair mechanisms and is an important mechanism in the development of cancer.[14] In addition to malignant processes, an imbalance of Cu in the body affects the development and progression of chronic, inflammatory and neurodegenerative diseases. Cu deficiency leads to lower overall energy levels, abnormal glucose and cholesterol metabolism, increased oxidative damage, and changes in the function and structure of circulating blood and immune cells.[14] Cu deficiency is associated with a higher incidence of infections and an increased risk of cardiovascular disease.[14]

Specifically, due to its role in inflammatory and antioxidant processes, Cu has an important role in the development of various cancers, such as gynecological cancers, lung cancer, colorectal cancer and other cancers of the digestive tract.[14] Recent preclinical and clinical data confirmed that Cu concentrations are abnormal in malignant tissues of mice and in cancer patients. Namely, in humans, elevated Cu concentrations have been found in malignant tissues of the breast, ovary, lung and stomach. Cu is being investigated as a potential target for cancer treatment due to its elevated levels in malignant tissues and its ability to promote angiogenesis, cancer growth and metastasis.[14-17] In addition to malignant tissues, the concentration of Cu is also elevated in the serum of cancer patients. Elevated levels of Cu have been measured in the serum of patients with lung cancer, colorectal cancer, epithelial ovarian cancer and biliary tract cancers, and decreased levels in adrenocortical and hepatocellular carcinoma.[14-19]

When Cu regulation is disrupted, the quantities and proportions of other essential trace elements may also be altered. Among these, the normal Cu/Zn ratio is known to be disturbed. An imbalance of Cu in the body affects the development and progression of chronic, inflammatory and neurodegenerative diseases and malignant processes. It has been found that high dietary intake of Zn can reduce intestinal absorption of Cu.[20] Altered intakes of only one of the two (similar observations are also made for the other essential trace elements) may cause an imbalance of the other. For example, relatively low levels of Zn and elevated levels of Cu can increase oxidative stress and impair the antioxidant activity of many enzymes.[20] Increased Cu/Zn ratios have been found in a wide variety of malignancies, including gastrointestinal cancers, gynecological cancers, breast cancer, and lung cancer, and have been correlated with the stage or condition of the disease at the time of treatment.[14-20] It has been suggested that the Cu/Zn ratio could be used for clinical diagnosis and as a prognostic biomarker to track response to treatment.

Trace element disorders

Trace element disorders (TEDs) are well established in diseases of genetic origin for which the levels of physiologically relevant metals in the blood are controlled by specific proteins. Inherited TED can result in protein malfunction and therefore, deficiency or toxic accumulation of metal in the body. Well-known examples are Wilson's disease and hemochromatosis.[21-22] Diagnosis usually involves gene mutation testing, clinical observations and biochemical testing. Examples of such biochemical tests are determinations of non-Cp-bound Cu, exchangeable Cu, total blood Fe and serum ferritin (light chain). Despite growing evidence that TED are also associated with many types of cancer, knowledge in this field of research is still relatively scarce.[11,12,14-20] It requires highly sophisticated interdisciplinary investigations, which promises to provide very useful information on the role of trace metals in cancers.

Trace element disorders as potential biomarkers in oncology

Several new findings have shown the potential to use TED identification as a biomarker for cancer.[23] It has been suggested that the imbalance in the Cu/Zn ratio could be used for clinical diagnosis and as a predictive biomarker to track the response to treatment. Cp correlates with immune infiltration and serves as a prognostic biomarker in breast cancer. Elevated serum Cu-Cp levels have been found in lung cancer, colon carcinoma, epithelial ovarian cancer and bile duct cancer, while the expression of Cu-Cp is significantly downregulated in adrenocortical carcinoma and hepatocellular carcinoma.[24] Serum Cu levels increase in several types of cancer. It was experimentally determined that in hepatocellular carcinoma patients, blood Cu and sulphur (S) are enriched in light isotopes compared with healthy individuals. Isotopic ratios of Cu ($^{65}Cu/^{63}Cu$) and S ($^{34}S/^{32}S$) were measured to elucidate their use as potential biomarkers of disease.[25] Changes in the isotopic compositions of Fe, Cu and Zn and their corresponding concentrations in plasma from hematological malignancy patients can be measured to assess their prognostic capability. Imbalances in trace metal concentrations, changes in their speciation and isotopic fractionation need to be further investigated to fully evaluate their emerging biomarker potential.[26]

Analytical methods

For improvements in cancer therapy efficacy, reliable and optimized analytical and imaging methods using contemporary instrumental techniques that allow investigations on the role of trace elements in cancer, quantitative determination of established or emerging biomarkers (exchangeable and Cp-bound Cu, stable isotope ratio of trace metals, such as Cu, Zn or Fe), as well as the monitoring of penetration, distribution and metabolism of a metallodrug within the target tissue/tumor are needed.[27-31]

Cu toxicity is strongly related to its free (exchangeable) fraction, which is not bound to Cp.[32-34] Due to the important physiological functions and role of Cu in various diseases, it is necessary to quantify its exchangeable and bound to Cp fractions. In clinical practice, Cp in serum or plasma is commonly determined by nephelometry or turbidimetry. They unspecifically measure both holoCp (Cp with Cu) and apoCp (Cp without Cu). The latter Cp form (apoCp) is not relevant for medical diagnosis. To overcome this disadvantage, monolithic chromatography coupled to inductively coupled plasma–mass spectrometry, which allows simultaneous quantification of exchangeable Cu, Cu bound to human albumin and holoCp, can be used. The chromatographic column used in this method comprises convective interaction media (CIM) affinity and weak anion-exchange disks (Protein G and diethylamine (DEAE) disks) assembled into a single housing forming a CLC monolithic column.[35-39]

Isotopic fractionation of stable isotopes of essential metals was proposed as an emerging predictive biomarker for cancer diagnosis. In hepatocellular carcinoma patients, blood Cu and sulphur (S) are enriched in light isotopes compared with control subjects. Isotopic ratios of Cu ($^{65}Cu/^{63}Cu$) and S ($^{34}S/^{32}S$) were measured to elucidate their use as potential biomarkers of disease.[31,32] Changes in the isotopic compositions of Fe, Cu and Zn and their corresponding concentrations in plasma from hematological malignancy patients can be measured to assess their prognostic capability.[31,32]

Trace elements as biomarkers in oncology are promising fields for detecting, diagnosing and predicting responses to treatment. To date, there has been no published clinical trial investigating copper as a predictive biomarker of response to systemic therapy. In this context, we selected patients with locoregional advanced, inoperable or metastatic BTCs for this noninterventional non-

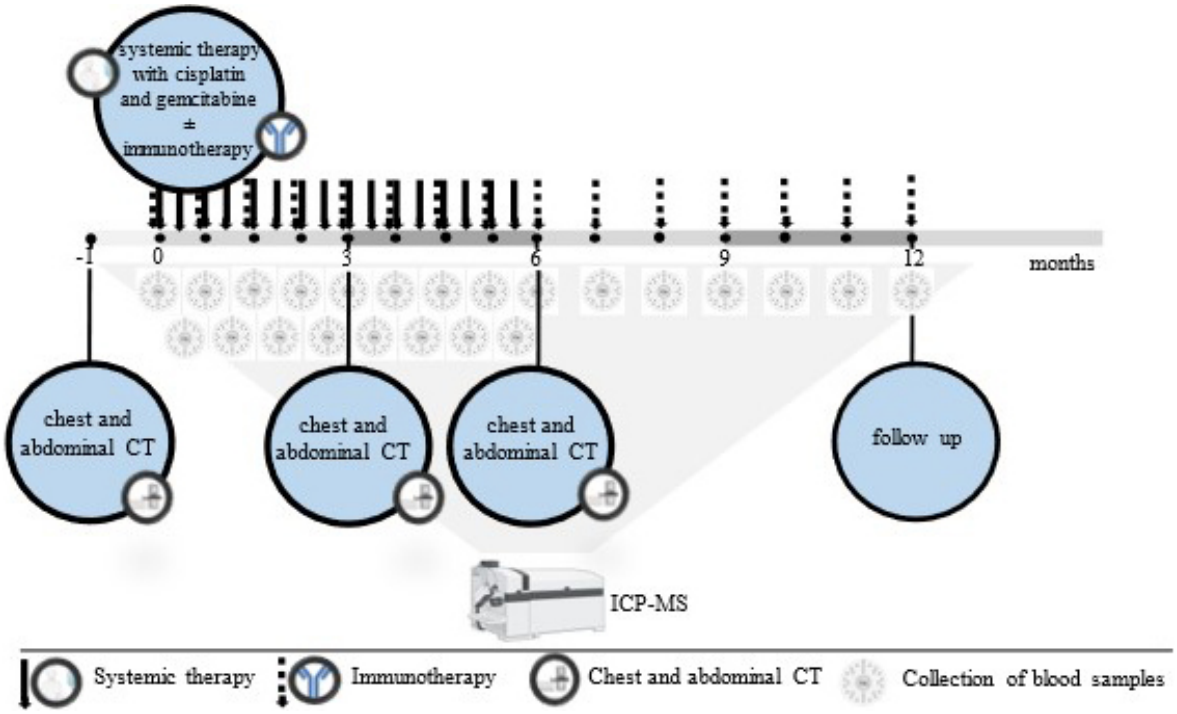


FIGURE 1. Study flow chart (created with BioRender.com.)

ICP-MS = inductively coupled plasma-mass spectrometry-based methods

randomized prospective clinical trial, treated with first-line systemic chemotherapy or immunochemotherapy, to identify serum Cu levels, its speciation and/or isotopic fractionation as a predictive biomarker of response to systemic therapy in correlation with radiological CT evaluation for response to systemic therapy. The proportion of free Cu and Cu bound to Cp and the isotopic ratio of $^{65}Cu/^{63}Cu$ in serum samples will be determined in enrolled healthy volunteers to establish reference values for the general population and to provide the foundation for the development of BTC cancer screening methodology. Determination of the total concentration of trace elements and speciation analysis will be carried out on a quadrupole inductively coupled plasma mass spectrometer (ICP-MS), while isotopic ratios will be precisely determined by multicollector ICP-MS.

Methods / design

Study setting

In a prospective, noninterventional, nonrandomized clinical study started in 2023 at the Institute of Oncology Ljubljana, 20 patients with BTC and 20 healthy volunteers are planning to enroll to provide the development of a BTC cancer screening methodology. The proportion of free Cu and Cu bound to Cp and the isotopic ratio of $^{65}Cu/^{63}Cu$ in serum samples will be determined in enrolled healthy volunteers to establish reference values. The inclusion of 20 BTC patients will enable the identification of serum Cu levels as a potential predictive biomarker of response to systemic therapy in correlation with radiological CT evaluation for response to systemic therapy. The clinical study was approved by the Ethics Committee ERIDEK-0095/2022 and the Institutional Review Board ERID-KSOPKR-0091/2022 at the Institute of Oncology Ljubljana, approved 13.12.2022 by the Commission of the Republic of Slovenia for Medical Ethics (0120-472/2022/3). It was registered with ClinicalTrials.gov under the registration number NCT06060990. All patients entering the study signed informed consent forms. Monitoring will be carried out throughout the study. The flow diagram of the study is presented in Figure 1.

Study outcomes

The main purpose of the research is to determine the total concentration of selected trace elements (Cu, Zn, Fe), the proportion of free Cu and

Cu bound to Cp, and the isotopic ratio between $^{65}Cu/^{63}Cu$ in blood serum samples of healthy volunteers and BTC cancer patients using methods based on ICP-MS. We will statistically evaluate the results and evaluate the possibilities of using the used analytical methods and the results of these clinical trials in cancer diagnostics and therapy.

Our hypothesis is that serum Cu levels and the $^{65}Cu/^{63}Cu$ isotope ratio in cancer patients differ significantly from those in healthy volunteers and that these levels vary according to the response to systemic chemotherapy or chemoimmunotherapy.

Primary objectives

The first primary objective is to establish reference levels of Cu, Zn and Fe in the serum of healthy volunteers and their levels in locally advanced inoperabile and metastatic BTC patients to establish a framework for reference Cu, Zn and Fe values.

The second primary objective of the study is to identify serum Cu levels, its speciation and/or isotopic fractionation as a predictive biomarker of response to systemic therapy in correlation with radiological CT evaluation for response to systemic therapy.

Secondary objectives

The secondary objective of the study is to apply newly developed analytical methods based on ICP-MS to metal-based biomarker research in oncology.

Patient population and recruitment

In this prospective, noninterventional, nonrandomized clinical study, we aim to include 20 patients with locoregionally advanced, inoperable or metastatic BTC who will start with first-line systemic chemotherapy or immunochemotherapy at the Institute of Oncology Ljubljana. Potential study participants will be identified at the institutional multidisciplinary tumor board, comprised of diagnostic radiologists, interventional radiologists, hepatobiliary surgeons, medical oncologists, and radiation oncologists. The study protocol will be explained to all eligible patients in detail. Only those who sign the consent form will enter the clinical study.

Patients' blood levels of Cu and other trace metals will be determined before starting systemic therapy and at least during one cycle of systemic therapy will be included in the analysis.

Twenty healthy volunteers will also be enrolled, after prior signed written consent to participate in the clinical study, for a single 7 ml blood draw for analysis.

Eligibility criteria and exclusion criteria

Inclusion criteria for patients

- aged ≥ 18 years;
- cytologically or histologically verified BTC;
- no prior systemic therapy and no radiation therapy for advanced, inoperable or metastatic disease;
- WHO performance status 0–2 (ECOG criteria);
- imaging diagnosis (CT of thoracic and abdominal organs) performed within 4 weeks prior to the first administration of systemic therapy;
- disease measurable by RECIST or ECOG criteria;
- signed Consent to Participate in Clinical Research form.

Exclusion criteria for patients

- prior systemic treatment and irradiation of inoperable, metastatic disease;
- WHO performance status > 2 (ECOG criteria);
- contraindications for treatment with immunotherapy (known deficiency of the immune system or active immunosuppressive treatment or active autoimmune disease requiring treatment);
- other malignancies, except cured basal cell or squamous cell carcinoma of the skin, carcinoma in situ of the cervix or other cured solid tumors without disease recurrence ≥ 3 years after treatment.

Inclusion criteria for healthy volunteers

- aged ≥ 18 years;
- signed Consent to Participate in Clinical Research form.

Exclusion criteria for healthy volunteers

- the presence of chronic internal diseases (neurodegenerative, cardiovascular, renal, lung, hematological, gastroenterological diseases) and autoimmune diseases.

Chemotherapy and immune-chemotherapy regimen

Patients will be followed for up to 12 consecutive months. All patients for whom the medical oncolo-

gist decides to be treated with first-line systemic therapy with combination chemotherapy with cisplatin and gemcitabine or combination chemotherapy combined with immunotherapy with the anti-PD-L1 inhibitor durvalumab during the course of treatment will be invited to participate in the clinical trial. Systemic chemotherapy and immunotherapy treatment, duration of treatment and other medical procedures will be performed independently of the study at the discretion of the medical oncologist and according to the recommendations of good clinical practice.

Systemic chemotherapy with cisplatin and gemcitabine will be administered in cycles every 3 weeks, with cisplatin and gemcitabine administered on days 1 and 8 of each cycle, for a total of 8 cycles, or systemic chemotherapy with cisplatin and gemcitabine administered in cycles every 3 weeks, with cisplatin and gemcitabine administered on days 1 and 8 of each cycle, in combination with immunotherapy with durvalumab administered on day 1 of each cycle for a total of 8 cycles, followed by maintenance treatment with durvalumab immunotherapy every 4 weeks until disease progression, unacceptable toxicity or a decision to discontinue treatment by the patient or treating medical oncologist. Standard chest and abdomen CT imaging with contrast will be performed before the start of systemic treatment as baseline imaging to delineate the extent of disease, then 3 months after the start of systemic treatment to assess the efficacy of treatment, and finally 6 months after the start of treatment. All further diagnostic and therapeutic procedures will be part of the standard management of patients undergoing systemic chemotherapy and immunotherapy. All decisions on additional treatment, either surgery or radiotherapy during systemic therapy, will be made by the multidisciplinary gastrointestinal cancer consortium. In the case of adverse events of systemic chemotherapy and immunotherapy, actions will follow standard recommendations for the treatment of complications and discontinuation of systemic treatment. Despite discontinuation of systemic treatment, patients will continue with the planned investigations in accordance with good clinical practice and according to the protocol of the clinical trial, in line with the primary and secondary objectives of the trial.

Collection of blood samples

All patients will give blood for laboratory tests, blood counts and biochemical tests, including a blood draw to determine the serum Cu level, its speciation and isotopic fractionation before starting treatment and then before each application of systemic therapy. A blood sample will be obtained before the start of systemic therapy and then on days 1 and 8 of each cycle at a regular outpatient check-up at the Institute of Oncology Ljubljana. Patients will additionally have 7 ml of blood drawn into a standard serum tube. The blood sample will be send to the Department of Experimental Oncology of the Institute of Oncology Ljubljana, where the sample will be centrifuged (1300 × g, 10 min, 4°C) and the serum needed for the analysis will be stored at -20°C until the analysis is performed by ICP-MS-based techniques at the Jožef Stefan Institute.

Once patient enrollment in the clinical study has been completed and 20 patients have been enrolled, this will be followed by the enrollment of 20 age- and sex-matched healthy volunteers. The blood sample will be prepared, stored, and analyzed as for the patients.

Assessment of objective response to the treatment

All patients will undergo diagnostic imaging (chest and abdominal CT with contrast) up to 4 weeks prior to enrollment in the clinical trial and then at 12 (± 7 days) and 24 weeks (± 7 days) after initiation of treatment and if disease progression or adverse events of systemic therapy are suspected. CT scans will be evaluated according to RECIST (response evaluation criteria in solid tumors) and irRECIST criteria (immune-related response evaluation criteria in solid tumors).[40,41] The IrRECIST criteria divide the response to treatment into different groups: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Pseudoprogression is defined as transient radiological disease progression in the absence of clinical progression and a progressive reduction in the burden of the underlying disease according to irRECIST criteria in patients who will receive durvalumab immunotherapy in addition to systemic chemotherapy.

Safety and management of adverse events

In case of adverse events of treatment with systemic chemotherapy and immunotherapy, measures will follow standard recommendations for treatment of complications and interruption of

systemic treatment. Adverse events of systemic therapy will be treated in accordance with the recommendations of the NCI Common Terminology Criteria for Adverse Events (CTCAE) v 5.0 and in accordance with good clinical practice.[42] Despite discontinuation of systemic therapy, patients will continue with planned investigations in accordance with good clinical practice and according to the protocol in the clinical trial according to the primary and secondary objectives of the trial.

Before each cycle of systemic therapy, a laboratory blood sample will be taken as a standard before the decision to continue treatment. Standard peripheral blood sampling will follow hygiene protocols. At the same time, we will add an additional peripheral blood sample to determine Cu in the serum. The collection of additional samples does not pose a major health risk, and the possible complications of blood collection are mainly local: the appearance of a hematoma or infection.[42] Imaging evaluation poses potential hazards due to contrast agent administration, anaphylactic reaction, and ionizing radiation.[42]

CT imaging diagnostics will take place at the same time intervals as planned for the evaluation of the effectiveness of systemic treatment. In this way, the subjects will not be exposed to additional imaging tests. In the case of a known allergy to the contrast agent, the procedure will be performed with appropriate premedication or with other methods. Hydration and other measures will be taken before the planned imaging diagnostics in case of deterioration of renal function.

Analytical methodology and blood sample analysis

Total concentrations of trace metals and quantitative determination of relevant species (Cu-Cp, exchangeable Cu) will be determined by ICP-MS or high-performance liquid chromatography coupled to inductively coupled plasmamass spectrometry (HPLC-ICPMS), respectively.[36,37,39] Emerging metal-based biomarkers (stable isotope fractionation of Cu, and, if relevant, Zn and S) will be followed by multicollector ICP-MS.

Data analysis

Data from the determination of relevant trace elements (with special attention to Cu) before systemic therapy and at least during one cycle of systemic therapy will be included in the analysis. The association between the change in, for example,

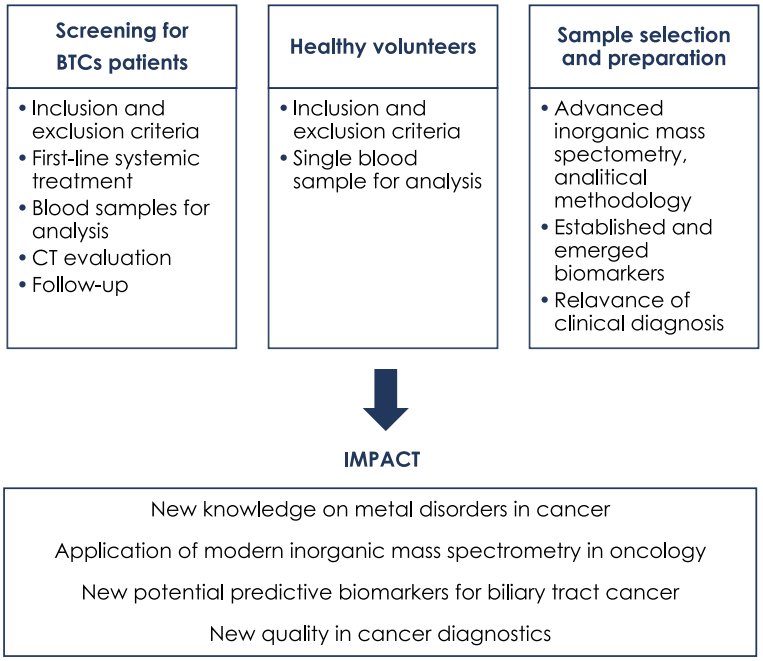


FIGURE 2. Schematic outline of the proposed clinical protocol.

Cu concentration, speciation, and/or isotopic fractionation between healthy individuals and those suffering from biliary tract cancer will be statistically analyzed to evaluate the applicability of such an approach as a diagnostic biomarker for disease. The same data will be used to assess response to treatment by a logistic regression model and a multivariate model including different variables. Paired t test or an appropriate nonparametric alternative will be used to compare values at different time points, and analysis of variance (ANOVA) or Kruskal – Wallis's test will be used when comparing several groups simultaneously. Statistical analysis will be performed using GraphPad Prism (GraphPad, San Diego, CA, USA), and differences will be considered statistically significant if $p<0.05$.

Follow-up

All patients are recommended to have a follow-up visit every 3 months in the first 2 years and every 6 months after 2 years after completion of first-line systemic therapy. Follow-up methods will be mainly outpatient visits and hospitalizations. Examinations to be performed on admission will include blood tests for the tumor markers CA19-9 and CEA and CT of the chest, abdomen, and pelvis. Overall survival (OS) will be defined as the time interval from treatment to cancer-related

death or final follow-up visit, and OS will be the preferred destination. Progression-free survival (PFS) will be measured from the time of treatment initiation to clinical or radiographic progression or death from any cause.

A schematic outline of the proposed clinical protocol is shown in Figure 2.

Statistical analysis

Response to treatment will be determined radiologically using RECIST or irRECIST criteria.[40,41] The objective response to treatment will be calculated as the percentage of patients who have a partial or complete response according to the RECIST or irRECIST criteria among all patients who will receive at least one cycle of systemic therapy and have at least one radiological assessment during systemic treatment. For patients who will not progress or die, the end date for analysis will be the date of last follow-up. Time to disease progression will be defined as the time interval from the date of first therapy administration to the date of disease progression or death, using the Kaplan–Meier method. Comparison of survival of several groups will be calculated using the log-rank test. The association between the change in Cu concentration, speciation and/or fractionation and response to treatment will be assessed by a logistic regression model and a multivariate model including different variables. Statistical analysis will be performed as previously described in the **Data analysis** chapter**.**

Ethics statement

The clinical protocol was approved by the Ethics Committee ERIDEK-0095/2022 and the Clinical Trials Protocol Review Committee ERID-KSOPKR-0091/2022 at the Institute of Oncology Ljubljana, and 13.12.2022 was approved by the Commission of the Republic of Slovenia for Medical Ethics (0120-472/2022/3). The clinical study will be performed in accordance with the ethical principles of the Declaration of Helsinki. The clinical trial number: NCT06060990. Informed consent will be obtained from each participating patient and healthy volunteer in written form.

Discussion

BTCs are rare tumors with poor prognosis. Most patients with BTCs have advanced disease at clinical presentation and relapse despite surgery. Metastatic disease is still incurable, with a 5% five-year OS without treatment. In recent years, the prognosis of metastatic patients has changed significantly, with longer median PFS and median OS, mainly due to the availability of systemic treatment, both adjuvant and, in particular, systemic treatment of metastatic disease with targeted drugs.[2-4,6,7] Specific serum biochemical tumor biomarkers are still lacking for the early detection of BTCs, and it is also difficult to distinguish them from metastatic diseases of other cancers.[3,4] Therefore, extensive efforts are underway to identify more precise biomarkers for the diagnosis, treatment response, and prognosis of BTCs.

Cu, Zn and Fe are among the trace metals that are essential for the normal functioning of the human body.[11-13] They are involved in many biochemical reactions, cofactors of enzymes, and regulate important biological processes by binding to specific receptors and transcription factors. Deregulation of trace metal homeostasis at the cellular and tissue level is a part of the pathology of many cancers. It accelerates the transformation of normal cells into cancerous cells and alters the inflammatory and antitumor responses of immune cells.[11-13]

Alterations in the concentrations of Cu and Zn in serum have been widely described in cancer patients.[14-20] It has been shown that for several types of cancer, the serum Cu concentration is significantly higher, while that of Zn is significantly lower in patients than in healthy individuals. These differences vary based on various factors (diet, sex, age, type of cancer, etc.) We will also focus on the isotopic fractionation of Cu and, if applicable, Zn in BTC patients to establish a stronger association between the alteration of isotope ratios of these elements and cancer and evaluate the applicability of isotope fractionation as a biomarker of cancer.[27-31] The hypothesis that the isotopic composition of Cu reflects changes in trace element homeostasis, with higher sensitivity than metal concentrations, will be tested. For this purpose, high-resolution multicollector ICP-MS will be used.[35-39]

Clinical studies with ethical approval will be carried out by a multidisciplinary team from the Institute of Oncology Ljubljana, Slovenia and Jožef Stefan Institute, Ljubljana, Slovenia. Its main objectives are to determine the total concentration of selected essential trace elements (Cu, Zn, Fe), the proportion of free Cu and Cu bound to Cp and the isotopic ratio of $^{65}Cu/^{63}Cu$ in blood serum samples from healthy volunteers and locally advanced in-

operable and metastatic BTC patients by ICP-MS-based methods.

Acknowledgement

The manuscript was edited by AJE Digital/Curie.

The clinical study is supported by the Slovenian Research and Innovation Agency (ARIS), programs P3-0321 of Institute of Oncology Ljubljana, Slovenia, program P1-0143 and Project No. J7-50128 of Jožef Stefan Institute, Slovenia. The funder was not involved in the study design, collection, analysis, interpretation of data, writing of this article or decision to submit it for publication.

References

1. Bertuccio P, Malvezzi M, Carioli G, Hashim D, Boffetta P, El-Serag HB, et al. Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. *J Hepatol* 2019; **71:** 104-14. doi: 10.1016/j.jhep.2019.03.013
2. Valle JW, Kelley RK, Nervi B, Oh DY, Zhu AX. Biliary tract cancer. *Lancet* 2021; **397:** 428-44. doi: 10.1016/S0140-6736(21)00153-7
3. Vogel A, Bridgewater J, Edeline J, Kelley RK, Klümpen HJ, Malka D, et al; ESMO Guidelines Committee. Biliary tract cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023; **34:** 127-40. doi: 10.1016/j.annonc.2022.10.506
4. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology: Biliary tract cancers. V 3. [online]. 2023. [cited 2024 Feb 18]. Available at: https://www.nccn.org/professionals/physician_gls/pdf/btc.pdf
5. *Cancer in Slovenia 2020*. Ljubljana: Institute of Oncology Ljubljana, Epidemiology and Cancer Registry, Slovenian Cancer Registry; 2023.
6. Đokic M, Stupan U, Licen S, Trotovsek B. Residual disease in lymph nodes has no influence on survival in patients with incidental gallbladder cancer – institution experience with literature review. *Radiol Oncol* 2021; **56:** 208-15. doi: 10.2478/raon-2021-0048
7. Fostea RM, Fontana E, Torga G, Arkenau HT. Recent progress in the systemic treatment of advanced/metastatic cholangiocarcinoma. *Cancers* 2020; **12:** 2599. doi: 10.3390/cancers12092599
8. Mirallas O, López-Valbuena D, García-Illescas D, Fabregat-Franco C, Verdaguer H, Tabernero, et al. Advances in the systemic treatment of therapeutic approaches in biliary tract cancer. *ESMO Open* 2022; **7:** 100503. doi: 10.1016/j.esmoop.2022.100503
9. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al; ABC-02 Trial Investigators. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; **362:** 1273-81. doi: 10.1056/NEJMoa0908721
10. Oh DY, He AR, Qin S, Chen T, Okusaka T, Arndt Vogel, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer. *NEJM* Evid 2022; **1:** EVID 0a2200015. doi: 10.1056/EVIDoa2200015
11. Ferreira CR, Gahl WA. Disorders of metal metabolism. *Transl Sci Rare Dis* 2017; **2**: 101-39. doi: 10.3233/TRD-170015
12. Morales M, Xue X. Targeting iron metabolism in cancer therapy. *Theranostics* 2021; **11:** 8412-29. doi: 10.7150/thno.59092
13. Stojsavljević, A, Vujotić L, Rovčanin B, Borković-Mitić S, Gavrović-Jankulović, Manojlović D. Assessment of trace metal alterations in the blood, cerebrospinal fluid and tissue samples of patients with malignant brain tumors. *Sci Rep* 2020; **10:** 3816. doi: 10.1038/s41598-020-60774-0
14. Lelièvre P, Sancey L, Coll JL, Deniaud A, Busser B. The multifaceted roles of copper in cancer: A trace metal element with dysregulated metabolism, but also a target or a bullet for therapy. *Cancers* 2020; **12:** 3594. doi: 10.3390/cancers12123594
15. Chen F, Han B, Meng Y, Han Y, Liu B, Zhang B, et al. Ceruloplasmin correlates with immune infiltration and serves as a prognostic biomarker in breast cancer. *Aging* 2021; **13:** 20438-67. doi: 10.18632/aging.203427
16. Wang B, Wang XP. Does ceruloplasmin defend against neurodegenerative diseases? *Curr Neuropharmacol* 2019; **17:** 539-49. doi: 10.2174/1570159X16666180508113025
17. Linder MC. Ceruloplasmin and other copper binding components of blood plasma and their functions: an update. *Metallomics* 2016; **8:** 887-905. doi: 10.1039/c6mt00103c
18. Mukae Y, Ito H, Miyata Y, Araki K, Matsuda T, Aibara N, et al. Ceruloplasmin levels in cancer tissues and urine are significant biomarkers of pathological features and outcome in bladder cancer. *Anticancer Res* 2021; **41:** 3815-23. doi: 10.21873/anticanres.15174
19. Sogabe M, Kojima S, Kaya T, Tomioka A, Kaji H, Sato T, et al. Sensitive new assay system for serum wisteria floribunda agglutinin-reactive ceruloplasmin that distinguishes ovarian clear cell carcinoma from endometrioma. *Anal Chem* 2022; **94:** 2476-84. doi: 10.1021/acs.analchem.1c04302
20. Michalczyk K, Cymbaluk-Płoska A. The role of zinc and copper in gynecological malignancies. *Nutrients* 2020; **12:** 3732. doi: 10.3390/nu12123732
21. Woimant F, Djebrani-Oussedik N, Poujois A. New tools for Wilson's disease diagnosis: exchangeablecopper fractio. *Ann Transl Med* 2019; **7(Suppl 2):** S70. doi: 10.21037/atm.2019
22. WHO Guidelines Review Committee, Nutrition and Food Safety (NFS). WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations [Internet]. Geneva: World Health Organization 2020. [cited 2024 Jan 15]. Available at: https://www.who.int/publications/i/item/9789240000124. PMID: 33909381
23. Lossow K, Schwarz M, Kipp AP. Are trace element concentrations suitable biomarkers for the diagnosis of cancer? *Redox Biol* 2021; **42:** 101900. doi: 10.1016/j.redox.2021.101900
24. Chen F, Han B, Meng Y, Han Y, Liu B, Zhang B, et al. Ceruloplasmin correlates with immune infiltration and serves as a prognostic biomarker in breast cancer. *Aging* 2021; **13:** 20438-67. doi: 10.18632/aging.203427
25. Balter V, Nogueira da Costa A, Bondanese VP, Jaouen K, Lamboux A, et al. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. *Proc Natl Acad Sci U S A* 2015; **112:** 982-5. doi: 10.1073/pnas.1415151112
26. Hastuti AAMB, Costas-Rodríguez M, Matsunaga A, Ichinose T, Hagiwara S, Shimura M, et al. Cu and Zn isotope ratio variations in plasma for survival prediction in hematological malignancy cases. *Sci Rep* 2020; **10:** 16389. doi: 10.1038/s41598-020-71764-7
27. Ge EJ, Bush AI, Casini A, Cobine PA, Cross JR, DeNicola GM, et al. Connecting copper and cancer: from transition metal signaling to metalloplasia. *Nat Rev Cancer* 2022; **22:** 102-13. doi: 10.1038/s41568-021-00417-2
28. Wang J, Zhao H, Xu Z, Cheng X. Zinc dysregulation in cancers and its potential as a therapeutic target. *Cancer Biol Med* 2020; **17:** 612-25. doi: 10.20892/j.issn.2095-3941.2020.0106
29. Krężel A, Maret W. The biological inorganic chemistry of zinc ions. *Arch Biochem Biophys* 2016; **611:** 3-19. doi: 10.1016/j.abb.2016.04.010
30. Infusino I, Valente C, Dolci A, Panteghini M. Standardization of ceruloplasmin measurements is still an issue despite the availability of a common reference material. *Anal Bioanal Chem* 2009; **397:** 521-5. doi: 10.1007/s00216-009-3248-0
31. Quarles CD, Macke M Jr, Michalke B, Zischka H, Karst U, Sullivan P, et al. LC-ICP-MS method for the determination of "extractable copper" in serum. *Metallomics* 2020; **12:** 1348-55. doi: 10.1039/d0mt00132e
32. Neselioglu S, Ergin M, Erel O. A new kinetic, automated assay to determine the ferroxidase activity of ceruloplasmin. *Anal Sci* 2017; **33:** 1339-44. doi: 10.2116/analsci.33.1339
33. Solovyev N, Ala A, Schilsky M, Mills C, Willis, K, Harrington C F. Biomedical copper speciation in relation to Wilson's disease using strong anion exchange chromatography coupled to triple quadrupole inductively coupled plasma-mass spectrometry. *Anal. Chim Acta* 2020; **1098:** 27-36. doi: 10.1016/j.aca.2019.11.033
34. Bernevic B, El-Khatib AH, Jakubowski N, Weller MG. Online immunocapture ICP-MS for the determination of the metalloprotein ceruloplasmin in human serum. *BMC Res Notes* 2018; **11:** doi:10.1186/s13104-018-3324-7

35. Marković K, Milačič R, Vidmar J, Marković S, Uršič K, Nikšić Žakelj M, et al. Monolithic chromatography on conjoint liquid chromatography columns for speciation of platinum-based chemotherapeutics in serum of cancer patients. *J Trace Elem Med Biol* 2020; **57:** 28-39. doi: 10.1016/j.jtemb.2019.09.011

36. Marković K, Milačič R, Marković S, Kladnik J, Turel I, Ščančar J. Binding kinetics of ruthenium pyrithione chemotherapeutic candidates to human serum proteins studied by HPLC-ICP-MS. *Molecules* 2020; **25:** 1512-3. doi: 10.3390/molecules25071512

37. Martinčič A, Čemažar M, Serša G, Kovač V, Milačič R, Ščančar J. A novel method for speciation of Pt in human serum incubated with cisplatin, oxaliplatin and carboplatin by conjoint liquid chromatography on monolithic disks with UV and ICP-MS detection. *Talanta* 2013; **116:** 141-8, doi: 10.1016/j.talanta.2013.05.016

38. Martinčič A, Milačič R, Vidmar J, Turel I, Keppler BK, Ščančar J. New method for the speciation of Ru-based chemotherapeutics in human serum by conjoint liquid chromatography on affinity and anion-exchange monolithic disks. *J Chromatogr A* 2014; **1371:** 168-76. doi: 10.1016/j.chroma.2014.10.054

39. Marković K, Cemazar M, Sersa G, Milačič R, Ščančar J. Speciation of copper in human serum using conjoint liquid chromatography on short-bed monolithic disks with UV and post column ID-ICP-MS detection. *J Anal At Spectrom* 2022; **37:** 1675-86. doi: 10.1039/D2JA00161F

40. Schwartz LH, Litière S, de Vries E, Ford R, Gwyther S, Mandrekar S, et al. RECIST 1.1-Update and clarification:from the RECIST committee. *Eur J Cancer* 2016; **62:** 132-7. doi: 10.1016/j.ejca.2016.03.081

41. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al; RECIST working group. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol* 2017; **18:** e143-e52. doi: 10.1016/S1470-2045(17)30074-8. Erratum in: *Lancet Oncol* 2019; **20:** e242.

42. National Cancer Institute (NCI). NCI common terminology criteria for adverse events (CTCAE). Version 5.0.2021. [cited 2024 Feb 18]. Available at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_8.5x11.pdf

correpondence

The influence of anaesthesia on cancer growth

Publish: March 1st, 2024.

Radiol Oncol 2024; 58(1): 9-14.

doi: 10.2478/raon-2024-0012

Radiol Oncol 2024; 58(2): 311-312.

To the editor

I read with a great interest the review recently published in Radiology and Oncology by Potocnik et al.[1] Upon examination, I have identified a critical discrepancy between the review's main text and the cited meta-analysis[2] results, which seems to have led to a significant misunderstanding in the presentation of the findings.

The review states that in a recent meta-analysis[2], patients with breast, esophageal, or non-small cell lung cancer had improved recurrence-free survival after receiving volatile anesthesia (VA) and that overall survival was longer after VA than after total intravenous anesthesia (TIVA). This statement contradicts the findings of the cited meta-analysis, which actually shows that TIVA is associated with improved outcomes both in terms of recurrence-free survival (pooled Hazard Ratio [HR], 0.78; 95% Confidence Interval [CI], 0.65 to 0.94; $P < 0.01$) and overall survival (pooled HR, 0.76; 95% CI, 0.63 to 0.92; $P < 0.01$) across several cancer types, including breast, esophageal, colorectal, gastric, and non-small cell lung cancer.

Interestingly, the conclusion section of the review correctly highlights the potential anti-inflammatory, antioxidant, and possibly antitumor effects of propofol (a common TIVA agent) compared to the proinflammatory effects of volatile anesthetics, which could accelerate metastasis. This conclusion aligns with the meta-analysis findings that favor TIVA over VA, suggesting a potential oversight or error in the review's main text.

Given the significance of these findings for clinical practice and the potential impact on patient care, I believe a clarification and correction of the discrepancy in the review's main text is crucial. Accurate representation of the meta-analysis results is essential for guiding future research and clinical decisions regarding anesthesia choice in cancer surgery.

Muhammet Selman Söğüt, M.D.
Koç University Hospital, Istanbul/Turkey
E-mail: ssogut@kuh.ku.edu.tr.

Notes

No potential conflict of interest relevant to this letter was report.

References

1. Potocnik I; Kerin-Povsic M, Markovic-Bozic J. The influence of anaesthetic technique on cancer growth. *Radiol Onc*ol 2024; **58(1):** 9–14. Available at: https://www.radioloncol.com/index.php/ro/article/view/4210
2. Yap A, Lopez-Olivo MA, Dubowitz J, Hiller J, Riedel B; Global OncoAnesthesia Research Collaboration Group. Anesthetic technique and cancer outcomes: a meta-analysis of total intravenous versus volatile anesthesia. Can J Anaesth 2019; 66: 546-61. doi: 10.1007/s12630-019-01330-x

Responses

The authors reply

While reviewing the article[1], we realised that we had made a mistake. Instead of VIMA, we should have written TIVA. Please, accept our apology. In the article, we also cited studies that concluded that volatile anaesthetics have anti-inflammatory action and so might act anti carcinogenic.[2-6] In the conclusion we also wrote that this area is still quite unexplored and that studies have led to very controversial results. Regarding that please, find enclosed an additional reference of Wang J *et al.*[7], who proved that volatile anaesthetics have a rule in the anti-cancer relevant signalling. Therefore, above mentioned mistake luckily did not have an effect on the message of the article.

Assist. Prof. Iztok Potocnik, M.D., Ph.D.
Institute of Oncology Ljubljana, Ljubljana, Slovenia
E-mail: vpotocnik@onko-i.si

Prof. Jasmina Markovic-Bozic, M.D., Ph.D.
University Clinical Centre Ljubljana, Ljubljana, Slovenia
E-mail: jasmina.markovicbozic@mf.uni-lj.si

Notes

No potential conflict of interest relevant to this letter was report.

References

3. Potocnik I; Kerin-Povsic M, Markovic-Bozic J. The influence of anaesthetic technique on cancer growth. *Radiol Onc*ol 2024; **58(1):** 9–14. Available at: https://www.radioloncol.com/index.php/ro/article/view/4210
4. El Azab SR, Rosseel PM, De Lange JJ, van Wijk EM, van Strik R, Scheffer GJ. Effect of VIMA with sevoflurane versus TIVA with propofol or midazolamsufentanil on the cytokine response during CABG surgery. *Eur J Anaesthesiol* 2002; **19:** 276-82. doi: 10.1017/s0265021502000443 41
5. Minou AF, Dzyadzko AM, Shcherba AE, Rummo OO. The influence of pharmacological preconditioning with sevoflurane on incidence of early allograft dysfunction in liver transplant recipients. *Anesthesiol Res Pract* 2012; **2012**: 930487. doi: 10.1155/2012/930487 42
6. Jerin A, Pozar-Lukanovic N, Sojar V, Stanisavljevic D, Paver-Erzen V, Osredkar J. Balance of pro- and anti-inflammatory cytokines in liver surgery. *Clin Chem Lab Med* 2003; **41:** 899-903. doi: 10.1515/CCLM.2003.136 43
7. Jabaudon M, Zhai R, Blondonnet R, Bonda WLM. Inhaled sedation in the intensive care unit. *Anaesth Crit Care Pain Med* 2022; **41:** 101133. doi: 10.1016/j.accpm.2022.101133 44
8. Song Z, Tan J. Effects of anesthesia and anesthetic techniques on metastasis of lung cancers: a narrative review. *Cancer Manag Res* 2022; **14:** 189-204. doi: 10.2147/CMAR.S343772 45
9. Oh CS, Park HJ, Piao L, Sohn KM, Koh SE, Hwang DY, et al. Expression profiles of immune cells after propofol or sevoflurane anesthesia for colorectal cancer surgery: a prospective double-blind randomized trial. *Anesthesiology* 2022; 136: 448-58. doi: 10.1097/ALN.0000000000004119
10. Wang J, Cheng CS, Lu Y, Sun S, Huang S. Volatile anesthetics regulate anti-cancer relevant signalling. *Front Oncol* 2021; **26:** 11: 610514. doi: 10.3389/fonc.2021.610514

Radiol Oncol 2024; 58(2): 153-169.
doi: 10.2478/raon-2024-0029

Endoskopska obravnava bolnikov z družinsko adenomatozno polipozo po preventivni kolektomiji ali obnovitveni proktokolektomiji. Sistematični pregled literature

Gavrić A, Rivero Sanchez L, Brunori A, Bravo R, Balaguer F, Pellisé M

Izhodišča. Pri bolnikih z družinsko adenomatozno polipozo (FAP) se zgodaj razvijejo adenomi debelega črevesa in danke in če jih ne zdravimo, bodo iz njih neizogibno nastale rakaste tvorbe. Profilaktična operacija ne prepreči nadaljnjega razvoja raka v rektalnem ostanku, rektalni manšeti pri bolnikih z analno anastomozo ilealne vrečke (angl. ileal pouch anal anastomosis, IPAA) in celo na ilealni sluznici telesa vrečke. Namen pričujočega pregleda je oceniti dolgoročni nastanek raka in adenomov pri bolnikih s FAP po preventivni operaciji ter povzeti trenutna priporočila za endoskopsko obravnavo in nadzor teh bolnikov.

Materiali in metode. S pomočjo navodil PRISMA smo sistematično iskali literaturo o raziskavah, ki so jih objavili od januarja 1946 do junija 2023. Uporabili smo elektronsko podatkovno zbirko PubMed.

Rezultati. Pregledali smo 54 člankov, ki so vključevali 5010 bolnikov. Delež raka v rektalnem ostanku je bil 8,8−16,7 % pri zahodni populaciji in 37 % pri vzhodni populaciji. Kumulativno tveganje za nastanek raka 30 let po operaciji je bilo 24 %. Umrljivost zaradi raka v ostanku danke je bilo 1,1−11,1 %, 5-letno preživetje pa 55 %. Delež adenomov po primarni IPAA je bil 9,4−85 % s kumulativnim tveganjem 85 % 20 let po operaciji in kumulativnim tveganjem 12 % za napredovale adenome 10 let po operaciji. Kumulativno tveganje za adenome po ileo-rektalni anastomozi (IRA) je bilo 85 % po 5 letih in 100 % po 10 letih. Adenomi so se pogosteje razvili po spenjani anastomozi (33,9−57 %) v primerjavi z ročno sešito anastomozo (0−33 %). Poročali so o 45 rakih pri bolnikih po IPAA, od katerih jih je bilo 30 v telesu vrečke, 15 pa v rektalni manšeti ali na anastomozi.

Zaključki. Pri bolnikih s FAP se med dolgotrajnim spremljanjem pogosto pojavijo rak in adenomi v rektalnem ostanku in ilealni vrečki. Redno endoskopsko spremljanje je priporočljivo ne le pri bolnikih z IRA, temveč tudi pri bolnikih z ilealno vrečko po proktokolektomiji.

Radiol Oncol 2024; 58(2): 170-178.
doi: 10.2478/raon-2024-0027

Potencialno resni zapleti novih sistemskih terapij proti raku. Zgodnja diagnoza in zgodnje ustrezno zdravljenje sta ključna

Blaž Kovač M, Šeruga B

Izhodišča. Različne oblike imunoterapije, kot so zaviralci imunskih nadzornih točk (*ang. immune checkpoint inhibitors*, ICI), modificirane celice T z izraženim himernim receptorjem za tumorske antigene (*ang. chimeric antigen receptor T-cells*, CAR-T) ter bispecifična monoklonska protitelesa (*ang. bispecific T-cell engagers*, BiTE]) in konjugati protitelo-zdravilo, vse pogosteje uporabljamo za zdravljenje solidnih rakov, limfomov in levkemij. Zdravniki različnih specialnosti se lahko srečajo z bolniki, ki razvijejo resne zaplete po tovrstnih zdravljenjih. Namen pričujočega pripovednega preglednega članka je predstaviti potencialno smrtne toksične zaplete novih protirakavih sistemskih zdravljenj ter njihove diagnostične obravnave in začeto zdravljenje.

Rezultati. Pojav toksičnih zapletov novih protirakavih sistemskih zdravljenj je lahko nepredvidljiv in nespecifičen. Klinična slika tovrstnih zapletov je lahko podobna ostalim, bolj pogostim stanjem, kot so npr. okužbe ali možganska kap. Če jih ne prepoznamo in zdravimo pravočasno, lahko hitro napredujejo v življenjsko nevarna stanja. Medtem ko ICI lahko povzročijo imunsko povzročeno vnetje različnih organov (npr. pnevmonitis ali kolitis), se lahko po zdravljenju s CAR-T ali BiTE pojavita sindrom sproščanja citokinov (*ang. cytokine release syndrome*, CRS) in sindrom nevrotoksičnosti, povezan z imunskimi efektorskimi celicami (*ang. immune effector cell-associated neurotoxicity syndrome*, ICANS). Temelja zdravljenja teh vnetnih zapletov sta ustrezna podporna terapija in sistemsko imunosupresivno zdravljenje. S slednjim je pogosto potrebno pričeti že pri blago do zmerno izraženih toksičnih zapletih. Tudi pri nekaterih hudih zapletih, povzročenih s konjugati protitelo-zdravilo, je potrebno uporabiti imunosupresivnega zdravljenja. Že zgodaj moramo v obravnavo tovrstnih zapletov vključiti multidisciplinarni tim, ki poleg onkologa oz. hematologa vključuje tudi ustreznega zdravnika specialista (npr. gastroenterologa v primeru kolitisa).

Zaključki. Zaposleni v zdravstvu, vključno s tistimi zunaj onkoloških centrov, bi morali biti seznanjeni s potencialno resnimi zapleti novih protirakavih sistemskih zdravljenj. Zgodnja diagnoza ter zdravljenje z zadostno podporno in imunosupresivno terapijo sta ključna za optimalni izhod bolnikov s temi zapleti.

Radiol Oncol 2024; 58(2): 179-185.
doi: 10.2478/raon-2024-0022

Kolitis zaradi zdravljenja raka z zaviralci imunskih kontrolnih točk. Pregled literature in opis kliničnih primerov

Ocepek A

Zdravljenje z zaviralci imunskih kontrolnih točk je učinkovito pri različnih oblikah raka, a je lahko povezano s pojavom imunsko pogojenih neželenih učinkov na drugih organih. Med pogostejše sodi prizadetost prebavne cevi, predvsem kolitis. Pri večini bolnikov kolitis poteka blago, ali pa se odzove na kortikosteroidno zdravljenje. Manjši delež bolnikov, pogosteje tistih, ki smo jih zdravili z zaviralci citotoksičnega T limfocitnega antigena-4, ima lahko težji potek kolitisa, pojavijo se lahko celo za življenje nevarni zapleti. Pri teh bolnikih je potrebno hitro ukrepanje, pravočasna postavitev diagnoze z endoskopsko oceno in zgodnje zdravljenje z visokimi odmerki kortikosteroidov ter ob njihovi neučinkovitosti reševalno zdravljenje z biološkimi zdravili kot sta infliksimab in vedolizumab. Predstavljamo tri primere iz klinične prakse, podatke o incidenci in klinični sliki ter trenutna priporočila glede diagnostičnega postopka in zdravljenja kolitisa, ki ga povzročajo zaviralci imunski kontrolnih točk.

Radiol Oncol 2024; 58(2): 186-195.
doi: 10.2478/raon-2024-0028

Patogeneza in potencialna reverzibilnost intestinalne metaplazije – mejnik v karcinogenezi želodca

Drnovšek J, Homan M, Zidar N, Šmid LM

Izhodišča. Rak želodca brez kardije ostaja glavni vzrok umrljivosti zaradi raka po vsem svetu, kljub temu, da incidenca v številnih industrializiranih državah upada. Razvoj raka želodca intestinalnega tipa poteka skozi večstopenjski proces, v katerem se normalna sluznica postopno preoblikuje v hiperproliferativni epitelij, čemur sledijo metaplastični procesi, ki vodijo do karcinogeneze. Kronična okužba s Helicobacter pylori je primarni etiološki povzročitelj, ki povzroči kronično vnetje želodčne sluznice, vodi do atrofičnega gastritisa in lahko povzroči intestinalno metaplazijo in displazijo. Tako intestinalna metaplazija kot displazija sta predrakavi spremembi, pri katerih obstaja večja verjetnost za pojav raka želodca. Atrofični gastritis se pogosto izboljša po eradikaciji Helicobacter pylori; vendar je pojav intestinalne metaplazije tradicionalno obravnavan kot "točka brez vrnitve" v zaporedju karcinogeneze. Po eradikaciji bakterije Helicobacter pylori se neatrofični kronični gastritis bodisi pozdravi, ali pa povzroči regresijo atrofičnega gastritisa in s tem zmanjša tveganje za nastanek raka želodca. V članku razpravljamo o patogenezi, epigenomiki in reverzibilnosti intestinalne metaplazije ter se na kratko predstavimo možne strategije zdravljenja.

Zaključki. Zdi se, da želodčna intestinalna metaplazija ni več ireverzibilna predrakava lezija. Vendar pa je še vedno veliko polemik glede izboljšanja intestinalne metaplazije po eradikaciji Helicobacter pylori.

Radiol Oncol 2024; 58(2): 196-205.
doi: 10.2478/raon-2024-0024

Uporabnost kliničnih parametrov in slikovnih parametrov MR za napovedovanje in spremljanje odgovora na zdravljenje s capecitabinom in temozolomidom (CAPTEM) pri bolnikih z jetrnimi zasevki nevroendokrinih tumorjev

Ingenerf M, Auernhammer C, Lorbeer R, Winkelmann M, Mansournia S, Mansour N, Hesse N, Heinrich K, Ricke J, Berger F, Schmid-Tannwald C

Izhodišča. Namen raziskave je bil proučiti možnosti napovedovanja in spremljanja kliničnih in multiparametričnih parametrov MR pri ocenjevanju odgovora na zdravljenje s capecitabinom in temozolomidom (CAPTEM) pri bolnikih z nevroendokrinimi tumorji (NET).

Bolniki in metode. V retrospektivni raziskavi (n = 44) smo ocenili odgovor na zdravljenje CAPTEM pri bolnikih z nevroendokrinimi zasevki v jetrih (*angl. neuroendocrine liver metastases,* NELM). Med 33 vključenimi bolniki, ki je predstavljala podskupino celotne kohorte raziskave, smo analizirali MR podatke in podatke pridobljene ob sledenju bolnikov (velikost tumorske spremermbe, vrednosti difuzijskega koeficienta [*angl. apparent diffusion coefficient ADC*] in intenziteto signala) ter klinične parametre (kromogranin A [CgA] in Ki-67 %). Tiste bolnike, ki so brez napredovanja bolezni preživeli več kot 6 mesecev smo opredelili, da so odgovorili na zdravljenje oz. kot odzivne bolnike.

Rezultati. Večina bolnikov je bila moških (75 %) in je imela tumorje G2 (76 %) trebušne slinavke (84 %). Srednje preživetje brez napredovanja bolezni je bilo 5,7 meseca; celotno srednje preživetje pa 25 mesecev. Neodzivni bolniki so imeli v primerjavi z odzivnimi višji Ki-67 pri primarnih tumorjih (16,5 % *vs.* 10 %, p = 0,01) in večjo obremenitev jeter (20 % *vs.* 5 %, p = 0,007). Neodzivni bolniki so imeli povišan CgA po zdravljenju, medtem ko je bil CgA pri odzivnih bolnikih blago zmanjšan. Spremembe ADC so se razlikovale med skupinami, neodzivni bolniki so imeli znižan ADC_{min} (-23 %) in jetrno prilagojen $ADC_{povpr.}/ADC_{jetrni\ povpr.}$ (-16 %), v primerjavi z odzivnimi, ki so imeli povečan ADC_{min} (50 %) in $ADC_{povpr.}/ADC_{jetrni\ povpr.}$ (30 %). Analiza ROC je pokazala najvišjo območje pod krivuljo (*angl. area under the curve*, AUC) (0,76) za posamezen parameter za Δ $ADC_{povpr.}/ADC_{jetrni\ povpr.}$ pri mejni vrednosti < 6,9 (občutljivost 76 % in specifičnost 75 %). Kombinacija Δ velikosti NELM in Δ ADC_{min} je dosegla najboljše rezultate (88 % občutljivost, 60 % specifičnost) in je bila boljša od samega Δ velikosti NELM (69 % občutljivost, 65 % specifičnost). Kaplan-Meierjeva analiza je pokazala pomembno daljše preživetje brez napredovanja bolezni za Δ $ADC_{povpr.}/ADC_{jetrni\ povpr.}$ < 6,9 (p = 0,024) in Δ velikost NELM > 0 % + Δ ADC_{min} < -2,9 % (p = 0,021).

Zaključki. Analiza preživetja bolnikov z NET in zasevki v jetrih, ki smo jih zdravili s CAPTEM, je pokazala potrebo po prilagojenih merilih ocenjevanja odgovora na zdravljenje. Ti vključujejo kombinirano vrednotenje CgA, vrednosti ADC in velikosti tumorja.

Radiol Oncol 2024; 58(2): 206-213.
doi: 10.2478/raon-2024-0021

Dolgoročna učinkovitost večplastnih modulatorjev pretoka za zdravljenje aortnih anevrizem

Pintarić K, Bolteżar L, Umek N, Kuhelj D

Izhodišča. V retrospektivni raziskavi smo preučevali učinkovitost znotrajžilnega zdravljenja z večplastnimi modulatorji pretoka (*angl. multilayer flow modulator*, MFM) pri zdravljenju aortnih anevrizem pri pacientih z visokim tveganjem, ki niso bili primerni za standardno zdravljenje.

Bolniki in metode. V raziskavo smo vključili 17 bolnikov z anevrizmo torakalne ali abdominalne aorte, ki smo jih med letoma 2011 in 2019 zdravili z vstavitvijo MFM-ja. Pri teh bolnikih bi klasično endovaskularno ali kirurško zdravljenje anevrizme predstavljalo preveliko tveganje. Po vstavitvi MFM-jev smo bolnike redno spremljali z računalniško tomografijo, kjer smo poleg premera anevrizmatske vreče merili tudi prostornino anevrizmatske vreče ter volumenske spremembe pretoka v anevrizmi. Poleg tega smo beležili tudi tehnično izvedljivost vstavitve MFM-jev in zaplete po posegu.

Rezultati. Tehnična izvedljivost je bila 100 %, pogostost zapletov v 30 dneh po posegu pa 17,6 %. Ob spremljanju smo pri 11 od 17 bolnikih ugotovili zmanjšanje prostornine pretoka znotraj anevrizme, kar kaže na ugoden hemodinamski odziv. Srednja vrednost zmanjšanja prostornine pretoka je bila 12 mL, relativo zmanjšanje 8 %. Hkrati pa nismo ugotovili doslednega zmanjšanja velikosti anevrizmatske vreče; večina anevrizmatskih vreč se je povečala za 46 ml oz. za 18 mm.

Zaključki. Čeprav MFM-ji ponujajo možno alternativno zdravljenje za bolnike z visokim tveganjem in anevrizmami aorte, je njihova učinkovitost pri preprečevanju večanja anevrizme omejena. Rezultati kažejo, da lahko MFM-ji zagotovijo stabilno hemodinamsko okolje, vendar ne povzročijo stabilnega zmanjšanja anevrizmatske vreče. Uporaba opisane tehnologije tako zahteva stalno pazljivost in dolgoročno spremljanje bolnikov.

Radiol Oncol 2024; 58(2): 214-220.
doi: 10.2478/raon-2024-0023

Napovedni dejavniki preživetja in varnost transarterijske kemoembolizacije (TACE) z irinotekanom, vezanim na delce (DEBIRI), pri bolnikih z jetrnimi zasevki pri raku debelega črevesja

Šljivić M, Sever M, Ocvirk J, Mesti T, Brecelj E, Popović P

Izhodišča. Pri bolnikih z neresektabilnimi jetrnimi zasevki raka debelega črevesa in danke veljavne smernice priporočajo transarterijsko kemoembolizacijo z irinotekanom, vezanim na delce (*angl. transarterial chemoembolisation with rinotecan-loaded drug-eluting beads*, DEBIRI TACE), kot eno izmed možnosti zdravljenja, ko so izkoriščeni vsi drugi načini sistemskega in kirurškega zdravljenja.

Bolniki in metode. V retrospektivno raziskavo smo med septembrom 2010 in marcem 2020 vključili 30 bolnikov (22 moških in 8 žensk; s povprečno starostjo 66,8 ± 13,2 let). Med njimi smo jih 57 % predhodno zdravili s sistemsko kemoterapijo, ostalih 43 % pa kemoterapije ni prejelo. Pri vseh je bila bolezen omejena na jetra. Dva posega v štiri-tedenskih intervalih smo naredili v primerih z unilobarno boleznijo ter štiri posege v dvo-tedenskih intervalih v primerih z bilobarno boleznijo. Vsi bolniki so prejeli premedikacijo in bili opazovani po posegu. Neželene dogodke smo ocenjevali po klasifikaciji Kardiovaskularne in intervencijske radiološke evropske zveze (CIRSE).

Rezultati. Srednje celokupno preživetje od začetka zdravljenja z DEBIRI TACE je bilo 17,4 mesecev (95 % interval zaupanja [IZ]: 10,0–24,7 mesecev), srednje preživetje brez napredovanja bolezni pa 4,2 meseca (95 % IZ: 0,9–7,4 mesecev). Srednje celokupno preživetje v skupini, ki je predhodno prejemala sistemsko kemoterapijo je bilo 17,4 mesecev, v skupini brez predhodne sistemske kemoterapije pa 21,6 mesecev. Univariatna analiza je pokazala boljše preživetje pri bolnikih z manj kot pet zasevki (p = 0,002). Neželenih dogodkov gradusa 4 in 5 ni bilo, prav tako ne smrti, povezanih s posegom. Neželeni dogodki gradusov 1 in 2 so bili prisotni pri 53 % bolnikih, gradusa 3 pa pri 6 %.

Zaključki. DEBIRI TACE je varna oblika zdravljenja jetrnih zasevkov pri raku debelega črevesja. Bolniki z manj kot petimi zasevki so imeli daljše preživetje, kot tisti s pet ali več.

Radiol Oncol 2024; 58(2): 221-233.
doi: 10.2478/raon-2024-0019

Terapevtski učinek tarčnega ultrazvočnega uničenja kontrastnih mikromehurčkov šisandrina A pri raku na jetrih in njihov mehanizem

Wang X, Wang F, Dong P, Zhou L

Izhodišča. Namen študije je bil raziskati terapevtski učinek tarčnega ultrazvočnega uničenja kontrastnih mikromehurčkov šisandrina A pri raku na jetrih in z njim povezan mehanizem.

Materiali in metode. Mikro-mehurčke Span-PEG, napolnjene s šisandrinom A, smo pripravili za uporabo s Span60, NaCl, PEG-1500 in šisandrina A. Stopnjo napolnjenosti mikromehurčkov Apna-PEG s šisandrinom A smo določili z metodo ultravijolične spektrofotometrije. Stopnjo preživetja celic Walker-256 s šisandrinom A pa smo ugotovili s testom MTT. Vsebnost šisandrina A v celicah smo izmerili s tekočinsko kromatografijo visoke ločljivosti. Za oceno terapevtskega učinka *in situ* smo uporabili ultrazvočno slikanje. Vsebnosti vnetnih dejavnikov v serumu smo izmerili z metodo ELISA. Patološke spremembe pri poskusnih živalih v vsaki skupini smo opazovali s pomočjo barvanja s hematoksilin-eosinom. Izražanja HIF-1a, VEGF in VEGFR-2 v tumorskih tkivih smo ugotavljali z imunohistokemičnim barvanjem, izražanja beljakovin signalne poti PI3K/AKT/mTOR v tumorskih tkivih pa s postopkom *Western blot*.

Rezultati. Sestavljeni mikromehurčki so bili enakomerne velikosti, porazdelitev velikosti delcev je bila enolična in stabilna, kar ustreza zahtevam za ultrazvočna kontrastna sredstva. Stopnja polnjenja šisandrina A v mikromehurčkih Span-PEG je bila 8,84 ± 0,14 %, učinkovitost enkapsulacije pa 82,24 ± 1,21 %. Vrednost IC50 šisandrina A je bila 2,87 μg/ml. Skupina zdravilo+mikromehurčki+ultrazvok je imela najbolj očiten zaviralni učinek na rakave celice Walker-256, najvišjo znotrajcelično koncentracijo zdravila, največje zmanjšanje prostornine tumorja, najbolj očitno zmanjšanje vnetnih dejavnikov v serumu in najbolj očitno izboljšanje patoloških vrednosti. Rezultati imunohistokemičnega barvanja so pokazali, da so se beljakovine HIF-1a, VEGF in VEGFR-2 najbolj zmanjšale v skupini zdravilo+mikromehurčki+ultrazvok ($P < 0,01$). Rezultati testov *Western blot* so pokazali, da je omenjena skupina najbolj zavirala signalno pot PI3K/AKT/mTOR ($P < 0,01$).

Zaključki. Šisandrin A je imel protitumorski učinek, njegov mehanizem pa je lahko povezan z zaviranjem signalne poti PI3K/AKT/mTOR. Mikromehurčki šisandrina A bi lahko spodbujali vnos šisandrina A v tumorske celice, potem ko so bili uničeni v tumorju zaradi delovanja ultrazvoka, in tako imeli najboljši protitumorski učinek.

Radiol Oncol 2024; 58(2): 234-242.
doi: 10.2478/raon-2024-0020

Povezanost ekspresije podenote alfa 3 laminina v duktalnem adenokarcinomu trebušne slinavke z zasevki v jetrih in s preživetjem

Xing Y, Jing X, Qing G, Jang Y

Izhodišča. Visoka stopnja umrljivosti zaradi duktalnega adenokarcinoma trebušne slinavke (*angl. pancreatic ductal adenocarcinoma*, PDAC) pripisujemo predvsem zasevkom. Znano je, da alfa 3 podenota laminina (LAMA3) vpliva na napredovanje tumorja. Vendar ostaja nejasen vpliv LAMA3 na jetrne zasevke pri PDAC. Namen raziskave je bil razjasniti, ali je pri PDAC z jetrnimi zasevki ekspresija LAMA3 povečana.

Bolniki in metode. Iz atlasa genoma raka (*angl. The Cancer Genome Atlas*, TCGA) in štirih naborov podatkov iz omnibusa genskega izražanja (*angl. Gene Expression Omnibus*, GEO) smo pridobili podatke o nivojih izražanja LAMA3 in povezanimi klinično-patološkimi parametri. Za oceno napovedne moči LAMA3 pri PDAC smo uporabili Kaplan-Meierjevo analizo. Retrospektivno smo zbrali klinično-patološke podatke in vzorce tkiv 117 kirurško zdravljenih bolnikih s PDAC v bolnišnici Univerze Qingdao. Ocenili smo izražanje LAMA3 in raziskali njegovo povezavo s klinično-patološkimi lastnostmi, kliničnim potekom bolezni in zasevki v jetrih.

Rezultati. Baze podatkov TCGA in GEO so pokazale, da je v tkivu PDAC povečano izražanje LAMA3 v primerjavi z normalnim tkivom. Pri bolnikih s PDAC je bila povišana ekspresija LAMA3 povezana s slabšim celokupnim preživetjem in preživetjem brez ponovitve bolezni. Ekspresija LAMA3 je bila znatno višja v tkivu PDAC kot v sosednjih tkivih. V tumorskem tkivu bolnikov s PDAC in jetrnimi zasevki je bila ekspresijo LAMA3 višja, kot pri bolnikih brez jetrnih zasevkov. Visoka ekspresija LAMA3 je sovpadala z velikostjo tumorja in stadijem TNM. Ekspresija LAMA3 in zasevki v jetrih sta bila neodvisna napovedna dejavnika za celokupno preživetje; ekspresija LAMA3 je bila neodvisno povezana s prisotnostjo jetrnih zasevkov.

Zaključki. Ekspresija LAMA3 je pri bolnikih s PDAC in jetrnimi zasevki povišana in napoveduje potek bolezni.

Radiol Oncol 2024; 58(2): 243-257.
doi: 10.2478/raon-2024-0016

Vpliv zgodnje celostne rehabilitacije na utrudljivost pri 600 bolnicah z rakom dojke. Prospektivna raziskava

Auprih M, Žagar T, Kovačevič N, Škufca Smrdel AC, Bešić N, Homar V

Izhodišča. Utrudljivost po zdravljenju raka dojke je pogosta težava bolnic, ki jo je težko zdraviti. Namen raziskave je bil proučiti, ali individualizirana celostna rehabilitacija zmanjša pogostost kronične utrudljivosti v primerjavi z neorganizirano rehabilitacijo.

Bolnice in metode. V prospektivno raziskavo smo vključili 600 bolnic z rakom dojke (stare 29–65 let, povprečno 52), ki so sodelovale v pilotni raziskavi o individualizirani celostni rehabilitaciji bolnic z rakom dojke v letih 2019–2021 in smo jih spremljali leto dni. V kontrolni skupini je bilo 301 bolnic, v intervencijski skupini pa 299. Bolnice so izpolnili tri vprašalnike (EORTC QLQ-C30, -BR23 in NCCN): pred zdravljenjem raka, ter šest in dvanajst mesecev po začetku zdravljenja raka. Pri kontrolni skupini smo izvajali standardno neorganizirano rehabilitacijo, medtem ko je bila intervencijska skupina deležne zgodnje, individualizirane multidisciplinarne in celostne rehabilitacije. Koordinatorka za celostno rehabilitacijo je bolnice napotila na dodatne intervencije (npr. psiholog, ginekolog, tim za obvladovanje bolečine, fizioterapija, tim za klinično prehrano, fizična vadba pod vodstvom kineziologa preko spleta, poklicna rehabilitacija, družinski zdravnik). Zbrali in analizirali smo podatke o demografskih značilnostih bolnic, razširjenosti bolezni, zdravljenju raka in težavah bolnic, o katerih so poročale v odgovorih na vprašanja iz vprašalnikov.

Rezultati. Med kontrolno in intervencijsko skupino bolnic ni bilo razlik glede starosti, izobrazbe, razširjenosti bolezni, kirurških posegov, sistemskega zdravljenja raka ali obsevanja. Prav tako ni bilo razlik glede utrudljivosti pred začetkom zdravljenja. Bolnice iz kontrolne skupine pa so imele pol leta ($p = 0,018$) in leto dni ($p = 0,001$) po začetku zdravljenja višjo stopnjo stalne utrudljivosti kot bolnice iz intervencijske skupine. Poleg tega je večji delež bolnic iz kontrolne skupine občutil pomembne motnje pri svojih običajnih dejavnostih zaradi utrudljivosti kot iz intervencijske skupine pol leta ($p = 0,042$) in leto dni ($p = 0,001$) po začetku zdravljenja. Multivariatna logistična regresija je pokazala, da je leto dni po začetku zdravljenja edini neodvisni dejavnik, povezan z utrudljivostjo, vključitev v intervencijsko skupino ($p = 0,044$). Vključitev v intervencijsko skupino je bila koristna. Bolnice iz kontrolne skupine so imele po zdravljenju 1,5-krat večjo verjetnost za utrudljivost kot bolnice iz intervencijske skupine.

Zaključki. Zgodnja individualizirana celostna rehabilitacija je povezana z manjšo prevalenco kronične utrudljivosti ali utrudljivosti, ki moti običajne aktivnosti pri bolnicah z rakom dojke, v primerjavi s kontrolno skupino bolnic.

Radiol Oncol 2024; 58(2): 258-267.
doi: 10.2478/raon-2024-0030

Napredek pri obvladovanju raka dojk z nizkim izraženjem receptorja HER2. Izboljšanje strategij diagnosticiranja in zdravljenja

Borštnar S, Bozović-Spasojević I, Cvetanović A, Dedić Plavetić N, Konsoulova A, Matos E, Popović L, Popovska S, Tomić S, Vrdoljak E

Izhodišča. Nedavni dokazi učinkovitosti konjugatov protitelesa in zdravila proti receptorju za epidermalni rastni dejavnik 2 (*angl. human epidermal growth factor receptor 2*, HER2), so omogočili pomembne spremembe pri obravnavi HER2-negativnega raka dojke. Novo ciljno entiteto, rak dojke z nizkim izražanjem receptorja HER2, so prepoznali pri tumorjih, ki smo jih prej razvrščali kot HER2-negativne. Pričakujemo, da se bo vsakodnevna praksa v patologiji in klinični onkologiji uskladila s trenutnimi priporočili, vendar bi lahko bil dostop bolnikov do novih protirakavih zdravil na različnih geografskih območjih oviran zaradi lokalnih izzivov.

Materiali in metode. Na strokovnem srečanju se je zbralo deset ekspertov, mnenjskih voditeljev s področja patologije in klinične onkologije z izkušnjami pri zdravljenju bolnikov z rakom dojke iz štirih držav Centralne in Vzhodne Evrope, iz Bolgarije, Hrvaške Srbije in Slovenije. Povzeli smo trenutno stanje razsejanega raka dojke z nizkim izražanjem receptorja HER2, lokalne izzive in akcijske načrte za preprečevanje zamud pri dostopu bolnikov do testiranja in zdravljenja na podlagi strokovnega mnenja.

Rezultati. Ugotovili smo vrzeli in razlike med državami na več ravneh. Ti so vključevali variabilnost (1) lokalnih epidemioloških podatkov o raku dojke z nizkim izražanjem receptorja HER2, (2) različno certificiranje patoloških laboratorijev, (3) različen nadzor kakovosti ter (4) različne pogoje za povračilo stroškov testiranja in zdravil proti raku za HER2-negativni razsejani rak dojke. Medtem ko so bile klinične odločitve usklajene z sodobnimi mednarodnimi smernicami, je bil optimalen dostop do testiranja in inovativnega zdravljenja omejen zaradi znatnih zamud pri povračilu stroškov ali omejenih pogojev povračila stroškov.

Zaključki. Preprečevanje zamud pri dostopu bolnikov z razsejanim rakom dojke, ki imajo nizko izraženost HER2, do diagnoze in novih zdravljenj, je ključnega pomena za optimizacijo rezultatov. Potrebna so multidisciplinarna skupna prizadevanja in proaktivne razprave med zdravniki in odločevalci za izboljšanje oskrbe bolnikov z razsejanim rakom dojke, ki imajo nizko izražanje receptorja HER2, v državah centralne in Vzhodne Evrope.

Radiol Oncol 2024; 58(2): 268-278.
doi: 10.2478/raon-2024-0025

Razkrivanje diagnostične poti pljučnega raka. Prepoznavanje izzivov in priložnosti za izboljšave

Marc Malovrh M, Adamič K

Izhodišča. Za večjo uspešnost obravnave bolnikov s pljučnim rakom je pomemben hiter in dobro organiziran celovit diagnostični postopek. Namen pričujoče raziskave je bil razkriti pomanjkljivosti in premajhne učinkovitosti diagnostičnega postopka ter predlagati strategije za izboljšave. Raziskava je potekala v enem od terciarnih centrov v Sloveniji.

Bolniki in metode. Uporabili smo celovit pristop, da smo skrbno razčlenili vse korake v diagnostičnem postopku za posameznike, pri katerih smo sumili, da so zboleli za pljučnim rakom. Z namenskimi vprašalniki smo zbrali in analizirali informacije od zaposlenih in od bolnikov, vključenih v obravnavo. Nadalje smo analizirali podatke bolnikov in izračunali časovne diagnostične intervale za te bolnike v dveh različnih obdobjih opazovanja.

Rezultati. Zaposlene sta najbolj obremenjevala stres in pretirani administrativni postopki. Pomemben rezultat vizualizacije diagnostične poti in priporočil zaposlenih je bila zasnova elektronske diagnostične klinične poti (*angl. electronic diagnostic clinical pathway,* eDCP), ki bo lahko bistveno zmanjšala administrativno obremenitev zaposlenih. Bolniki so bili na splošno zelo zadovoljni z diagnostično potjo, poročali pa so o predolgih čakalnih dobah. Z analizo dveh časovnih obdobij smo ugotovili, da so diagnostični intervali v večini primerov presegali priporočene časovnice. Z dvema ukrepoma smo diagnostične intervale znatno skrajšali: okrepili smo diagnostični tim in pridobili v uporabo dodatni aparat PET-CT. Povprečni čas od napotitve osebnega zdravnika do odločitve o zdravljenju na multidisciplinarnem konziliju je bil 50,8 (± 3,0) dni pred in 37,1 (± 2,3) dni po uvedbi teh ukrepov.

Zaključki. Raziskava je razkrila priložnosti za izboljšave diagnostične poti za bolnike s pljučnim rakom. Največji vpliv so imele organizacijsko-administrativne izboljšave in izboljšave, povezane z zmogljivostjo.

Radiol Oncol 2024; 58(2): 279-288.
doi: 10.2478/raon-2024-0015

Vpliv različnega intraoperativnega spremljanja tekočin na pooperativni izid po resekciji abdominalnih tumorjev

Jenko M, Mencin K, Novak-Janković V, Spindler-Vesel A

Izhodišča. Intraoperativno spremljanje tekočin je pomemben vidik kirurgije raka, vključno s kolorektalno kirurgijo in pankreatoduodenektomijo. Namen raziskave je bil preizkusiti ali intraoperativno multimodalno spremljanje tekočin zmanjša pooperativno obolevnost in trajanje hospitalizacije pri bolnikih z abdominalnimi operacijami, ob uporabi istih anestetičnih protokolov z epiduralno analgezijo.

Bolniki in metode. Prospektivno raziskavo smo izvedli pri dveh skupinah bolnikov. Kirurške bolnike z visokim tveganjem, ki so bili podvrženi večji abdominalni operaciji, smo naključno izbrali in vključili v kontrolno skupino. Pri njih (44 bolnikov) smo spremljali tekočine na standardni način. V drugo, protokolno skupino pa smo vključili bolnike, kjer smo uporabili cerebralno oksigenacijo in razširjeno hemodinamsko spremljanje s protokolom za intraoperativne posege (44 bolnikov).

Rezultati. Dolžini hospitalizacije se med skupinama nista razlikovali. Srednja vrednost je bila pri kobntrolni skupini 9 dni (interkvartilni razpon [IQR] 8 dni), v protokolni skupini pa 9 dni (IQR 5,5 dni; p = 0,851). Prav tako ni bilo razlik pri pojavu pooperativnih ledvičnih ali srčnih okvar. Prokalcitonin pa je bil značilno višji (najvišja pooperativna vrednost v prvih 3 dneh) v kontrolni skupini 0,75 mcg/L (IQR 3,19 mcg/L), v protokolni skupini 0,3 mcg/L (0,88 mcg/L; p = 0,001). Obratno so bolniki v protokolni skupini prejeli večji volumen intraoperativne tekočine, srednja vrednost intraoperativne bilance tekočin je bila +1300 ml (IQR 1063 ml) pri kontrolni skupini pa +375 ml (IQR 438 ml; p < 0,001).

Zaključki. Pri intraoperativnem spremljanju tekočin in uporabi vazopresorjev so bile pomembne razlike. Srednja pooperativna vrednost prokalcitonina je bila bistveno višja pri bolnikih v kontrolni skupini, kar kaže na razlike v imunskem odzivu na poškodbo tkiva pri različnih intraoperativnih statusih tekočine, ni pa bilo razlike v pooperativni obolevnosti ali dolžini hospitalizacije .

Radiol Oncol 2024; 58(2): 289-299.
doi: 10.2478/raon-2024-0018

Primerjava dozimetrije in učinkovitosti na znanju temelječega in ročnega načrtovanja ob uporabi volumetrične modulirane obločne terapije pri kraniospinalnem obsevanju

Tsai WT, Hsieh HL, Hung SK, Zeng CF, Lee MF, Lin PH, Lin CY, Li WC, Chiou WY, Wu TH

Izhodišča. Kraniospinalno obsevanje predstavlja izziv pri načrtovanju zdravljenja zaradi velikega tarčnega volumna, stikanja polj in več ogroženih organov. Namen raziskave je bil oceniti učinkovitost načrtovanja na podlagi znanja (*angl. knowledge-based planning*, KBP) pri kraniospinalnem obsevanju. Primerjali smo izvirne ročne načrte, začetne načrte KBP *RapidPlan* in končne načrte KBP *RapidPlan*, ki smo jih dodatno optimizirali, da bi zadostili omejitvam doze.

Bolniki in metode. Porazdelitev doze v tarči smo ocenjevali glede na pokritost tarčnih volumnov, povprečno dozo, indeks skladnosti in indeks homogenosti. Ocenili smo dozimetrične rezultate v področju ogroženih organov, čas načrtovanja obsevanja in monitorsko enoto.

Rezultati. Vsi izvirni ročni načrti in končni načrti KBP *RapidPlan* so v celoti dosegli cilje načrtovanja, začetni načrti KBP *RapidPlan* pa v 89,36 %. Wilcoxonovi testi so pokazali primerljivo pokritost tarčnih volumnov, indeks skladnosti in indeks homogenosti v skupini ročnega načrtovanja in skupini končnih načrtov KBP *RapidPlan*; najslabša kakovost načrtovanja pa se je pokazala v skupini začetnih načrtov KBP *RapidPlan*. V področju ogroženih organov sta imeli skupini končnih načrtov KBP *RapidPlan* in začetnih načrtov KBP *RapidPlan* boljše dozimetrične rezultate kot skupina z ročnimi načrti ($P < 0,05$ za optične živce, oči, parotidne žleze in srce). Čas načrtovanja se je bistveno skrajšal, povprečno 677,80 min; v skupini z ročnimi načrti 227,66 min ($P < 0,05$) oziroma v ostalih dveh skupinah KBP 307,76 min ($P < 0,05$); čas načrtovanja se med njimi ni značilno razlikoval.

Zaključki. KBP lahko bistveno skrajša čas načrtovanja pri kraniospinalnem obsevanju. Za izboljšanje kakovosti obsevalnega načrta priporočamo ponovno ročno optimiziranje po začetni KBP.

Radiol Oncol 2024; 58(2): 300-310.
doi: 10.2478/raon-2024-0026

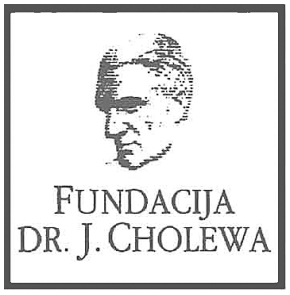
Določanje bakra in drugih elementov v sledovih v serumskih vzorcih bolnikov z raki biliarnega trakta. Protokol prospektivne neintervencijske nerandomizirane klinične raziskave

Reberšek M, Hribernik N, Marković K, Marković S, Uršič Valentinuzzi K, Čemažar M, Zuliani T, Milačič R, Ščančar J

Izhodišča. Rake biliarnega trakta običajno odkrijemo v napredovali stopnji, ko je bolezen neozdravljiva. Tumorski označevalci v krvi, ki jih trenutno določamo, imajo omejeno diagnostično vrednost pri rakih biliarnega trakta, zato nujno potrebujemo občutljive in specifične označevalce za zgodnejše diagnosticiranje. Deregulacija homeostaze elementov v sledovih je vključena v kancerogenezo različnih vrst raka, vključno z raki biliarnega trakta. Cilj raziskave je določiti in primerjati skupne koncentracije bakra (Cu), cinka (Zn) in železa (Fe) ter deleže prostega Cu in Cu, vezanega na ceruloplazmin (Cp), ter izotopsko razmerje $^{65}Cu/^{63}Cu$ v serumskih vzorcih zdravih prostovoljcev in bolnikov z rakom ob uporabi metod na osnovi induktivno sklopljene plazme in masne spektrometrije (*angl. inductively coupled plasma-mass spectrometry-based methods*, ICP-MS).

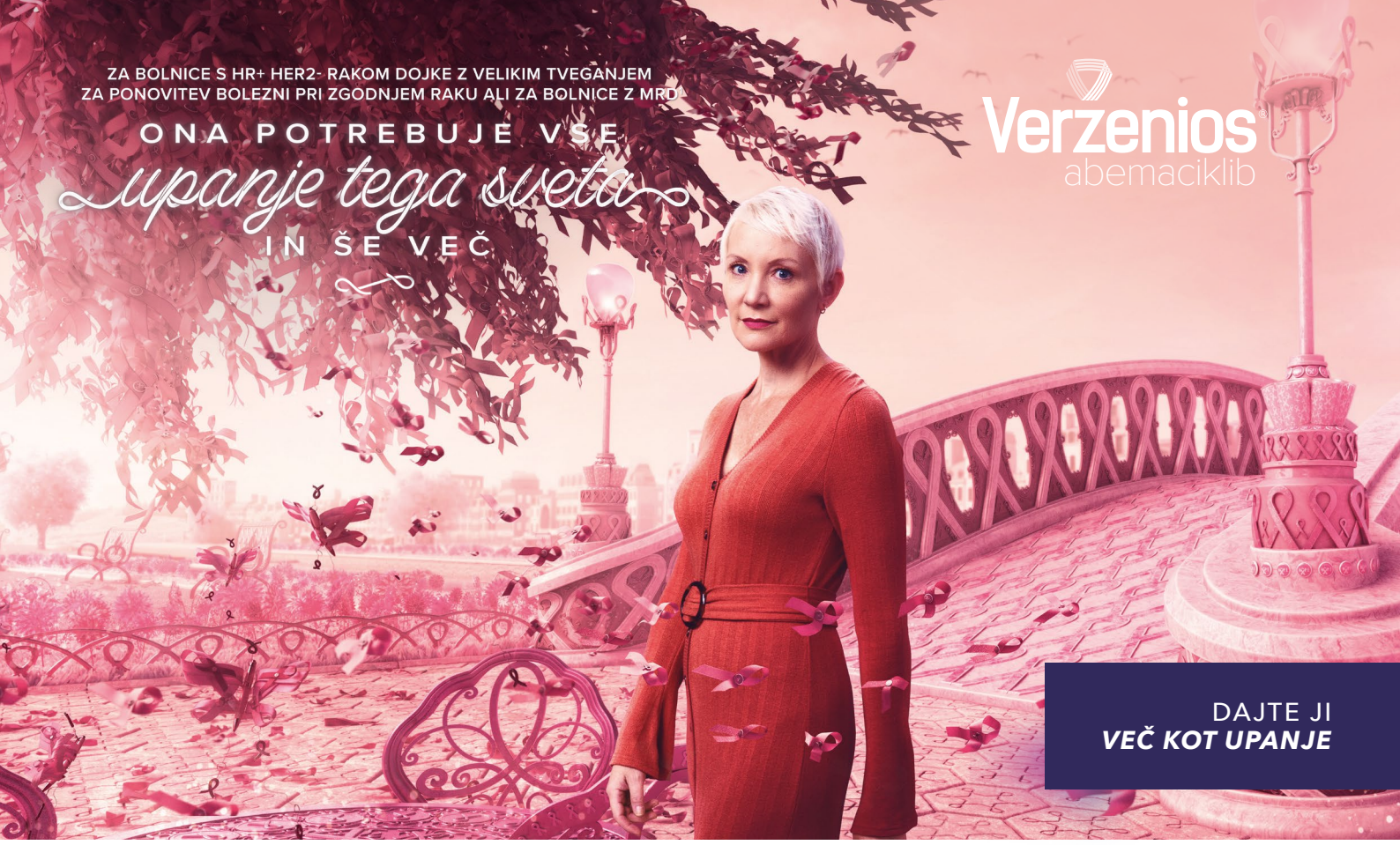
Bolniki in metode. V prospektivno, neintervencijsko, nerandomizirano raziskavo bomo vključili 20 bolnikov in 20 zdravih prostovoljcev, da bi ugotovili ravni Cu, Zn in Fe v serumu ter izotopsko rakcionacijo Cu, ki bi lahko predstavljali označevalec odgovora na sistemsko zdravljenje rakov biliarnega trakta, kar bomo ocenjevali z računalniško tomografijo. Uporabili bomo novo razvite analitične metode, ki temeljijo na ICP-MS.

Zaključki. V raziskavi bomo primerjali skupne koncentracije izbranih elementov v sledovih, deleža prostega Cu in Cu, vezanega na Cp, ter izotopskega razmerja $^{65}Cu/^{63}Cu$ v serumskih vzorcih zdravih prostovoljcev in vzorcih bolnikov z raki biliarnega trakta. Želimo zagotovili osnovo za razvoj metodologije presejanja in pridobiti podatke za možnega napovednega označevalca odgovora na sistemsko zdravljenje pri rakih biliarnega trakta.



Fundacija ''Docent dr. J. Cholewa''
je neprofitno, neinstitucionalno in nestrankarsko
združenje posameznikov, ustanov in organizacij, ki želijo
materialno spodbujati in poglabljati raziskovalno
dejavnost v onkologiji.

Dunajska 106
1000 Ljubljana
IBAN: SI56 0203 3001 7879 431



SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

IME ZDRAVILA Verzenios 50 mg/100 mg/150 mg filmsko obložene tablete **KAKOVOSTNA IN KOLIČINSKA SESTAVA** Ena filmsko obložena tableta vsebuje 50 mg/100 mg/150 mg abemacikliba. Ena filmsko obložena tableta vsebuje 14 mg/28 mg/42 mg laktoze (v obliki monohidrata). **Terapevtske indikacije** Zgodnji rak dojk Zdravilo Verzenios je v kombinaciji z endokrinim zdravljenjem indicirano za adjuvantno zdravljenje odraslih bolnikov z na hormonske receptorje (HR) pozitivnim, na receptorje humanega epidermalnega rastnega faktorja 2 (HER2) negativnim zgodnjim rakom dojk s pozitivnimi bezgavkami, pri katerih obstaja veliko tveganje za ponovitev. Pri ženskah v pred- ali perimenopavzi je treba endokrino zdravljenje z zaviralcem aromataze kombinirati z agonistom gonadoliberina LHRH – luteinizing hormone–releasing hormone). Napredovali ali metastatski rak dojk Zdravilo Verzenios je indicirano za zdravljenje žensk z lokalno napredovalim ali metastatskim, na hormonske receptorje (HR) pozitivnim in na receptorje humanega epidermalnega rastnega faktorja 2 (HER2) negativnim rakom dojk v kombinaciji z zaviralcem aromataze ali s fulvestrantom kot začetnim endokrinim zdravljenjem ali pri ženskah, ki so prejele predhodno endokrino zdravljenje. Pri ženskah v pred- ali perimenopavzi je treba endokrino zdravljenje kombinirati z agonistom LHRH. **Odmerjanje in način uporabe** Zdravljenje z zdravilom Verzenios mora uvesti in nadzorovati zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje rakavih bolezni. Priporočeni odmerek abemaciklibа je 150 mg dvakrat na dan, kadar se uporablja v kombinaciji z endokrinim zdravljenjem. *Zgodnji rak dojk* Zdravilo Verzenios je treba jemati neprekinjeno dve leti, ali do ponovitve bolezni ali pojava nesprejemljive toksičnosti. *Napredovali ali metastatski rak dojk* Zdravilo Verzenios je treba jemati, dokler ima bolnica od zdravljenja klinično korist ali do pojava nesprejemljive toksičnosti. Če bolnica bruha ali izpusti odmerek zdravila Verzenios, ji je treba naročiti, da naj naslednji odmerek vzame ob predvidenem času; dodatnega odmerka ne sme vzeti. Obvladovanje nekaterih neželenih učinkov lahko zahteva prekinitev in/ali zmanjšanje odmerka. Zdravljenje z abemaciklibom prekinite v primeru povišanja vrednosti AST in/ali ALT >3 x ZMN SKUPAJ s celokupnim bilirubinom > 2,0 x ZMN v odsotnosti holestaze ter pri bolnicah z intersticijsko pljučno boleznijo (ILD)/pnevmonitis stopnje 3 ali 4. Sočasni uporabi močnih zaviralcev CYP3A4 se je treba izogibati. Če se uporabi močnih zaviralcev CYP3A4 ni mogoče izogniti, je treba odmerek abemaciklibа znižati na 100 mg dvakrat na dan. Pri bolnicah, pri katerih je bil odmerek znižan na 100 mg abemaciklibа dvakrat na dan in pri katerih se sočasnemu dajanju močnega zaviralca CYP3A4 ni mogoče izogniti, je treba odmerek abemaciklibа dodatno znižati na 50 mg dvakrat na dan. Pri bolnicah, pri katerih je bil odmerek znižan na 50 mg abemaciklibа dvakrat na dan in pri katerih se sočasnemu dajanju močnega zaviralca CYP3A4 ni mogoče izogniti, je mogoče z odmerkom abemaciklibа nadaljevati ob natančnem spremljanju znakov toksičnosti. Alternativno je mogoče odmerek abemaciklibа znižati na 50 mg enkrat na dan ali prekiniti dajanje abemaciklibа. Če je uporaba zaviralca CYP3A4 prekinjena, je treba odmerek abemaciklibа povečati na odmerek, kakršen je bil pred uvedbo zaviralca CYP3A4 (po 3–5 razpolovnih časih zaviralca CYP3A4). Prilagajanje odmerka glede na starost in pri bolnicah z blago ali zmerno ledvično okvaro ter z blago (Child Pugh A) ali zmerno (Child Pugh B) jetrno okvaro ni potrebno. Pri dajanju abemaciklibа bolnicam s hudo ledvično okvaro sta potrebna previdnost in skrbno spremljanje glede znakov toksičnosti. Način uporabe Zdravilo Verzenios je namenjeno za peroralno uporabo. Odmerek se lahko vzame s hrano ali brez nje. Zdravila se ne sme jemati z grenivko ali grenivkinim sokom. Bolnice naj odmerke vzamejo vsak dan ob približno istem času. Tableto je treba pogoltniti celo (bolnice tablet pred zaužitjem ne smejo gristi, drobiti ali deliti). **Kontraindikacije** Preobčutljivost na učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi** Pri bolnicah, ki so prejemale abemaciklib, so poročali o nevtropeniji, o večji pogostnosti okužb kot pri bolnicah, zdravljenih s placebom in endokrinim zdravljenjem, o povečanih vrednostih ALT in AST. Pri bolnicah, pri katerih se pojavi nevtropenija stopnje 3 ali 4, je priporočljivo prilagoditi odmerek. Do primerov nevtropenične sepse s smrtnim izidom je prišlo pri < 1 % bolnic z metastatskim rakom dojk. Bolnicam je treba naročiti, naj o vsaki epizodi povišane telesne temperature poročajo zdravstvenemu delavcu. Bolnice je treba spremljati za znake in simptome globoke venske tromboze (VTE) in pljučne embolije ter jih zdraviti, kot je medicinsko utemeljeno. Glede na stopnjo VTE bo morda treba spremeniti odmerek abemaciklibа. Pri bolnikih, pri katerih se pojavi resni arterijski trombembolični dogodek (ATE), je treba oceniti koristi in tveganja nadaljnjega zdravljenja z abemaciklibom. Glede na povečanje vrednosti ALT ali AST je mogoče potrebna prilagoditev odmerka. Driska je najpogostejši neželeni učinek. Bolnice je treba ob prvem znaku tekočega blata začeti zdraviti z antidiaroiki, kot je loperamid, povečati vnos peroralnih tekočin in obvestiti zdravnika. Sočasni uporabi induktorjev CYP3A4 se je treba izogibati zaradi tveganja za zmanjšano učinkovitost abemaciklibа. Bolnice z redkimi dednimi motnjami, kot so intoleranca za galaktozo, popolno pomanjkanje laktaze ali malapsorpcija glukoze/galaktoze, tega zdravila ne smejo jemati. Bolnice je treba spremljati glede pljučnih simptomov, ki kažejo na ILD/pnevmonitis, in jih ustrezno zdraviti. Glede na stopnjo ILD/pnevmonitisa je morda potrebno prilagajanje odmerka abemaciklibа. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij** Abemaciklib se primarno presnavlja s CYP3A4. Sočasna uporaba abemaciklibа in zaviralcev CYP3A4 lahko poveča plazemsko koncentracijo abemaciklibа. Uporabi močnih zaviralcev CYP3A4 sočasno z abemaciklibom se je treba izogibati. Če je močne zaviralce CYP3A4 treba dajati sočasno, je treba odmerek abemaciklibа zmanjšati, nato pa bolnico skrbno spremljati glede toksičnosti. Pri bolnicah, zdravljenih z zmernimi ali šibkimi zaviralci CYP3A4, ni potrebno prilagajanje odmerka, vendar jih je treba skrbno spremljati za znake toksičnosti. Sočasni uporabi močnih induktorjev CYP3A4 (vključno, vendar ne omejeno na: karbamazepin, fenitoin, rifampicin in šentjanževko) se je treba izogibati zaradi tveganja za zmanjšano učinkovitost abemaciklibа. Abemaciklib in njegovi glavni aktivni presnovki zavirajo prenašalce v ledvicah, in sicer kationski organski prenašalec 2 (OCT2) ter prenašalca MATE1. *In vivo* lahko pride do medsebojnega delovanja abemaciklibа in klinično pomembnih substratov teh prenašalcev, kot je dofelitid ali kreatinin. Trenutno ni znano, ali lahko abemaciklib zmanjša učinkovitost sistemskih hormonskih kontraceptivov, zato se ženskam, ki uporabljajo sistemske hormonske kontraceptive, svetuje, da hkrati uporabljajo tudi mehansko metodo. **Neželeni učinki** Najpogostejši neželeni učinki so driska, okužbe, nevtropenija, levkopenija, anemija, utrujenost, navzea, bruhanje in zmanjšanje apetita. *Zelo pogosti:* okužbe, nevtropenija, levkopenija, anemija, trombocitopenija, limfopenija, zmanjšanje apetita, glavobol, disgevzija, omotica, driska, bruhanje, navzea, stomatitis, alopecija, pruritus, izpuščaj, pireksija, utrujenost, povečana vrednost alanin-aminotransferaze, povečana vrednost aspartat-aminotransferaze. *Pogosti:* povečano solzenje, venska trombembolija, ILD/pnevmonitis, dispepsija, spremembe na nohtih, suha koža, mišična šibkost. *Občasni:* febrilna nevtropenija **Rok uporabnosti** 3 leta **Posebna navodila za shranjevanje** Za shranjevanje zdravila niso potrebna posebna navodila. **Imetnik dovoljenja za promet z zdravilom**: Eli Lilly Nederland B.V., Papendorpseweg 83, 3528BJ, Utrecht, Nizozemska. Datum prve odobritve dovoljenja za promet: 27. september 2018 Datum zadnjega podaljšanja: 23. junij 2023 **Datum zadnje revizije besedila**: 9.11.2023 **Režim izdaje:** Rp/Spec - Predpisovanje in izdaja zdravila je le na recept zdravnika specialista ustreznega področja medicine ali od njega pooblaščenega zdravnika.

Reference: 1. Povzetek glavnih značilnosti zdravila Verzenios, zadnja odobrena verzija.

Pomembno: Predpisovanje in izdaja zdravila je le na recept zdravnika specialista ustreznega področja medicine ali od njega pooblaščenega zdravnika. Pred predpisovanjem zdravila Verzenios si preberite zadnji veljavni Povzetek glavnih značilnosti zdravil. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu

Eli Lilly farmacevtska družba, d.o.o., Dunajska cesta 167, 1000 Ljubljana, telefon 01 / 580 00 10, faks 01 / 569 17 05

PP-AL-SI-0268, 5.2.2024, Samo za strokovno javnost.

PR/BSI/BEN/2022/017

TANTUM VERDE®

benzidaminijev klorid

Za lajšanje bolečine in oteklin v ustni in žrelu, ki so posledica radiomukozitisa

Bistvene informacije iz Povzetka glavnih značilnosti zdravila

Tantum Verde 1,5 mg/ml oralno pršilo, raztopina
Tantum Verde 3 mg/ml oralno pršilo, raztopina

Sestava: 1,5 mg/ml: 1 ml raztopine vsebuje 1,5 mg benzidaminijevega klorida, kar ustreza 1,34 mg benzidamina. V enem razpršku je 0,17 ml raztopine. En razpršek vsebuje 0,255 mg benzidaminijevega klorida, kar ustreza 0,2278 mg benzidamina. **Sestava 3 mg/ml:** 1 ml raztopine vsebuje 3 mg benzidaminijevega klorida, kar ustreza 2,68 mg benzidamina. V enem razpršku je 0,17 ml raztopine. En razpršek vsebuje 0,51 mg benzidaminijevega klorida, kar ustreza 0,4556 mg benzidamina. **Terapevtske indikacije:** Samozdravljenje: Lajšanje bolečine in oteklin pri vnetju v ustni votlini in žrelu, ki so lahko posledica okužb in stanj po operaciji. Po nasvetu in navodilu zdravnika: Lajšanje bolečine in oteklin v ustni votlini in žrelu, ki so posledica radiomukozitisa. **Odmerjanje in način uporabe:** Uporaba: 2- do 6-krat na dan (vsake 1,5 do 3 ure). Odmerjanje 1,5 mg/ml: Odrasli: 4 do 8 razprškov 2- do 6-krat na dan. Pediatrična populacija: Mladostniki, stari od 12 do 18 let: 4-8 razprškov 2- do 6-krat na dan. Otroci od 6 do 12 let: 4 razprški 2- do 6-krat na dan. Otroci, mlajši od 6 let: 1 razpršek na 4 kg telesne mase; do največ 4 razprške 2- do 6-krat na dan. Odmerjanje 3 mg/ml: Odrasli: 2 do 4 razprški 2- do 6-krat na dan. Pediatrična populacija: Mladostniki, stari od 12 do 18 let: 2 do 4 razprški 2- do 6-krat na dan. Otroci od 6 do 12 let: 2 razprška 2- do 6-krat na dan. Otroci, mlajši od 6 let: 1 razpršek na 8 kg telesne mase; do največ 2 razprška 2- do 6-krat na dan. Starejši bolniki, bolniki z jetrno okvaro in bolniki z ledvično okvaro: niso potrebni posebni previdnostni ukrepi. Trajanje zdravljenja ne sme biti daljše od 7 dni. Način uporabe: Za orofaringealno uporabo. Zdravilo se razprši v usta in žrelo. **Kontraindikacije:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Pri nekaterih bolnikih lahko resne bolezni povzročijo ustne/žrelne ulceracije. Če se simptomi v treh dneh ne izboljšajo, se mora bolnik posvetovati z zdravnikom ali zobozdravnikom, kot je primerno. Uporaba benzidamina ni priporočljiva za bolnike s preobčutljivostjo na salicilno kislino ali druga nesteroidna protivnetna zdravila. Pri bolnikih, ki imajo ali so imeli bronhialno astmo, lahko pride do bronhospazma. Pri takih bolnikih je potrebna previdnost. To zdravilo vsebuje 13,6 mg alkohola (etanola) v enem razpršku (0,17 ml), kar ustreza manj kot 0,34 ml piva oziroma 0,14 ml vina. Majhna količina alkohola v zdravilu ne bo imela nobenih opaznih učinkov. To zdravilo vsebuje metilparahidroksibenzoat (E218). Lahko povzroči alergijske reakcije (lahko zapoznele). To zdravilo vsebuje manj kot 1 mmol (23 mg) natrija v enem razpršku (0,17 ml), kar v bistvu pomeni 'brez natrija'. Zdravilo vsebuje aromo poprove mete z benzilalkoholom, cinamilalkoholom, citralom, citronelolom, geraniolom, izoevgenolom, linalolom, evgenolom in D-limonen, ki lahko povzročijo alergijske reakcije. Zdravilo z jakostjo 3 mg/ml vsebuje makrogolglicerol hidroksistearat 40. Lahko povzroči želodčne težave in drisko. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Študij medsebojnega delovanja niso izvedli. **Nosečnost in dojenje:** O uporabi benzidamina pri nosečnicah in doječih ženskah ni zadostnih podatkov. Uporaba zdravila med nosečnostjo in dojenjem ni priporočljiva. **Vpliv na sposobnost vožnje in upravljanja strojev:** Zdravilo v priporočenem odmerku nima vpliva na sposobnost vožnje in upravljanja strojev. **Neželeni učinki:** Neznana pogostnost (ni mogoče oceniti iz razpoložljivih podatkov): anafilaktične reakcije, preobčutljivostne reakcije, odrevenelost, laringospazem, suha usta, navzea in bruhanje, oralna hipestezija, angioedem, fotosenzitivnost, pekoč občutek v ustih. Neposredno po uporabi se lahko pojavi občutek odrevenelosti v ustih in v žrelu. Ta učinek se pojavi zaradi načina delovanja zdravila in po kratkem času izgine. **Način in režim izdaje zdravila:** BRp-Izdaja zdravila je brez recepta v lekarnah in specializiranih prodajalnah. **Imetnik dovoljenja za promet:** Aziende Chimiche Riunite Angelini Francesco – A.C.R.A.F. S.p.A., Viale Amelia 70, 00181 Rim, Italija **Datum zadnje revizije besedila:** 05. 04. 2022

Pred svetovanjem ali izdajo preberite celoten Povzetek glavnih značilnosti zdravila.

Samo za strokovno javnost.

Datum priprave informacije: april 2022

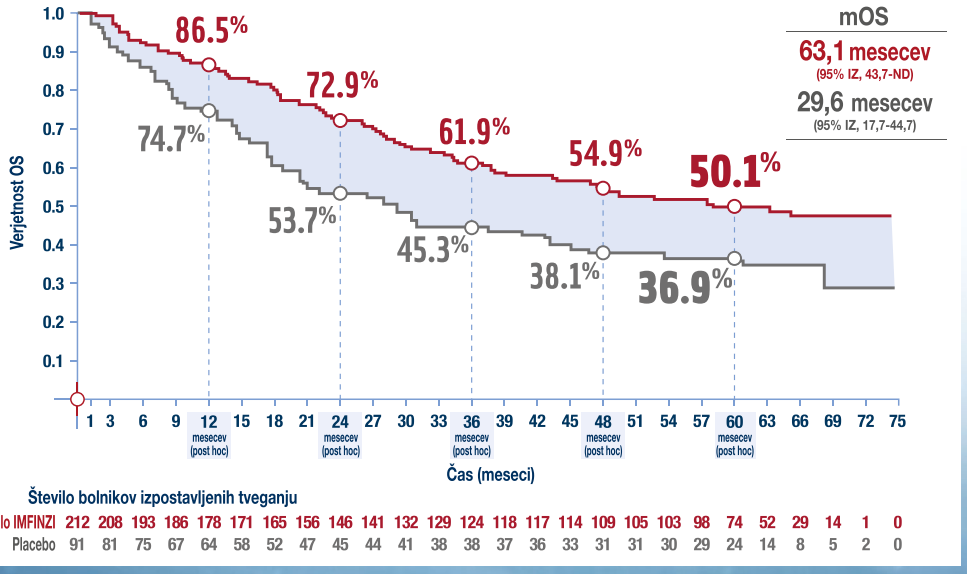
ANGELINI

Odgovoren za trženje: Bonifar d.o.o.

IMFINZI™
durvalumab
50 mg/ml koncentrat za raztopino za infundiranje

AKTIVIRA IMUNSKI SISTEM.
PREPOZNA. **REAGIRA.**

5-LETNO CELOKUPNO PREŽIVETJE BOLNIKOV Z LOKALNO NAPREDOVALIM, NEOPERABILNIM NSCLC S PDL-1 ≥ 1%[1]



Po petih letih je bilo živih še 50% bolnikov zdravljenih z zdravilom Imfinzi po zaključeni sočasni kemoradioterapiji na osnovi platine.

NSCLC... nedrobnocelični rak pljuč (non small cell lung cancer), mOS...mediano celokupno preživetje

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Imfinzi 50 mg/ml koncentrat za raztopino za infundiranje

SESTAVA: 1 ml koncentrata za raztopino za infundiranje vsebuje 50 mg durvalumaba. Ena viala z 2,4 ml koncentrata vsebuje 120 mg durvalumaba. Ena viala z 10 ml koncentrata vsebuje 500 mg durvalumaba. **INDIKACIJE:** *Neoperabilni nedrobnocelični rak pljuč (NSCLC).* Zdravilo IMFINZI je kot samostojno zdravljenje indicirano za zdravljenje lokalno napredovalega, neoperabilnega nedrobnoceličnega raka pljuč (NSCLC – non small cell lung cancer) pri odraslih, ki imajo tumorje z ≥ 1 % izraženostjo PD L1 na tumorskih celicah in pri katerih bolezen ni napredovala po kemoradioterapiji na osnovi platine. Zdravilo IMFINZI je v kombinaciji s tremelimumabom in kemoterapijo na osnovi platine indicirano za prvo linijo zdravljenja odraslih z metastatskim NSCLC brez senzibilizirajočih mutacij EGFR ali pozitivnih mutacij ALK. *Drobnocelični rak pljuč (SCLC).* Zdravilo IMFINZI je v kombinaciji z etopozidom in bodisi karboplatinom bodisi cisplatinom indicirano za prvo linijo zdravljenja odraslih z razsejanim drobnoceličnim rakom pljuč (ES SCLC – extensive stage small cell lung cancer). *Rak biliarnega trakta (RBT).* Zdravilo IMFINZI je v kombinaciji z gemcitabinom in cisplatinom indicirano za prvo linijo zdravljenja odraslih z neoperabilnim ali metastatskim rakom biliarnega trakta (RBT). *Hepatocelularni karcinom (HCK).* Zdravilo IMFINZI je kot samostojno zdravljenje indicirano za prvo linijo zdravljenja odraslih z napredovalim ali neoperabilnim hepatocelularnim karcinomom (HCK). Zdravilo IMFINZI je v kombinaciji s tremelimumabom indicirano za prvo linijo zdravljenja odraslih z napredovalim ali neoperabilnim karcinomom jetrnih celic (hepatocelularnim karcinomom – HCK). **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje mora uvesti in nadzorovati zdravnik, ki ima izkušnje na področju zdravljenja raka. Bolnike z lokalno napredovalim nedrobnoceličnim rakom pljuč je treba za zdravljenje izbrati na podlagi izraženosti PD-L1, ugotovljene z validirano testno metodo. Odmerjanje: Priporočeni odmerek zdravila Imfinzi pri samostojnem zdravljenju pri lokalno napredovalem NSCLC je 10 mg/kg na 2 tedna ali 1500 mg na 4 tedne do napredovanja bolezni, nesprejemljivih toksičnih učinkov ali največ 12 mesecev. Pri HCK je priporočeni odmerek zdravila Imfinzi 1500 mg na 4 tedne do napredovanja bolezni ali do nesprejemljivih toksičnih učinkov. Priporočeni odmerek zdravila Imfinzi pri kombinaranem zdravljenju pri metastatskem NSCLC je med kemoterapijo s platino: 1500 mg v kombinaciji s 75 mg tremelimumaba in kemoterapijo na osnovi platine na 3 tedne (21 dni) 4 cikle (12 tednov), po kemoterapiji s platino: 1500 mg na 4 tedne kot monoterapija in vzdrževalno zdravljenje s pemetreksedom (na podlagi histologije) na 4 tedne, peti odmerek 75 mg tremelimumaba je treba dati 16. teden skupaj z zdravilom Imfinzi. Trajanje zdravljenja je do napredovanja bolezni ali nesprejemljivih toksičnih učinkov. Pri ES-SCLC je priporočeni odmerek zdravila Imfinzi 1500 mg v kombinaciji s kemoterapijo na 3 tedne (21 dni) 4 cikluse in nato 1500 mg na 4 tedne kot samostojno zdravljenje do napredovanja bolezni ali nesprejemljivih toksičnih učinkov. Pri RBT je priporočeni odmerek zdravila Imfinzi 1500 mg v kombinaciji s kemoterapijo na 3 tedne (21 dni) do 8 ciklusov, nato po 1500 mg na 4 tedne kot samostojno zdravljenje do napredovanja bolezni ali nesprejemljivih toksičnih učinkov. Pri HCK je priporočeni odmerek 1500 mg zdravila IMFINZI v kombinaciji s 300 mg tremelimumaba v enem odmerku 1. dan 1. cikla, nato zdravilo Imfinzi kot monoterapija na 4 tedne do napredovanja bolezni ali nesprejemljivih toksičnih učinkov. Povečevanje ali zmanjševanje odmerka ni priporočljivo. Glede na individualno varnost in prenašanje je lahko potrebna odložitev zdravljenja ali trajno prenehanje uporabe zdravila. V primeru domnevnih imunsko pogojenih neželenih učinkov je treba opraviti ustrezno ovrednotenje za potrditev etiologije ozioroma izključitev druge etiologije. Glede na resnost neželenega učinka je treba prenehati uporabljati zdravilo Imfinzi in/ali tremelimumab in pričeti z dajanjem kortikosteroidov. Če se stanje ne izboljša ali se poslabša, pride v poštev povečanje odmerka kortikosteroidov in/ali dodatna uporaba sistemskih imunosupresivov. Po izboljšanju na ≤ 1. stopnjo je treba začeti s postopnim zmanjševanjem kortikosteroida in ga zmanjševati v obdobju vsaj 1 meseca. Po odložitvi uporabe je mogoče zdravilo Imfinzi in/ali tremelimumab znova začeti uporabljati v času 12 tednov, če se neželeni učinki izboljšajo na ≤ 1. stopnjo in je odmerek kortikosteroida zmanjšan na ≤ 10 mg prednizona ali ekvivalenta na dan. Zdravilo Imfinzi in tremelimumab je treba dokončno ukiniti, če se imunsko pogojeni neželeni učinki 3. stopnje (močno izraženi) ponavljajo in pri katerih koli imunsko pogojenih neželenih učinkih 4. stopnje (življenjsko nevarni), razen pri endokrinopatijah, ki jih nadziramo z nadomestnimi hormoni. Način uporabe: Zdravilo Imfinzi je namenjeno za intravensko uporabo. Dati ga je treba kot raztopino za intravensko infundiranje v obdobju 1 ure. Če je zdravilo Imfinzi uporabljeno v kombinaciji s kemoterapijo, je treba dati zdravilo Imfinzi pred kemoterapijo na isti dan. Če je zdravilo Imfinzi uporabljeno v kombinaciji s tremelimumabom in kemoterapijo na osnovi platine, je treba najprej dati tremelimumab, ki mu v istem dnevu sledita zdravilo Imfinzi in nato kemoterapija na osnovi platine. Če je zdravilo Imfinzi uporabljeno v kombinaciji s petim odmerkom tremelimumaba in vzdrževalnim zdravljenjem s pemetreksedom 16. teden, je treba najprej dati tremelimumab, ki mu v istem dnevu sledita zdravilo Imfinzi in potem vzdrževalno zdravljenje s pemetreksedom. Zdravilo Imfinzi, tremelimumab in kemoterapijo na osnovi platine je treba dati v ločenih intravenskih infuzijah. Dajanje zdravila Imfinzi in tremelimumaba vsako traja po 1 uro. Če je zdravilo Imfinzi uporabljeno v kombinaciji s tremelimumabom, je treba dati tremelimumab pred zdravilom Imfinzi na isti dan. Zdravilo Imfinzi in tremelimumab je treba dati v ločenih intravenskih infuzijah. **KONTRAINDIKACIJE:** Preobčutljivost na učinkovino (učinkovine) ali katero koli pomožno snov. **OPOZORILA IN PREVIDNOSTNI UKREPI:** Za izboljšanje sledljivosti bioloških zdravil je treba jasno zabeležiti lastniško ime in številko serije uporabljenega zdravila. Imunsko pogojeni pnevmonitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, sta se pojavila imunsko pogojeni pnevmonitis ali intersticijska bolezen pljuč, opredeljeno kot potreba po uporabi sistemskih kortikosteroidov in brez jasne druge etiologije. Pnevmonitis in radiacijski pnevmonitis: Pri bolnikih, zdravljenih z radioterapijo pljuč, je pogost radiacijski pnevmonitis in klinična slika pnevmonitisa in radiacijskega pnevmonitisa je zelo podobna. V študiji PACIFIC sta se pri bolnikih, ki so opravili zdravljenje z najmanj 2 cikloma sočasne kemoradioterapije od 1 do 42 dni pred začetkom preskušanja, pnevmonitis ali radiacijski pnevmonitis pojavila pri 161 (33,9 %) bolnikih v skupini z zdravilom Imfinzi in pri 58 (24,8 %) bolnikih v skupini s placebom, vključno s 3. stopnjo (3,4 % in 3,0 %) in 5. stopnjo (1,1 % in 1,7 %). Bolnike je treba spremljati glede znakov in simptomov pnevmonitisa ali radiacijskega pnevmonitisa. Imunsko pogojeni hepatitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, se je pojavil imunsko pogojeni hepatitis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Alanin-aminotransferazo, aspartat-aminotransferazo, celokupni bilirubin in alkalno fosfatazo kontrolirajte pred začetkom zdravljenja in pred vsako nadaljnjo infuzijo. Dodaten nadzor pride v poštev na podlagi klinične ocene. Imunsko pogojeni kolitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, sta se pojavila imunsko pogojeni kolitis ali driska, opredeljeno kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Pri bolnikih, ki so prejemali zdravilo Imfinzi v kombinaciji s tremelimumabom, so poročali o neželenih učinkih perforacije črevesa in perforacije debelega črevesa. Imunsko pogojene endokrinopatije: Imunsko pogojeni hipotiroidizem, hipertiroidizem in tiroiditis: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, so se pojavili imunsko pogojeni hipotiroidizem, hipertiroidizem in tiroiditis; hipertiroidizmu lahko sledi hipotiroidizem. Bolnike je treba spremljati glede nenormalnih izvidov delovanje ščitnice pred zdravljenjem in redno med zdravljenjem ter kot je potrebno glede na klinično oceno. Imunsko pogojena adrenalna insuficienca: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, se je pojavila imunsko pogojena adrenalna insuficienca. Bolnike je treba spremljati glede kliničnih znakov in simptomov adrenalne insuficience. Imunsko pogojena sladkorna bolezen tipa 1: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, se je pojavila imunsko pogojena sladkorna bolezen tipa 1, ki se lahko najprej kaže kot diabetična ketoacidoza, ki je lahko smrtno nevarna, če je dovolj zgodaj ne odkrijemo. Bolnike je treba spremljati glede kliničnih znakov in simptomov sladkorne bolezni tipa 1. Imunsko pogojeni hipofizitis/hipopituitarizem: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, sta se pojavila imunsko pogojena hipofizitis ali hipopituitarizem. Bolnike je treba spremljati glede kliničnih znakov in simptomov hipofizitisa ali hipopituitarizma. Imunsko pogojeni nefritis: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, se je pojavil imunsko pogojeni nefritis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojeni izpuščaj: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, se je pojavil imunsko pogojeni izpuščaj ali dermatitis (vključno s pemfigoidom), opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Pri bolnikih, ki so bili zdravljeni z zaviralci PD-1, so poročali o pojavljanju Stevens-Johnsonovega sindroma ali toksične epidermalne nekrolize. Imunsko pogojeni miokarditis: Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, se je pojavil imunsko pogojeni miokarditis, ki je lahko usoden. Imunsko pogojeni pankreatitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi v kombinaciji s tremelimumabom in kemoterapijo, se je pojavil imunsko pogojeni pankreatitis. Drugi imunsko pogojeni neželeni učinki: Glede na mehanizem delovanja zdravila Imfinzi ali zdravila Imfinzi v kombinaciji s tremelimumabom se lahko pojavijo še drugi potencialno imunsko pogojeni učinki. Naslednji imunsko pogojeni neželeni učinki so bili opaženi pri bolnikih, ki so prejemali samostojno zdravljenje z zdravilom Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom: miastenija gravis, transverzni mielitis, miozitis, polimiozitis, meningitis, encefalitis, Guillain Barréjev sindrom, imunska trombocitopenija, neinfektivni cistitis. Z infundiranjem povezane reakcije: Bolnike je treba spremljati glede znakov in simptomov z infundiranjem povezanih reakcij. Pri bolnikih, ki so prejemali zdravilo Imfinzi ali zdravilo Imfinzi v kombinaciji s tremelimumabom, so bile opisane hude z infundiranjem povezane reakcije. Za bolezen specifični previdnostni ukrepi (RBT): Holangitis in okužbe biliarnega trakta pri bolnikih z napredovalim RBT niso redke. V študiji TOPAZ-1 so o primerih holangitisa poročali v obeh terapevtskih skupinah (14,5 % v skupini z zdravilom Imfinzi + kemoterapijo, 8,2 % v skupini s placebom + kemoterapijo); ti primeri so bili večinoma povezani z biliarnimi opornicami in niso imeli imunsko posredovane etiologije. Bolnike z RBT (zlasti tiste z biliarno opornico) je treba natančno nadzorovati glede holangitisa ali okužb biliarnega trakta tako pred uvedbo zdravljenja kot redno med zdravljenjem. Metastatski NSCLC: Podatkov o starejših bolnikih (≥ 75 let), zdravljenih z zdravilom Imfinzi v kombinaciji s tremelimumabom in kemoterapijo na osnovi platine, je malo. Morebitno korist/tveganje te sheme je priporočljivo skrbno pretehtati za vsakega bolnika posebej. Bolniki, ki niso bili vključeni v klinične študije: V klinične študije niso bili vključeni bolniki z naslednjimi značilnostmi: izhodiščno oceno zmogljivosti ECOG ≥ 2; aktivno ali predhodno dokumentirano avtoimunsko boleznijo v 2 letih pred začetkom študije; anamnezo imunske pomanjkljivosti; anamnezo hudih imunsko pogojenih neželenih učinkov; boleznimi, ki so zahtevale sistemsko imunosupresijo, razen fiziološkega odmerka sistemskih kortikosteroidov (≤ 10 mg na dan prednizona ali ekvivalenta); neobvladanimi sočasnimi boleznimi; aktivno tuberkulozo ali okužbo s hepatitisom B ali C ali HIV; bolniki, ki so prejeli živo oslabljeno cepivo v 30 dneh pred začetkom zdravljenja z zdravilom Imfinzi ali v 30 dneh po začetku. Dokler takšnih podatkov ni, je treba durvalumab v teh skupinah bolnikov uporabljati previdno ter po skrbnem individualnem pretehtanju možnih koristi in tveganj za posameznega bolnika. Varnost sočasnega profilaktičnega kranialnega obsevanja obenem z zdravilom Imfinzi pri bolnikih z ES SCLC ni znana. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI:** Razen fizioloških odmerkov sistemskih kortikosteroidov (≤ 10 mg na dan prednizona ali ekvivalenta) pred uvedbo durvalumaba ni priporočljivo uporabljati sistemskih kortikosteroidov ali imunosupresivov, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost durvalumaba. Vendar pa je mogoče kortikosteroide ali druge imunosupresive uporabiti po začetku zdravljenja z durvalumabom za zdravljenje imunsko pogojenih neželenih učinkov. Z durvalumabom niso izvedli formalnih farmakokinetičnih (PK) študij medsebojnega delovanja zdravil. Primarni poti odstranjevanja durvalumaba sta katabolizem beljakovin preko retikuloendotelijskega sistema oziroma tarčno posredovano odstranjevanje, zato ni pričakovati presnovnih medsebojnih delovanj med zdravili. Farmakokinetična medsebojna delovanja med durvalumabom in kemoterapijo so ocenjevali v študiji CASPIAN; izkazalo se je, da sočasno zdravljenje z durvalumabom ne vpliva na farmakokinetiko etopozida, karboplatina ali cisplatina. Poleg tega populacijska farmakokinetična analiza kaže, da sočasno zdravljenje s kemoterapijo ne vpliva pomembno na farmakokinetiko durvalumaba. Farmakokinetična medsebojna delovanja med durvalumabom v kombinaciji s tremelimumabom in kemoterapijo na osnovi platine so ocenili v študiji POSEIDON. Izkazalo se je, da med sočasnim zdravljenjem ni bilo klinično pomembnih farmakokinetičnih medsebojnih delovanj med tremelimumabom, durvalumabom, nab-paklitakselom, gemcitabinom, pemetreksedom, karboplatinom ali cisplatinom. **PLODNOST, NOSEČNOST IN DOJENJE:** Ženske v rodni dobi morajo med zdravljenjem z durvalumabom in vsaj še 3 mesece po zadnjem odmerku durvalumaba uporabljati učinkovito kontracepcijo. Podatkov o uporabi durvalumaba pri nosečnicah ni. Glede na mehanizem delovanja durvalumab lahko vpliva na vzdrževanje nosečnosti; v alogenskem modelu nosečnosti pri miših je bilo ugotovljeno, da moteno signaliziranje PD-L1 poveča izgubo plodov. Pri nosečnici uporabljeni durvalumab lahko škoduje plodu in ga ni priporočljivo uporabljati med nosečnostjo in pri ženskah v rodni dobi, ki ne uporabljajo učinkovite kontracepcije med zdravljenjem in vsaj še 3 mesece po zadnjem odmerku. Ni znano, ali se durvalumab pri človeku izloča v materinem mleku. Pri človeku protitelesa lahko prehajajo v materino mleko, a možnost absorpcije in škode za novorojenčka ni znana. Toda možnega tveganja za dojenega otroka ni mogoče izključiti. Odločiti se je treba, ali naj ženska prekine z dojenjem ali naj prekine zdravljenje z durvalumabom oziroma sploh ne začne zdravljenja z njim, pri čemer je treba upoštevati koristi dojenja za otroka in koristi zdravljenja za žensko. Podatkov o možnih vplivih durvalumaba na plodnost pri človeku ali živalih ni. **NEŽELENI UČINKI:** Ugotovitve o varnosti zdravila Imfinzi pri samostojnem zdravljenju temeljijo na kumulativnih podatkih 4045 bolnikov z več vrstami tumorjev. Zdravilo Imfinzi so uporabljali v odmerku 10 mg/kg na 2 tedna, 20 mg/kg na 4 tedne ali 1500 mg/kg na 4 tedne. Najpogostejši neželeni učinki (> 10 %) so bili kašelj/produktiven kašelj (18,7 %), driska (16,1 %), izpuščaj (15,5 %), artralgija (13,8 %), zvišana telesna temperatura (13,0 %), bolečine v trebuhu (13,0 %), okužbe zgornjih dihal (12,1 %), srbenje (11,4 %) in hipotiroidizem (10,9 %). Podatki o varnosti zdravila Imfinzi v kombinaciji s kemoterapijo temeljijo na kumulativnih podatkih 603 bolnikov iz 2 študij (TOPAZ-1 in CASPIAN). Najpogostejši (> 10 %) neželeni učinki so bili nevtropenija (53,1 %), anemija (43,9 %), navzea (37,5 %), utrujenost (36,8 %), trombocitopenija (28,0 %), zaprtje (25,4 %), zmanjšan apetit (22,6 %), bolečine v trebuhu (18,4 %), alopecija (18,4 %), levkopenija (17,2 %), bruhanje (16,9 %), zvišana telesna temperatura (15,1 %), izpuščaj (14,8 %), driska (13,8 %), zvišana aspartat-aminotransferaza ali zvišana alanin-aminotransferaza (10,9 %), kašelj/produktiven kašelj (10,8 %) in srbenje (10,4 %). Izsledki o varnosti zdravila Imfinzi v kombinaciji s 75 mg tremelimumaba in kemoterapijo temeljijo na podatkih 330 bolnikov z metastatskim NSCLC. Najpogostejši (> 20 %) neželeni učinki so bili anemija (49,7 %), navzea (41,5 %), nevtropenija (41,2 %), utrujenost (36,1 %), izpuščaj (25,8 %), trombocitopenija (24,5 %) in driska (21,5 %). Izsledki o varnosti zdravila Imfinzi v kombinaciji z enkratnim odmerkom 300 mg tremelimumaba temeljijo na združenih podatkih 462 bolnikov v HCK iz študije HIMALAYA in še ene študije pri bolnikih s HCK, študija 22. Najpogostejši neželeni učinki (> 10 %) so bili izpuščaj (32,5 %), srbenje (25,5 %), driska (25,3 %), bolečine v trebuhu (19,7 %), zvišana aspartat aminotransferaza/zvišana alanin aminotransferaza (18,0 %), zvišana telesna temperatura (13,9 %), hipotiroidizem (13,0 %), kašelj/produktiven kašelj (10,8 %), periferni edemi (10,4 %) in zvišana lipaza (10,0 %). **VRSTA IN VSEBINA OVOJNINE:** 2,4 ml (skupno 120 mg durvalumaba) koncentrata v stekleni viali iz stekla tipa 1 z elastomernim zamaškom in sivo snemno aluminijsko zaporo. Pakiranje vsebuje 1 vialo. 10 ml (skupno 500 mg durvalumaba) koncentrata v stekleni viali iz stekla tipa 1 z elastomernim zamaškom in belo snemno aluminijsko zaporo. Pakiranje vsebuje 1 vialo. **NAČIN IZDAJANJA ZDRAVILA:** H - Predpisovanje in izdaja zdravila je le na recept. **DATUM REVIZIJA BESEDILA:** 14.12.2023 (ŠI-3708). **IMETNIK DOVOLJENJA ZA PROMET:** AstraZeneca AB, S-151 85, Sodertalje, Švedska

Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila.
Dodatne informacije so na voljo pri družbi AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, Ljubljana, telefon +386 1 51 35 600.

Samo za strokovno javnost. Informacija pripravljena januarja 2024.

Reference: 1.Spigel DR, Faivre-Finn C, Gray JE, et al. Five-year survival outcomes from the PACIFIC trial: durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. J Clin Oncol. Forthcoming 2021.

AstraZeneca

SI-3715

DOVOLI SI VERJETI

Lynparza®
olaparib
tablete 100 mg
tablete 150 mg

***Prvi in edini zaviralec PARP odobren za 4 različne lokalizacije tumorjev*[1-5]**

RAK JAJČNIKOV

Prvi zaviralec PARP odobren za vzdrževalno zdravljenje napredovalega raka jajčnikov v monoterapiji (v 1L pri bolnicah z mutacijo *gena BRCA1/2* in 2L) ali kombinaciji z bevacizumabom (pri bolnicah s HRD).[1-3, 5]

RAK DOJK

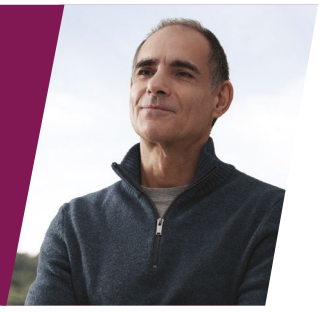
Prvi zaviralec PARP odobren za zdravljenje, pri bolnikih z zarodno mutacijo gena *BRCA1/2*, ki imajo HER2-negativni zgodnji, lokalno napredovali ali razsejan rak dojk.[1-2, 4]

RAK TREBUŠNE SLINAVKE

Edini zaviralec PARP odobren za vzdrževalno zdravljenje bolnikov z zarodno mutacijo gena *BRCA1/2*, ki imajo razsejani adenokarcinom trebušne slinavke in jim bolezen ni napredovala po najmanj 16 tednih prvega reda zdravljenja s kemoterapijo na osnovi platine.[1-4]

RAK PROSTATE

Edini zaviralec PARP odobren za zdravljenje bolnikov z razsejanim KORP v monoterapiji za bolnike z mutacijami gena *BRCA1/2*, ki jim je bolezen napredovala po zdravljenju z novim hormonskim zdravilom, in v kombinaciji z abirateronom ne glede na status mutacij.[1-4]



PARP – poli (ADP-riboza) polimeraza, **1L** – v prvem redu zdravljenja, **2L** – v drugem redu zdravljenja, **HRD** – pomanjkanje homologne rekombinacije, **KORP** – na kastracijo odporen rak prostate

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

LYNPARZA 100 mg filmsko obložene tablete
LYNPARZA 150 mg filmsko obložene tablete

SESTAVA: Ena filmsko obložena tableta vsebuje 100 mg olapariba ali 150 mg olapariba.
INDIKACIJE:
Rak jajčnikov: 1) *zdravilo Lynparza je indicirano kot monoterapija za*:
- vzdrževalno zdravljenje odraslih bolnic z napredovalim (stadij III in IV po FIGO) epitelijskim rakom visokega gradusa jajčnikov, jajcevodov ali primarnim peritonealnim rakom z mutacijo gena BRCA1/2 (germinalno in/ali somatsko), ki so v odzivu (popolnem ali delnem) po zaključeni prvi liniji kemoterapije na osnovi platine.
- vzdrževalno zdravljenje odraslih bolnic, pri katerih je prišlo do ponovitve epitelijskega raka visokega gradusa jajčnikov, jajcevodov ali primarnega peritonealnega raka, občutljivega na platino, ki so v popolnem ali delnem odzivu na kemoterapijo na osnovi platine.

2) *zdravilo Lynparza je v kombinaciji z bevacizumabom indicirano za*:
- vzdrževalno zdravljenje odraslih bolnic z napredovalim (stadij III in IV po FIGO) epitelijskim rakom visokega gradusa jajčnikov, jajcevodov ali primarnim peritonealnim rakom, ki so v popolnem ali delnem odzivu po zaključeni prvi liniji kemoterapije na osnovi platine v kombinaciji z bevacizumabom, pri katerih je rak povezan s pozitivnim stanjem pomanjkanja homologne rekombinacije (HRD – homologous recombination deficiency), opredeljenim z mutacijo gena *BRCA1/2* in/ali genomsko nestabilnostjo.

Rak dojk: *zdravilo Lynparza je indicirano kot*:
- monoterapija ali v kombinaciji z endokrinim zdravljenjem za adjuvantno zdravljenje odraslih bolnikov z germinalnimi mutacijami gena *BRCA1/2*, ki imajo HER2-negativnega zgodnjega raka dojk z velikim tveganjem in so bili predhodno zdravljeni z neoadjuvantno ali adjuvantno kemoterapijo.
- monoterapija za zdravljenje odraslih bolnikov z germinalno mutacijo gena *BRCA1/2*, ki imajo HER2-negativnega lokalno napredovalega ali metastatskega raka dojk. Bolniki morajo biti predhodno zdravljeni z antraciklinom in taksanom v okviru (neo)adjuvantnega zdravljenja ali zdravljenja metastatske bolezni, razen če za ti zdravljenji niso primerni. Pri bolnikih, ki imajo raka dojk s pozitivnimi hormonskimi receptorji (HR), je morala bolezen prav tako napredovati med predhodnim hormonskim zdravljenjem ali po njem, ali morajo bolniki veljati za neprimerne za hormonsko zdravljenje.

Adenokarcinom trebušne slinavke: zdravilo Lynparza je kot monoterapija indicirano za vzdrževalno zdravljenje odraslih bolnikov z germinalno mutacijo gena BRCA1/2, ki imajo metastatski adenokarcinom trebušne slinavke in njihova bolezen ni napredovala po najmanj 16 tednih zdravljenja s platino v shemi prve linije kemoterapije.
Rak prostate: *zdravilo Lynparza je indicirano:*
- kot monoterapija za zdravljenje odraslih bolnikov z metastatskim, na kastracijo odpornim rakom prostate (mKORP) in mutacijami gena *BRCA1/2* (germinalnimi in/ali somatskimi), pri katerih je bolezen napredovala po predhodni terapiji, ki je vsebovala novo hormonsko zdravilo.
- v kombinaciji z abirateronom in prednizonom ali prednizolonom za zdravljenje odraslih bolnikov z mKORP, pri katerih kemoterapija ni klinično indicirana

ODMERJANJE IN NAČIN UPORABE: Priporočeni odmerek zdravila Lynparza pri monoterapiji ali v kombinaciji z bevacizumabom pri raku jajčnikov ali v kombinaciji z abirateronom in prednizonom ali prednizolonom pri raku prostate ali z endokrinim zdravljenjem je 300 mg dvakrat na dan (to ustreza celotnemu dnevnemu odmerku 600 mg). 100-mg tablete so na voljo za zmanjšanje odmerka. Bolnice s ponovitvijo raka jajčnikov morajo začeti zdravljenje z zdravilom Lynparza najpozneje v 8 tednih po zadnjem odmerku sheme zdravljenja na osnovi platine. Če je zdravilo Lynparza uporabljeno v kombinaciji z bevacizumabom za prvo linijo vzdrževalnega zdravljenja po dokončanju prve linije zdravljenja na osnovi platine in z bevacizumabom, je odmerek bevacizumaba 15 mg/kg enkrat na 3 tedne. Glejte celotne informacije o zdravilu za bevacizumab. Za priporočeno odmerjanje partnerskega zdravila/partnerskih zdravil (zaviralec aromataze/antiestrogen in/ali LHRH) v kombinaciji endokrinega zdravljenja glejte celotne informacije o zadevnem zdravilu. Če je zdravilo Lynparza uporabljeno v kombinaciji z abirateronom za zdravljenje bolnikov z mKORP, je odmerek abiraterona 1000 mg peroralno enkrat na dan. Abirateron je treba dajati s 5 mg prednizona ali prednizolona peroralno dvakrat na dan. Glejte celotne informacije o zdravilu za abirateron. Prvo linijo vzdrževalnega zdravljenja napredovalega raka jajčnikov z mutacijo gena BRCA in prvo linijo vzdrževalnega zdravljenja HRD-pozitivnega napredovalega raka jajčnikov v kombinaciji z bevacizumabom je priporočljivo nadaljevati do radiološkega napredovanja bolezni ali nesprejemljive toksičnosti ali do največ 2 leti, če po 2 letih ni radioloških znakov bolezni. V primeru znakov bolezni po 2 letih, se lahko zdravljenje nadaljuje, če bi le to po mnenju zdravnika bilo koristno za bolnico. Glejte informacije o zdravilu bevacizumab za priporočeno celotno trajanje zdravljenja največ 15 mesecev, vključno z obdobji v kombinaciji s kemoterapijo in kot vzdrževalno zdravljenje. Pri adjuvantnem zdravljenju zgodnjega raka dojk je prporočljivo, da bolniki prejemajo zdravljenje do 1 leto ali do ponovitve bolezni ali do nesprejemljive toksičnosti, kar od tega se zgodi najprej. Zdravljenje ponovitve raka jajčnikov, raka dojk, adenokarcinoma trebušne slinavke in raka prostate je priporočljivo nadaljevati do napredovanja osnovne bolezni ali nesprejemljive toksičnosti. Učinkovitost in varnost ponovnega vzdrževalnega zdravljenja z zdravilom Lynparza po prvi ali poznejši ponovitvi bolezni pri bolnicah z rakom jajčnikov nista bili dokazani. Podatkov o učinkovitosti in varnosti ponovnega zdravljenja pri bolnicah z rakom dojk ni. Pri raku prostate je treba pri bolnikih, ki niso bili kirurško kastrirani, nadaljevati z medicinsko kastracijo z analogom luteinizirajočega hormona sproščajočega hormona. Če je zdravilo Lynparza uporabljeno v kombinaciji z abirateronom in prednizonom ali prednizolonom, je zdravljenje priporočljivo nadaljevati do napredovanja osnovne bolezni ali do nesprejemljivih toksičnih učinkov. Pri vseh bolnikih je treba med zdravljenjem še naprej uporabljati analoge GnRH (gonadotropin sproščajočega hormona) ali pa morajo bolniki pred tem opraviti obojestransko orhidektomijo. Glejte informacije o zdravilu za abirateron. Podatkov o učinkovitosti ali varnosti ponovnega zdravljenja z zdravilom Lynparza pri bolnikih z rakom prostate ni. V primeru potrebe po zmanjšanju odmerka zaradi neželenih učinkov je priporočeno zmanjšanje odmerka na 250 mg dvakrat na dan (to ustreza celotnemu dnevnemu odmerku 500 mg). Če je potrebno še dodatno zmanjšanje odmerka, je priporočljivo zmanjšanje odmerka na 200 mg dvakrat na dan (to ustreza celotnemu dnevnemu odmerku 400 mg). Zdravljenje z zdravilom Lynparza mora uvesti in nadzorovati zdravnik, ki ima izkušnje z uporabo zdravil proti raku. Mutacijsko stanje *BRCA* in/ali genomsko nestabilnost morajo imeti bolniki potrjeno z validiranim testom. Pred uporabo zdravila Lynparza v kombinaciji z abirateronom in prednizonom ali prednizolonom za zdravljenje bolnikov z mKORP genomsko testiranje ni potrebno. Genetsko svetovanje bolnikom z mutacijami *BRCA* je treba opraviti v skladu z lokalnimi predpisi. Zdravilo Lynparza se lahko pri bolnikih z blago okvaro ledvic (očistek kreatinina 51 do 80 ml/min) uporablja brez prilagoditve odmerka. Pri bolnikih z zmerno okvaro ledvic (očistek kreatinina 31 do 50 ml/min) je priporočen odmerek 200 mg dvakrat na dan. Uporaba zdravila se pri bolnikih s hudo okvaro ali končno odpovedjo ledvic (očistek kreatinina ≤ 30 ml/min) ne priporoča, ker varnost in farmakokinetika pri tej skupini bolnikov nista bili raziskani. Zdravilo Lynparza se lahko daje bolnikom z blago ali zmerno okvaro jeter (klasifikacija Child-Pugh A ali B) brez prilagoditve odmerka. Uporabe zdravila Lynparza se ne priporoča pri bolnikih s hudo okvaro jeter (klasifikacija Child-Pugh C), ker varnost in farmakokinetika pri tej skupini bolnikov nista bili raziskani. Zdravilo Lynparza je za peroralno uporabo. Tablete zdravila Lynparza je treba pogoltniti cele in se jih ne sme gristi, drobiti, raztapljati ali lomiti. Lahko se jih jemlje ne glede na obroke. **KONTRAINDIKACIJE:** Preobčutljivost na učinkovino ali katero koli pomožno snov. Dojenje med zdravljenjem in en mesec po zadnjem odmerku. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** *Hematološki toksični učinki:* Pri bolnikih, zdravljenih z zdravilom Lynparza, so bili opisani hematološki toksični učinki, vključno s klinično diagnozo in/ali laboratorijskimi izsledki, na splošno blage ali zmerne (stopnja 1 ali 2 po CTCAE) anemije, nevtropenije, trombocitopenije in limfopenije. Bolniki ne smejo začeti zdravljenja z zdravilom Lynparza, dokler ne okrevajo po hematoloških toksičnih učinkih predhodnega zdravljenja proti raku. Preiskava celotne krvne slike je priporočljiva na začetku zdravljenja, potem vsak mesec prvih 12 mesecev zdravljenja in pozneje redno. Če se pri bolniku pojavijo hudi hematološki toksični učinki ali je odvisen od transfuzij krvi, je treba zdravljenje z zdravilom Lynparza prekiniti in uvesti ustrezno hematološko testiranje. Če krvne vrednosti ostanejo klinično nenormalne še 4 tedne po prekinitvi uporabe zdravila Lynparza, je priporočljivo opraviti preiskavo kostnega mozga in/ali krvno citogenetsko analizo. *Mielodisplastični sindrom/akutna mieloična levkemija (MDS/AML):* Celokupna pojavnost MDS/AML je bila pri bolnikih, ki so v kliničnih preizkušanjih prejemali monoterapijo z zdravilom Lynparza, vključno v obdobju dolgoročnega spremljanja preživetja, < 1,5 %, z večjo pojavnostjo pri bolnicah z *BRCA*m, pri katerih je prišlo do ponovitve na platino občutljivega raka jajčnikov, ki so predhodno prejele vsaj dve liniji kemoterapije s platino in so jih spremljali 5 let. Večina teh primerov je bila s smrtnim izidom. Če obstaja sum na MDS/AML, je potrebno bolnico napotiti na nadaljnje preiskave k hematologu, vključno z analizo kostnega mozga in odvzemom krvi za citogenetiko. Če se po preiskavi dolgotrajne hematološke toksičnosti potrdi MDS/AML, je treba uporabo zdravila Lynparza prekiniti in bolnico ustrezno zdraviti. *Venski trombembolični dogodki:* Med zdravljenjem z zdravilom Lynparza so poročali o venskih trombemboličnih dogodkih, predvsem o pljučni emboliji, vendar ti dogodki niso imeli kakšnega doslednega kliničnega vzorca. V primerjavi z drugimi odobrenimi indikacijami so opažali večjo pojavnost pri bolnikih z metastatskim, na kastracijo odpornim rakom prostate, ki so prejemali tudi androgeno deprivacijsko zdravljenje. Bolnike spremljajte glede kliničnih znakov in simptomov venske tromboze in pljučne embolije, ter jih zdravite kot je medicinsko ustrezno. Bolniki z anamnezo VTE imajo morda večje tveganje za njeno ponovitev in jih je treba ustrezno spremljati. *Pnevmonitis:* V kliničnih študijah je bil pnevmonitis, vključno s smrtnim izidom, opisan pri < 1,0 % bolnikov, ki so prejemali zdravilo Lynparza, spremljali pa so jih številni predispozicijski dejavniki. Če se pri bolniku pojavijo novi ali poslabšajo obstoječi dihalni simptomi, npr. dispneja, kašelj in zvišana telesna temperatura, ali je ugotovljen nenormalen radiološki izvid prsnih organov, je treba zdravljenje z zdravilom Lynparza prekiniti in takoj opraviti preiskave. Če je pnevmonitis potrjen, je treba zdravljenje z zdravilom Lynparza prekiniti in bolnika ustrezno zdraviti. *Hepatotoksičnost:* Če se pojavijo klinični simptomi ali znaki, ki kažejo na razvoj hepatotoksičnosti, je treba takoj izvesti klinično oceno bolnika in preiskave delovanja jeter. V primeru suma na z zdravilom povzročeno okvaro jeter (DILI - drug-induced liver injury) je treba zdravljenje prekiniti. V primeru hude DILI je treba razmisliti o ukinitvi zdravljenja, kot je klinično primerno. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Zdravilo Lynparza se uporablja kot monoterapija in ni primerno za uporabo v kombinaciji z mielosupresivnimi zdravili proti raku, vključno z zdravili, ki poškodujejo DNA. Sočasna uporaba olapariba s cepivi ali imunosupresivnimi zdravili ni raziskana. Za presnovni očistek olapariba so pretežno odgovorni izoencimi CYP3A4/5. Sočasna uporaba zdravila Lynparza z znanimi močnimi ali zmernimi zaviralci tega izoencima ni priporočljiva. Če je treba sočasno uporabiti močne ali zmerne zaviralce CYP3A, je treba odmerek zdravila Lynparza zmanjšati. Prav tako med zdravljenjem z zdravilom Lynparza ni priporočljivo pitje grenivkinega soka. Prav tako olapariba ni priporočljivo uporabljati z znanimi močnimi ali zmernimi do močnimi induktorji tega izoencima, ker obstaja možnost, da se učinkovitost zdravila Lynparza bistveno zmanjša. Olaparib *in vitro* zavira CYP3A4 ter *in vivo* predvidoma blago zavira CYP3A. Zato je potrebna previdnost pri sočasni uporabi olapariba z občutljivimi substrati CYP3A4 ali substrati, ki imajo ozko terapevtsko okno. Bolnike, ki sočasno z olaparibom prejemajo substrate CYP3A z ozkim terapevtskim oknom, je priporočljivo ustrezno klinično spremljati. *In vitro* so ugotovili indukcijo CYP1A2, 2B6 in 3A4, prav tako ni mogoče izključiti možnosti, da olaparib inducira CYP2C9, CYP2C19 in P-gp, zato lahko olaparib po sočasni uporabi zmanjša izpostavljenost substratom teh presnovnih encimov in prenašalne beljakovine. Učinkovitost nekaterih hormonskih kontraceptivov se lahko zmanjša, če so uporabljeni sočasno z olaparibom. *In vitro* olaparib zavira efluksni prenašaleca P-gp, zato je potrebno bolnike, ki sočasno prejemajo substrate P-gp, ustrezno klinično spremljati. *In vitro* olaparib zavira BCRP, OATP1B1, OCT1, OCT2, OAT3, MATE1 in MATE2K. Ni mogoče izključiti možnosti, da olaparib poveča izpostavljenost BCRP, OATP1B1, OCT1, OCT2, OAT3, MATE1 in MATE2K. Še zlasti je previdnost potrebna, če se olaparib uporablja v kombinaciji s katerim koli statinom. Izvedli so klinično študijo za oceno kombinacije olapariba z anastrozolom, letrozolom in tamoksifenom, vendar klinično pomembnih medsebojnih delovanj niso opazili. **NEŽELENI UČINKI:** Zdravilo Lynparza je bilo povezano z neželenimi učinki, ki so bili na splošno blage ali zmerne resnosti (stopnja po CTCAE 1 ali 2) in na splošno niso zahtevali prekinitve zdravljenja. Če je zdravilo Lynparza uporabljeno v kombinaciji z bevacizumabom pri raku jajčnikov ali v kombinaciji z abirateronom in prednizonom ali prednizolonom pri raku prostate, se varnostni profil na splošno sklada z varnostnim profilom vsakega posameznega zdravila. Varnostni profil temelji na kumulativnih podatkih 4499 bolnikov s solidnimi tumorji, ki so bili v kliničnih preskušanjih zdravljeni z monoterapijo z zdravilom Lynparza v priporočenem odmerku. *Zelo pogosti neželeni učinki:* anemija, nevtropenija, levkopenija, zmanjšanje apetita, omotica, glavobol, spremenjen okus, kašelj, dispneja, bruhanje, driska, navzea, dispepsija in utrujenost (vključno z astenijo). *Pogosti neželeni učinki:* limfopenija, trombocitopenija, zvišanje transaminaz, stomatitis, bolečine v zgornjem delu trebuha, izpuščaj, zvišanje kreatinina v krvi in venska trombembolija. **PLODNOST, NOSEČNOST IN DOJENJE:** Ženske v rodni dobi ne smejo biti noseče na začetku zdravljenja z zdravilom Lynparza in ne smejo zanositi med zdravljenjem in še 6 mesecev po prejetju zadnjega odmerka. Pri vseh ženskah v rodni dobi je potrebno pred zdravljenjem opraviti test nosečnosti in ga redno izvajati med celotnim zdravljenjem. Priporočljivi sta dve visoko učinkoviti in komplementarni obliki kontracepcije. Zaradi možnega medsebojnega delovanja olapariba s hormonsko kontracepcijo je treba razmisliti o dodatni nehormonski kontracepciji. Pri ženskah s hormonsko odvisnim rakom je treba razmisliti o dveh nehormonskih načinih kontracepcije. Zdravilo Lynparza je kontraindicirano med obdobjem dojenja in še en mesec po prejetju zadnjega odmerka. Moški bolniki morajo med zdravljenjem in še 3 mesece po prejetju zadnjega odmerka zdravila Lynparza med spolnimi odnosi z nosečo žensko ali žensko v rodni dobi uporabljati kondom. Tudi partnerke moških bolnikov morajo uporabljati visoko učinkovito kontracepcijo, če so v rodni dobi. Moški bolniki med zdravljenjem z zdravilom Lynparza in še 3 mesece po zadnjem odmerku tega zdravila ne smejo darovati sperme. **REŽIM PREDPISOVANJA IN IZDAJE ZDRAVILA:** Rp/Spec. **DATUM ZADNJE REVIZIJE BESEDILA:** 5. 10. 2023 (SI-3466). **IMETNIK DOVOLJENJA ZA PROMET**: AstraZeneca AB, SE-151 85 Södertälje , Švedska. Dodatne informacije so na voljo pri podjetju AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, 1000 Ljubljana, telefon: 01/51 35 600. **Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila.**

Literatura: 1. Povzetek glavnih značilnosti zdravila Lynparza, 5.10.2023, **2.** https://www.ema.europa.eu/en/medicines/human/EPAR/rubraca, dostopano 31.1.2024, **3.** https://www.ema.europa.eu/en/medicines/human/EPAR/zejula, dostopano 31.1.2024, **4.** https://www.ema.europa.eu/en/medicines/human/EPAR/talzenna, dostopano 31.1.2024, **5.** https://www.ema.europa.eu/en/news/lynparza-recommended-approval-ovarian-cancer, dostopano 31.1.2024

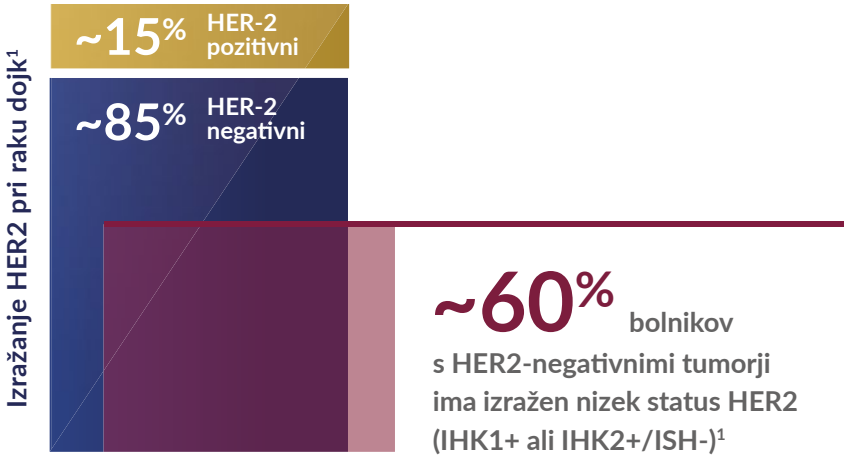
AstraZeneca
AstraZeneca UK Limited, Podružnica v Sloveniji,
Verovškova 55, 1000 Ljubljana, tel: 01/51 35 600

Samo za strokovno javnost. Datum priprave gradiva: februar 2024. SI-3729

STATUS HER2 NI EDINA KLASIFIKACIJA, NA KATERO POMISLIMO PRI RAZSEJANEMU RAKU DOJK

POMEN NIZKEGA IZRAŽANJA HER2 ŠE NI V CELOTI ODKRIT

Znotraj HER2-negativne klasifikacije, si različni statusi HER2 zaslužijo pozornost[1]



Po izčrpanju standardnih možnosti zdravljenja HER2-negativnega razsejanega raka dojk se odkrivajo dodatne možnosti zdravljenja za različne statuse izražanja HER2.

HER-2: receptor 2 za epidermalni rastni faktor, IHK: imunohistokemija, ISH: hibridizacija *in situ*

Literatura: 1. Tarantino P, Hamilton E, Tolaney SM, et al. HER2-low breast cancer: pathological and clinical landscape. J Clin Oncol. 2020;38(17):1951-1962.

Daiichi-Sankyo | AstraZeneca

Dodatne informacije so na voljo pri družbi AstraZeneca UK Limited,
Podružnica v Sloveniji, Verovškova 55, 1000 Ljubljana. Tel. št.: 01 513 56 00.
Datum priprave informacije: april 2023. Samo za strokovno javnost. SI-2980

KEYTRUDA® je odobrena za zdravljenje več kot 25 indikacij rakavih obolenj[1]

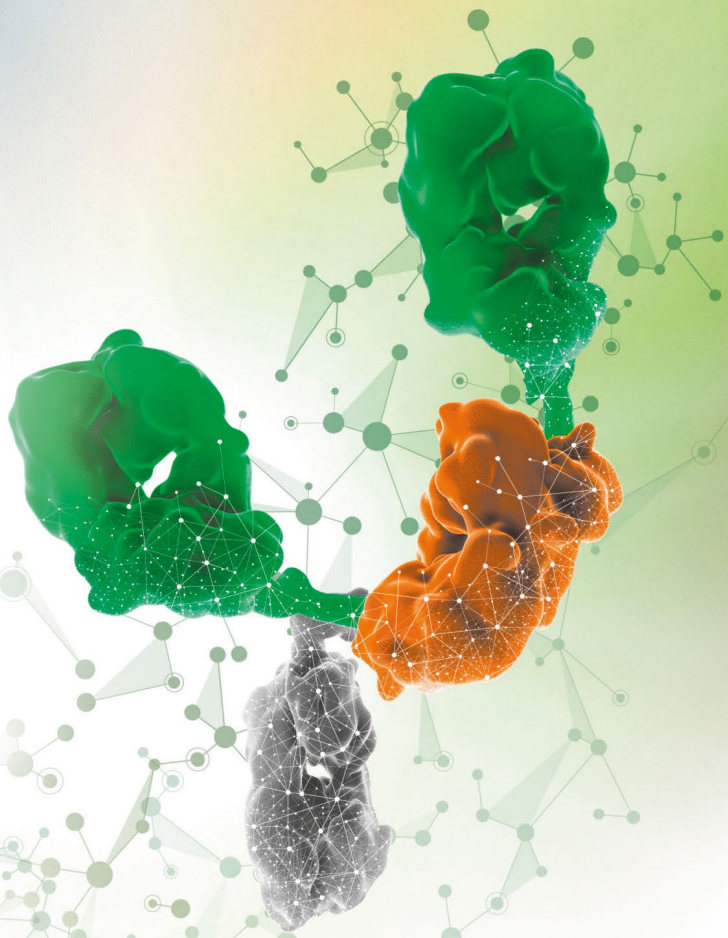
Referenca: 1. Keytruda EU SmPC

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA • Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila! • Ime zdravila: KEYTRUDA 25 mg/ml koncentrat za raztopino za infundiranje vsebuje pembrolizumab. • **Terapevtske indikacije:** Zdravilo KEYTRUDA je kot samostojno zdravljenje indicirano za zdravljenje: odraslih in mladostnikov, starih 12 let ali več, z napredovalim (neoperabilnim ali metastatskim) melanomom; za adjuvantno zdravljenje odraslih in mladostnikov, starih 12 let ali več, z melanomom v stadiju IIB, IIC ali III, in sicer po popolni kirurški odstranitvi; za adjuvantno zdravljenje odraslih z nedrobnoceličnim pljučnim rakom, ki imajo visoko tveganje za ponovitev bolezni po popolni kirurški odstranitvi in kemoterapiji na osnovi platine; metastatskega nedrobnoceličnega pljučnega raka (NSCLC) v prvi liniji zdravljenja pri odraslih, ki imajo tumorje z ≥ 50 % izraženostjo PD-L1 (TPS) in brez pozitivnih tumorskih mutacij EGFR ali ALK; lokalno napredovalega ali metastatskega NSCLC pri odraslih, ki imajo tumorje z ≥ 1 % izraženostjo PD-L1 (TPS) in so bili predhodno zdravljeni z vsaj eno shemo kemoterapije, bolniki s pozitivnimi tumorskimi mutacijami EGFR ali ALK so pred prejemom zdravila KEYTRUDA morali prejeti tudi tarčno zdravljenje; odraslih in pediatričnih bolnikov, starih 3 leta ali več, s ponovljenim ali neodzivnim klasičnim Hodgkinovim limfomom (cHL), pri katerih avtologna presaditev matičnih celic (ASCT) ni bila uspešna, ali po najmanj dveh predhodnih zdravljenjih kadar ASCT ne pride v poštev kot možnost zdravljenja; lokalno napredovalega ali metastatskega urotelijskega raka pri odraslih, predhodno zdravljenih s kemoterapijo, ki je vključevala platino; lokalno napredovalega ali metastatskega urotelijskega raka pri odraslih, ki niso primerni za zdravljenje s kemoterapijo, ki vsebuje cisplatin in imajo tumorje z izraženostjo PD-L1 ≥ 10, ocenjeno s kombinirano pozitivno oceno (CPS); ponovljenega ali metastatskega ploščatoceličnega raka glave in vratu (HNSCC) pri odraslih, ki imajo tumorje z ≥ 50 % izraženostjo PD-L1 (TPS), in pri katerih je bolezen napredovala med zdravljenjem ali po zdravljenju s kemoterapijo, ki je vključevala platino; za adjuvantno zdravljenje odraslih z rakom ledvičnih celic s povišanim tveganjem za ponovitev bolezni po nefrektomiji, ali po nefrektomiji in kirurški odstranitvi metastatskih lezij, za zdravljenje odraslih z MSI-H (microsatellite instability-high) ali dMMR (mismatch repair deficient) kolorektalnim rakom v naslednjih terapevtskih okoliščinah: prva linija zdravljenja metastatskega kolorektalnega raka; zdravljenje neoperabilnega ali metastatskega kolorektalnega raka po predhodnem kombiniranem zdravljenju, ki je temeljilo na fluoropirimidinu; in za zdravljenje MSI-H ali dMMR tumorjev pri odraslih z: napredovalim ali ponovljenim rakom endometrija, pri katerih je bolezen napredovala med ali po predhodnem zdravljenju, ki je vključevalo platino, v katerih koli terapevtskih okoliščinah, in ki niso kandidati za kurativno operacijo ali obsevanje; neoperabilnim ali metastatskim rakom želodca, tankega črevesa ali biliarnega trakta, pri katerih je bolezen napredovala med ali po vsaj enem predhodnem zdravljenju. Zdravilo KEYTRUDA je kot samostojno zdravljenje ali v kombinaciji s kemoterapijo s platino in 5-fluorouracilom (5-FU) indicirano za prvo linijo zdravljenja metastatskega ali neoperabilnega ponovljenega ploščatoceličnega raka glave in vratu pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1. Zdravilo KEYTRUDA je v kombinaciji s kemoterapijo, ki vključuje platino, indicirano za neoadjuvantno zdravljenje, in v nadaljevanju kot samostojno zdravljenje za adjuvantno zdravljenje odraslih z operabilnih nedrobnoceličnim pljučnim rakom, ki imajo visoko tveganje za ponovitev bolezni; v kombinaciji s pemetreksedom in kemoterapijo na osnovi platine je indicirano za prvo linijo zdravljenja metastatskega neploščatoceličnega NSCLC pri odraslih, pri katerih tumorji nimajo pozitivnih mutacij EGFR ali ALK; v kombinaciji s karboplatinom in bodisi paklitakselom bodisi nab-paklitakselom je indicirano za prvo linijo zdravljenja metastatskega ploščatoceličnega NSCLC pri odraslih; v kombinaciji z aksitinibom ali v kombinaciji z lenvatinibom je indicirano za prvo linijo zdravljenja napredovalega raka ledvičnih celic (RCC) pri odraslih; v kombinaciji s kemoterapijo s platino in fluoropirimidinom je indicirano za prvo linijo zdravljenja lokalno napredovalega neoperabilnega ali metastatskega raka požiralnika pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 10; v kombinaciji s kemoterapijo za neoadjuvantno zdravljenje, in v nadaljevanju kot samostojno adjuvantno zdravljenje po kirurškem posegu, je indicirano za zdravljenje odraslih z lokalno napredovalim trojno negativnim rakom dojk ali trojno negativnim rakom dojk v zgodnjem stadiju z visokim tveganjem za ponovitev bolezni; v kombinaciji s kemoterapijo je indicirano za zdravljenje lokalno ponovljenega neoperabilnega ali metastatskega trojno negativnega raka dojk pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 10 in predhodno niso prejeli kemoterapije za metastatsko bolezen; v kombinaciji z lenvatinibom je indicirano za zdravljenje napredovalega ali ponovljenega raka endometrija (EC) pri odraslih z napredovalo boleznijo med ali po predhodnem zdravljenju s kemoterapijo, ki je vključevala platino, v katerih koli terapevtskih okoliščinah, in ki niso kandidati za kurativno operacijo ali obsevanje; v kombinaciji s kemoterapijo, z bevacizumabom ali brez njega, je indicirano za zdravljenje persistentnega, ponovljenega ali metastatskega raka maternične vratu pri odraslih bolnicah, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1; v kombinaciji s trastuzumabom, fluoropirimidinom in kemoterapijo, ki vključuje platino, je indicirano za prvo linijo zdravljenja lokalno napredovalega neoperabilnega ali metastatskega HER2-pozitivnega adenokarcinoma želodca ali gastroezofagealnega prehoda pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1; v kombinaciji s fluoropirimidinom in kemoterapijo, ki vključuje platino, je indicirano za prvo linijo zdravljenja lokalno napredovalega neoperabilnega ali metastatskega HER2-negativnega adenokarcinoma želodca ali gastroezofagealnega prehoda pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1; v kombinaciji z gemcitabinom in cisplatinom je indicirano za prvo linijo zdravljenja lokalno napredovalega neoperabilnega ali metastatskega raka biliarnega trakta pri odraslih. • **Odmerjanje in način uporabe:** Testiranje PD-L1: Če je navedeno v indikaciji, je treba izbiro bolnika za zdravljenje z zdravilom KEYTRUDA na podlagi izraženosti PD-L1 tumorja potrditi z validirano preiskavo. Testiranje MSI/MMR: Če je navedeno v indikaciji, je treba izbiro bolnika za zdravljenje z zdravilom KEYTRUDA na podlagi MSI-H/dMMR statusa tumorja potrditi z validirano preiskavo. Odmerjanje: Priporočeni odmerek zdravila KEYTRUDA pri odraslih je bodisi 200 mg na 3 tedne ali 400 mg na 6 tednov, apliciran z intravensko infuzijo v 30 minutah. Priporočeni odmerek zdravila KEYTRUDA za samostojno zdravljenje pri pediatričnih bolnikih s cHL, starih 3 leta ali več, ali bolnikih z melanomom, starih 12 let ali več, je 2 mg/kg telesne mase (do največ 200 mg) na 3 tedne, apliciran z intravensko infuzijo v 30 minutah. Za uporabo v kombinaciji glejte povzetke glavnih značilnosti zdravil sočasno uporabljenih zdravil. Če se uporablja kot del kombiniranega zdravljenja skupaj z intravensko kemoterapijo, je treba zdravilo KEYTRUDA aplicirati prvo. Bolnike je treba zdraviti do napredovanja bolezni ali nesprejemljivih toksičnih učinkov (in do maksimalnega trajanja zdravljenja, če je le to določeno za indikacijo). Pri adjuvantnem zdravljenju melanoma, NSCLC ali RCC je treba zdravilo uporabljati do ponovitve bolezni, pojava nesprejemljivih toksičnih učinkov oziroma mora zdravljenje trajati do enega leta. Za neoadjuvantno in adjuvantno zdravljenje operabilnega NSCLC morajo bolniki neoadjuvantno prejeti zdravilo KEYTRUDA v kombinaciji s kemoterapijo, in sicer 4 odmerke po 200 mg na 3 tedne ali 2 odmerka po 400 mg na 6 tednov ali do napredovanja bolezni, ki izključuje definitivni kirurški poseg, ali do pojava nesprejemljivih toksičnih učinkov, čemur sledi adjuvantno zdravljenje z zdravilom KEYTRUDA kot samostojnim zdravljenjem, in sicer 13 odmerkov po 200 mg na 3 tedne ali 7 odmerkov po 400 mg na 6 tednov ali do ponovitve bolezni ali do pojava nesprejemljivih toksičnih učinkov. Bolniki, pri katerih pride do napredovanja bolezni, ki izključuje definitivni kirurški poseg, ali do nesprejemljivih toksičnih učinkov, povezanih z zdravilom KEYTRUDA kot neoadjuvantnim zdravljenjem v kombinaciji s kemoterapijo, ne smejo prejeti zdravila KEYTRUDA kot samostojnega zdravljenja za adjuvantno zdravljenje. Za neoadjuvantno in adjuvantno zdravljenje TNBC morajo bolniki neoadjuvantno prejeti zdravilo KEYTRUDA v kombinaciji s kemoterapijo, in sicer 8 odmerkov po 200 mg na 3 tedne ali 4 odmerke po 400 mg na 6 tednov, ali do napredovanja bolezni, ki izključuje definitivni kirurški poseg, ali do pojava nesprejemljivih toksičnih učinkov, čemur sledi adjuvantno zdravljenje z zdravilom KEYTRUDA kot samostojnim zdravljenjem, in sicer 9 odmerkov po 200 mg na 3 tedne ali 5 odmerkov po 400 mg na 6 tednov ali do ponovitve bolezni ali pojava nesprejemljivih toksičnih učinkov. Bolniki, pri katerih pride do napredovanja bolezni, ki izključuje definitivni kirurški poseg, ali do nesprejemljivih toksičnih učinkov povezanih z zdravilom KEYTRUDA kot neoadjuvantnim zdravljenjem v kombinaciji s kemoterapijo, ne smejo prejeti zdravila KEYTRUDA kot samostojnega zdravljenja za adjuvantno zdravljenje. Če je aksitinib uporabljen v kombinaciji s pembrolizumabom, se lahko razmisli o povečanju odmerka aksitiniba nad začetnih 5 mg v presledkih šest tednov ali več. V primeru uporabe v kombinaciji z lenvatinibom je treba zdravljenje z enim ali obema zdraviloma prekiniti, kot je primerno. Uporabo lenvatiniba je treba zadržati, odmerek zmanjšati ali prenehati z uporabo, v skladu z navodili v povzetku glavnih značilnosti zdravila za lenvatinib, in sicer za kombinacijo s pembrolizumabom. Pri bolnikih starih ≥ 65 let, bolnikih z blago do zmerno okvaro ledvic, bolnikih z blago ali zmerno okvaro jeter prilagoditev odmerka ni potrebna. Odložitev odmerka ali ukinitev zdravljenja: Zmanjšanje odmerka zdravila KEYTRUDA ni priporočljivo. Za obvladovanje neželenih učinkov je treba uporabo zdravila KEYTRUDA zadržati ali ukiniti, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. • **Kontraindikacije:** Preobčutljivost na učinkovino ali katero koli pomožno snov. • **Povzetek posebnih opozoril, previdnostnih ukrepov, interakcij in neželenih učinkov:** Imunsko pogojeni neželeni učinki (pnevmonitis, kolitis, hepatitis, nefritis, endokrinopatije, neželeni učinki na kožo in drugi): Pri bolnikih, ki so prejemali pembrolizumab, so se pojavili imunsko pogojeni neželeni učinki, vključno s hudimi in smrtnimi primeri. Večina imunsko pogojenih neželenih učinkov, ki so se pojavili med zdravljenjem s pembrolizumabom, je bila reverzibilnih in so jih obvladali s prekinitvami uporabe pembrolizumaba, uporabo kortikosteroidov in/ali podporno oskrbo. Pojavijo se lahko tudi po zadnjem odmerku pembrolizumaba in hkrati prizadanejo več organskih sistemov. V primeru suma na imunsko pogojene neželene učinke je treba poskrbeti za ustrezno oceno za potrditev etiologije oziroma izključitev drugih vzrokov. Glede na izrazitost neželenega učinka je treba zadržati uporabo pembrolizumaba in uporabiti kortikosteroide – za natančna navodila, prosimo, glejte Povzetek glavnih značilnosti zdravila Keytruda. Zdravljenje s pembrolizumabom lahko poveča tveganje za zavrnitev pri prejemnikih presadkov čvrstih organov. Pri bolnikih, ki so prejemali pembrolizumab, so poročali o hudih z infuzijo povezanih reakcijah, vključno s preobčutljivostjo in anafilaksijo. Pembrolizumab se iz obtoka odstrani s katabolizmom, zato presnovnih medsebojnih delovanj zdravil ni pričakovati. Uporabi sistemskih kortikosteroidov ali imunosupresivov pred uvedbo pembrolizumaba se je treba izogibati, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost pembrolizumaba. Vendar pa je kortikosteroide ali druge imunosupresive mogoče uporabiti za zdravljenje imunsko pogojenih neželenih učinkov. Kortikosteroide je mogoče uporabiti tudi kot premedikacijo, če je pembrolizumab uporabljen v kombinaciji s kemoterapijo, kot antiemetično profilakso in/ali za ublažitev neželenih učinkov, povezanih s kemoterapijo. Ženske v rodni dobi morajo med zdravljenjem s pembrolizumabom in vsaj še 4 mesece po zadnjem odmerku pembrolizumaba uporabljati učinkovito kontracepcijo, med nosečnostjo in dojenjem se ga ne sme uporabljati. Varnost pembrolizumaba pri samostojnem zdravljenju so v kliničnih študijah ocenili pri 7631 bolnikih, ki so imeli različne vrste raka, s štirimi odmerki (2 mg/kg telesne mase na 3 tedne, 200 mg na 3 tedne in 10 mg/kg telesne mase na 2 ali 3 tedne). V tej populaciji bolnikov je mediani čas opazovanja znašal 8,5 meseca (v razponu od 1 dneva do 39 mesecev), najpogostejši neželeni učinki zdravljenja s pembrolizumabom pa so bili utrujenost (31 %), diareja (22 %) in navzea (20 %). Večina poročanih neželenih učinkov pri samostojnem zdravljenju je bila po izrazitosti 1. ali 2. stopnje. Najresnejši neželeni učinki so bili imunsko pogojeni neželeni učinki in hude z infuzijo povezane reakcije. Pojavnost imunsko pogojenih neželenih učinkov pri uporabi pembrolizumaba samega za adjuvantno zdravljenje je znašala 37 % za vse stopnje in 9 % od 3. do 5. stopnje, pri metastatski bolezni pa 25 % za vse stopnje in 6 % od 3. do 5. stopnje. Pri adjuvantnem zdravljenju niso zaznali nobenih novih imunsko pogojenih neželenih učinkov. Varnost pembrolizumaba pri kombiniranem zdravljenju s kemoterapijo so ocenili pri 5183 bolnikih z različnimi vrstami raka, ki so v kliničnih študijah prejemali pembrolizumab v odmerkih 200 mg, 2 mg/kg telesne mase ali 10 mg/kg telesne mase na vsake 3 tedne. V tej populaciji bolnikov so bili najpogostejši neželeni učinki naslednji: anemija (52 %), navzea (52 %), utrujenost (35 %), diareja (33 %), zaprtost (32 %), bruhanje (28 %), zmanjšanje apetita (28 %), zmanjšano število nevtrofilcev (27 %) in nevtropenija (25 %). Pojavnost neželenih učinkov 3. do 5. stopnje je pri bolnikih z NSCLC pri kombiniranem zdravljenju s pembrolizumabom znašala 69 % in pri zdravljenju samo s kemoterapijo 61 %, pri bolnikih s HNSCC pri kombiniranem zdravljenju s pembrolizumabom 85 % in pri zdravljenju s kemoterapijo v kombinaciji s cetuksimabom 84 %, pri bolnikih z rakom požiralnika pri kombiniranem zdravljenju s pembrolizumabom 86 % in pri zdravljenju samo s kemoterapijo 83 %, pri bolnikih s TNBC pri kombiniranem zdravljenju s pembrolizumabom 80 % in pri zdravljenju samo s kemoterapijo 77 %, pri bolnicah z rakom materničnega vratu pri kombiniranem zdravljenju s pembrolizumabom 82 % in pri zdravljenju s kemoterapijo z ali brez bevacizumaba 75 %, pri bolnikih z rakom želodca pri kombiniranem zdravljenju s pembrolizumabom (kemoterapija z ali brez trastuzumaba) 74 % in pri kemoterapiji v kombinaciji z ali brez trastuzumaba 68 %, in pri bolnikih z rakom biliarnega trakta pri kombiniranem zdravljenju s pembrolizumabom 85 % in pri samostojni kemoterapiji 84 %. Varnost pembrolizumaba v kombinaciji z aksitinibom ali lenvatinibom pri napredovalem RCC in v kombinaciji z lenvatinibom pri napredovalem EC so ocenili pri skupno 1456 bolnikih z napredovalim RCC ali napredovalim EC, ki so v kliničnih študijah prejemali 200 mg pembrolizumaba na 3 tedne skupaj s 5 mg aksitiniba dvakrat na dan ali z 20 mg lenvatiniba enkrat na dan, kot je bilo ustrezno. V teh populacijah bolnikov so bili najpogostejši neželeni učinki diareja (58 %), hipertenzija (54 %), hipotiroidizem (46 %), utrujenost (41 %), zmanjšan apetit (40 %), navzea (40 %), artralgija (30 %), bruhanje (28 %), zmanjšanje telesne mase (28 %), disfonija (28 %), bolečine v trebuhu (28 %), proteinurija (27 %), sindrom palmarno-plantarne eritrodizestezije (26 %), izpuščaj (26 %), stomatitis (25 %), zaprtost (25 %), mišično-skeletna bolečina (23 %), glavobol (23 %) in kašelj (21 %). Neželenih učinkov od 3. do 5. stopnje je bilo pri bolnikih z RCC med uporabo pembrolizumaba v kombinaciji z aksitinibom ali lenvatinibom 80 % in med uporabo sunitiniba samega 71 %. Pri bolnicah z EC je bilo neželenih učinkov od 3. do 5. stopnje med uporabo pembrolizumaba v kombinaciji z lenvatinibom 89 % in med uporabo kemoterapije same 73 %. Za celoten seznam neželenih učinkov, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. Za dodatne informacije o varnosti v primeru uporabe pembrolizumaba v kombinaciji glejte povzetke glavnih značilnosti zdravila za posamezne komponente kombiniranega zdravljenja. • **Način in režim izdaje zdravila:** H – Predpisovanje in izdaja zdravila je le na recept, zdravilo se uporablja samo v bolnišnicah. • **Imetnik dovoljenja za promet z zdravilom:** Merck Sharp & Dohme B.V. , Waarderweg 39, 2031 BN Haarlem, Nizozemska.

Merck Sharp & Dohme inovativna zdravila d.o.o.
Ameriška ulica 2, 1000 Ljubljana; tel: +386 1/ 520 42 01, fax: +386 1/ 520 43 50

Samo za strokovno javnost. | H - Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila Keytruda, ki je na voljo pri naših strokovnih sodelavcih ali na lokalnem sedežu družbe.

Zdravilo Columvi▼ je kot monoterapija indicirano za zdravljenje odraslih bolnikov s ponovljenim ali neodzivnim difuznim velikoceličnim limfomom B, ki so prejeli vsaj dve predhodni liniji sistemskega zdravljenja.[1]



COLUMVI®
glofitamab

Reference: 1 Povzetek glavnih značilnosti zdravila Columvi, dostopano decembra 2023 na https://www.ema.europa.eu/sl/documents/product-information/columvi-epar-product-information_sl.pdf

▼Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila. Kako poročati o neželenih učinkih, si poglejte skrajšani povzetek glavnih značilnosti zdravila pod „Poročanje o domnevnih neželenih učinkih".

Ime zdravila: Columvi 2,5 mg in 10 mg koncentrat za raztopino za infundiranje **Kakovostna in količinska sestava:** Ena viala z 2,5 ml/10 ml koncentrata vsebuje 2,5 mg/10 mg glofitamaba v koncentraciji 1 mg/mL. Glofitamab je humanizirano bispecifično monoklonsko protitelo, usmerjeno proti CD20/proti CD3. **Terapevtske indikacije:** Zdravilo Columvi je kot monoterapija indicirano za zdravljenje odraslih bolnikov s ponovljenim ali neodzivnim difuznim velikoceličnim limfomom B (DVCLB), ki so prejeli vsaj dve predhodni liniji sistemskega zdravljenja. **Odmerjanje in način uporabe:** Zdravilo Columvi se sme uporabljati le pod nadzorom zdravnika, izkušenega na področju diagnosticiranja in zdravljenja onkoloških bolnikov, ki lahko zagotavlja ustrezno zdravstveno obravnavo za obvladovanje hudih reakcij, povezanih s sindromom sproščanja citokinov (cytokine release syndrome CRS). Odmerjanje: Zdravilo Columvi je treba dajati v intravenski infuziji po shemi za postopno povečevanje odmerka do doseženega priporočenega odmerka 30 mg (opisano v SmPC v preglednici 2), po končanem predhodnem zdravljenju z obinutuzumabom. Vsak cikel traja 21 dni. Vse bolnike je treba seznaniti s tveganjem za pojav CRS, jih seznaniti z njegovimi znaki in simptomi ter jim naročiti, naj se v primeru pojava teh znakov in simptomov nemudoma posvetujejo z lečečim zdravnikom. Trajanje zdravljenja: Zdravljenje z zdravilom Columvi je priporočljivo izvajati največ 12 ciklov ali do napredovanja bolezni ali do pojava nesprejemljivih toksičnih učinkov. Prilagoditev odmerka: Zmanjšanja odmerka zdravila Columvi niso priporočena. Način uporabe: Zdravilo Columvi je namenjeno le za intravensko uporabo. Zdravilo Columvi mora pred intravensko uporabo razredčiti zdravstveni delavec ob upoštevanju aseptičnega postopka. Zdravilo je treba dati v intravenski infuziji po namenski infuzijski liniji. Za navodila o redčenju zdravila Columvi pred uporabo glejte SmPC. **Kontraindikacije:** Preobčutljivost na učinkovino, obinutuzumab ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** CD20-negativna bolezen: Na voljo je malo podatkov o bolnikih s CD20-negativnim DVCLB, ki so se zdravili z zdravilom Columvi. Sindrom sproščanja citokinov: Pri bolnikih, ki so prejemali zdravilo Columvi, so poročali o pojavu CRS, vključno z življenje ogrožajočimi reakcijami. Večina primerov CRS se je pojavila po prvem odmerku zdravila Columvi. Pred infundiranjem zdravila Columvi v 1. in 2. ciklu mora biti na voljo vsaj 1 odmerek tocilizumaba za uporabo v primeru pojava CRS. Bolnike je treba spremljati ob vsakem infundiranju zdravila Columvi in še vsaj 10 ur po koncu prvega infundiranja. Pri bolnikih je treba izključiti druge morebitne vzroke zvišane telesne temperature, hipoksije in hipotenzije, na primer okužbe ali sepso. CRS je treba obravnavati glede na bolnikovo klinično sliko in v skladu s priporočili za vodenje CRS. Kartica za bolnika: Bolniku je treba izročiti kartico za bolnika in mu naročiti, naj jo ima vedno pri sebi. Medsebojno delovanje s substrati CYP450: Začetno sproščanje citokinov na začetku zdravljenja z zdravilom Columvi lahko zavira encime CYP450 in vodi v nihanja koncentracij sočasno uporabljenih zdravil. Bolnike, ki se zdravijo s substrati CYP450 z ozkim terapevtskim indeksom, je treba po uvedbi zdravljenja z zdravilom Columvi spremljati, saj lahko nihanja koncentracij sočasno uporabljenih zdravil vodijo v toksičnost, izgubo učinkovitost ali neželene dogodke. Resne okužbe: Pri bolnikih, ki so prejemali zdravilo Columvi, so se pojavile resne okužbe. Zdravila Columvi ne smejo prejeti bolniki z aktivno okužbo. Zdravilo Columvi je treba uporabljati previdno pri bolnikih z anamnezo kroničnih ali ponavljajočih se okužb, bolnikih s pridruženimi boleznimi, ki lahko povečajo nagnjenost k okužbam, in bolnikih z intenzivnim predhodnim imunosupresivnim zdravljenjem. Bolnike je treba pred in med zdravljenjem z zdravilom Columvi obravnavati glede morebitnih okužb ter jih ustrezno zdraviti. Bolniku je treba naročiti, naj poišče zdravniško pomoč, če se pri njem pojavijo znaki ali simptomi okužbe. Bolnike s febrilno nevtropenijo je treba obravnavati glede okužbe in jih takoj zdraviti. Zagon tumorja: Pri bolnikih, ki so prejemali zdravilo Columvi, so poročali o zagonu tumorja. Med znaki in simptomi sta bila navedena lokalna bolečina in oteklina. Zagon tumorja ne pomeni neuspeha zdravljenja ali napredovanja tumorja. Vendar pa obstaja tveganje za prizadetost in smrt bolnika zaradi učinka mase tumorja ob zagonu tumorja pri bolnikih z obsežnimi tumorji, ki se nahajajo v neposredni bližini dihalnih poti in/ali vitalnih organov. Pri bolnikih, ki prejemajo zdravilo Columvi, je priporočljivo spremljati in ocenjevati kritična anatomska mesta glede pojava zagona tumorja in jih zdraviti, kot je klinično indicirano. Za zdravljenje zagona tumorja pridejo v poštev kortikosteroidi in analgetiki. Sindrom razpada tumorja: Pri bolnikih, ki so prejemali zdravilo Columvi, so poročali o sindromu razpada tumorja. Bolniki z velikim tumorskim bremenom, hitro rastočimi tumorji, motenim delovanjem ledvic ali dehidracijo imajo večje tveganje za pojav sindroma razpada tumorja. Bolnike s tveganjem je treba natančno spremljati z ustreznimi preiskavami elektrolitskega stanja, hidracije in delovanja ledvic. Pred predhodnim zdravljenjem z obinutuzumabom in pred infundiranjem zdravila Columvi presodite o uporabi ustreznih preventivnih ukrepov. Med ukrepe v primeru sindroma razpada tumorja spadajo agresivna hidracija, korekcija elektrolitskih motenj, antihiperurikemiki in podporno zdravljenje. Cepljenje: Cepljenje z živimi cepivi med zdravljenjem z zdravilom Columvi ni priporočljivo. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Začetno sproščanje citokinov ob uvedbi zdravljenja z zdravilom Columvi lahko zavira delovanje encimov CYP450. Tveganje za medsebojno delovanje zdravil je največje znotraj enega tedna po vsakem od prvih 2 odmerkov zdravila Columvi pri bolnikih, ki sočasno prejemajo substrate CYP450 z ozkim terapevtskim indeksom. Bolnike, ki se zdravijo s substrati CYP450 z ozkim terapevtskim indeksom, je treba po uvedbi zdravljenja z zdravilom Columvi spremljati. **Neželeni učinki:** Najpogostejši neželeni učinki (> 20 %) so bili sindrom sproščanja citokinov, nevtropenija, anemija, trombocitopenija in izpuščaj. Najpogostejši resni neželeni učinki so bili sindrom sproščanja citokinov, sepsa, COVID-19, zagon tumorja, pljučnica COVID-19, febrilna nevtropenija, nevtropenija in plevralni izliv. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Javna agencija Republike Slovenije za zdravila in medicinske pripomočke, Sektor za farmakovigilanco, Nacionalni center za farmakovigilanco, Slovenčeva ulica 22, SI-1000 Ljubljana, Tel: +386 (0)8 2000 500, Faks: +386 (0)8 2000 510, e-pošta: h-farmakovigilanca@jazmp.si, spletna stran: www.jazmp.si. Za zagotavljanje sledljivosti zdravila je pomembno, da pri izpolnjevanju obrazca o domnevnih neželenih učinkih zdravila navedete številko serije biološkega zdravila. **Režim izdaje zdravila:** H **Imetnik dovoljenja za promet:** Roche Registration GmbH, Emil-Barell-Strasse 1, 79639 Grenzach-Wyhlen, Nemčija **Verzija: 1.0/23**

Roche

Samo za strokovno javnost
DODATNE INFORMACIJE SO NA VOLJO PRI: Roche farmacevtska družba d.o.o., Stegne 13G, 1000 Ljubljana

Datum priprave informacije: december 2023
M-SI-00001083(v1.0)

Širimo obzorja v 3. liniji zdravljenja metastatskega kolorektalnega raka (mCRC)

VEČ KOT 10-MESEČNO CELOKUPNO PREŽIVETJE

Lonsurf® v kombinaciji z bevacizumabom je pokazal edinstvene rezultate pri zdravljenju mCRC v 3. liniji, saj je bila prvič dosežena mediana celokupnega preživetja (mOS) 10,8 meseca, s skoraj polovico živih bolnikov po enem letu in v dobri kondiciji za nadaljnje zdravljenje.[1]

Lonsurf®
trifluridin/tipiracil

Usmerjen v prihodnost

Literatura: 1. Prager GW et al. N Engl J Med 2023;388:1657-67.

Družba Servier ima licenco družbe Taiho za zdravilo Lonsurf®. Pri globalnem razvoju zdravila sodelujeta obe družbi in ga tržita na svojih določenih področjih.

Skrajšan povzetek glavnih značilnosti zdravila: Lonsurf 15 mg/6,14 mg filmsko obložene tablete in Lonsurf 20 mg/8,19 mg filmsko obložene tablete

SESTAVA*: Lonsurf 15 mg/6,14 mg: Ena filmsko obložena tableta vsebuje 15 mg trifluridina in 6,14 mg tipiracila (v obliki klorida). **Lonsurf 20 mg/8,19 mg**: Ena filmsko obložena tableta vsebuje 20 mg trifluridina in 8,19 mg tipiracila (v obliki klorida). **TERAPEVTSKE INDIKACIJE*:** V kombinaciji z bevacizumabom za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (KRR), ki so prejeli dva predhodna režima zdravljenja raka, vključno s kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF – Vascular Endothelial Growth Factor) in/ali zaviralci receptorjev za epidermalni rastni dejavnik (EGFR – Epidermal Growth Factor Receptor). V monoterapiji za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom, ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci VEGF in zaviralci EGFR. V monoterapiji za zdravljenje odraslih bolnikov z metastatskim rakom želodca, vključno z adenokarcinomom gastro-ezofagealnega prehoda, ki so bili predhodno že zdravljeni z najmanj dvema sistemskima režimoma zdravljenja za napredovalo bolezen. **ODMERJANJE IN NAČIN UPORABE*:** Priporočeni začetni odmerek zdravila Lonsurf pri odraslih je 35 mg/m^2/odmerek peroralno dvakrat dnevno na 1. do 5. dan in 8. do 12. dan vsakega 28dnevnega cikla zdravljenja, najpozneje 1 uro po zaključku jutranjega in večernega obroka (20 mg/m^2/odmerek dvakrat dnevno pri bolnikih s hudo ledvično okvaro). Odmerek, izračunan glede na telesno površino, ne sme preseči 80 mg/odmerek. Možne prilagoditve odmerka glede na varnost in prenašanje zdravila pri posameznem bolniku: dovoljena so zmanjšanja odmerka na najmanjši odmerek 20 mg/m^2 dvakrat dnevno (oz. 15 mg/m^2/odmerek dvakrat dnevno pri bolnikih s hudo ledvično okvaro). Potem ko je bil odmerek zmanjšan, povečanje ni dovoljeno. Kadar se zdravilo Lonsurf uporablja v kombinaciji z bevacizumabom za zdravljenje metastatskega KRR, je odmerek bevacizumaba 5 mg/kg telesne mase enkrat na 2 tedna. **KONTRAINDIKACIJE*:** Preobčutljivost na učinkovini ali katero koli pomožno snov. **OPOZORILA IN PREVIDNOSTNI UKREPI*:** Supresija kostnega mozga: Pred uvedbo zdravljenja in po potrebi za spremljanje toksičnosti zdravila, najmanj pred vsakim ciklom zdravljenja, je treba pregledati celotno krvno sliko. Zdravljenja ne smete začeti, če je absolutno število nevtrofilcev < 1,5 x 10^9/l, če je število trombocitov < 75 x 10^9/l ali če se je pri bolniku zaradi predhodnih zdravljenj pojavila klinično pomembna nehematološka toksičnost 3. ali 4. stopnje, ki še traja. Bolnike je treba skrbno spremljati zaradi morebitnih okužb, uvesti je treba ustrezne ukrepe, kot je klinično indicirano. Toksičnost za prebavila: Potrebna je uporaba antiemetikov, antidiaroikov ter drugih ukrepov, kot je klinično indicirano. Če je potrebno, prilagodite odmerke. Ledvična okvara: Uporaba zdravila ni priporočljiva pri bolnikih s končno stopnjo ledvične bolezni. Bolnike z ledvično okvaro je potrebno med zdravljenjem skrbno spremljati; bolnike z zmerno ali hudo ledvično okvaro je treba zaradi hematološke toksičnosti bolj pogosto spremljati. Jetrna okvara: Uporaba zdravila Lonsurf pri bolnikih z obstoječo zmerno ali hudo jetrno okvaro ni priporočljiva. Proteinurija: Pred začetkom zdravljenja in med njim je priporočljivo spremljanje proteinurije z urinskimi testnimi lističi. Pomožne snovi: Zdravilo vsebuje laktozo. **INTERAKCIJE*:** Previdnost: Zdravila, ki medsebojno delujejo z nukleozidnimi prenašalci CNT1, ENT1 in ENT2, zaviralci OCT2 ali MATE1, substrati humane timidin-kinaze (npr. zidovudin), hormonski kontraceptivi. **PLODNOST*.** Bolnikom, ki želijo spočeti otroka, je treba svetovati, da se odločijo za svetovanje o reprodukciji ter shranjevanje jajčnih celic oz. sperme z zamrzovanjem pred začetkom zdravljenja z zdravilom Lonsurf. **NOSEČNOST IN DOJENJE*:** Ni priporočljivo. **KONTRACEPCIJA*:** Ženske in moški morajo uporabljati zelo učinkovite metode kontracepcije med zdravljenjem in do 6 mesecev po zaključku zdravljenja. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA STROJEV*:** Med zdravljenjem se lahko pojavijo utrujenost, omotica ali splošno slabo počutje. **NEŽELENI UČINKI*:** *Zelo pogosti:* nevtropenija, levkopenija, anemija, trombocitopenija, zmanjšan apetit, diareja, navzea, bruhanje, utrujenost, stomatitis. *Pogosti:* okužba spodnjih dihal, okužba, febrilna nevtropenija, limfopenija, hipoalbuminemija, disgevzija, omotica, glavobol, hipertenzija, dispneja, bolečina v trebuhu, zaprtje, razjede v ustih, bolezni ustne votline, hiperbilirubinemija, izpuščaj, artralgija, mialgija, alopecija, pruritus, suha koža, proteinurija, pireksija, edem, vnetje sluznice, splošno slabo počutje, zvišanje jetrnih encimov, zvišanje alkalne fosfataze v krvi, zmanjšanje telesne mase. *Občasni:* okužba žolčevoda, gripa, okužba sečil, gingivitis, herpes zoster, okužba s kandido, bakterijska okužba, nevtropenična sepsa, okužba zgornjih dihal, konjunktivitis, bolečina zaradi raka, pancitopenija, monocitopenija, eritropenija, levkocitoza, monocitoza, dehidracija, hiperglikemija, hiperkaliemija, hipokaliemija, hipofosfatemija, hiponatriemija, hipokalciemija, anksioznost, nespečnost, periferna nevropatija, nevrotoksičnost, parestezija, letargija, vrtoglavica, angina pektoris, aritmija, palpitacije, hipotenzija, vročinski oblivi, pljučna embolija, disfonija, epistaksa, izcedek iz nosu, kašelj, krvavitev v prebavilih, ileus, kolitis, gastritis, moteno praznjenje želodca, abdominalna distenzija, analno vnetje, dispepsija, gastroezofagealna refluksna bolezen, glositis, bolezen zob, siljenje na bruhanje, flatulenca, hepatotoksičnost, sindrom palmarne-plantarne eritrodisestezije, urtikarija, akne, hiperhidroza, bolezni nohtov, bolečina v kosteh, mišična oslabelost, mišični krči, bolečina v okončinah, ledvična odpoved, motnje mikcije, hematurija, motnje menstruacije, poslabšanje splošnega zdravstvenega stanja, bolečina, občutek spremembe telesne temperature, neugodje v okončinah, zvišanje kreatinina v krvi, povečanje mednarodnega umerjenega razmerja (INR), zvišanje sečnine v krvi, zvišanje laktatne dehidrogenaze v krvi, zvišanje C-reaktivnega proteina, zmanjšan hematokrit. *Redki:* infekcijski enteritis, tinea pedis, septični šok, granulocitopenija, putika, hipernatriemija, pekoč občutek, disestezija, hiperestezija, hipoestezija, sinkopa, katarakta, suho oko, zamegljen vid, diplopija, zmanjšana ostrina vida, neugodje v ušesu, embolija, orofaringealna bolečina, plevralni izliv, ascites, akutni pankreatitis, subileus, slab zadah, bukalni polip, hemoragični enterokolitis, krvavitev dlesni, ezofagitis, parodontalna bolezen, proktalgija, refluksni gastritis, razširitev žolčnih vodov, mehur, eritem, preobčutljivostne reakcije na svetlobo, luščenje kože, otekanje sklepov, neinfektivni cistitis, levkociturija, kseroza, podaljšanje aktiviranega parcialnega tromboplastinskega časa, podaljšanje intervala QT na elektrokardiogramu, znižanje celokupnih proteinov. *Post-marketinške izkušnje:* intersticijska bolezen pljuč. **PREVELIKO ODMERJANJE***: Neželeni učinki, o katerih so poročali v povezavi s prevelikim odmerjanjem, so bili v skladu z uveljavljenim varnostnim profilom. Glavni pričakovani zaplet prevelikega odmerjanja je supresija kostnega mozga. **FARMAKODINAMIČNE LASTNOSTI*:** *Farmakoterapevtska skupina:* zdravila z delovanjem na novotvorbe, antimetaboliti, oznaka ATC: L01BC59. Zdravilo Lonsurf sestavljata antineoplastični timidinski nukleozidni analog, trifluridin, in zaviralec timidin-fosforilaze (TPaze), tipiracilijev klorid. Po privzemu v rakave celice timidin-kinaza fosforilira trifluridin. Ta se v celicah nato presnovi v substrat deoksiribonukleinske kisline (DNA), ki se vgradi neposredno v DNA ter tako preprečuje celično proliferacijo. TPaza hitro razgradi trifluridin in njegova presnova po peroralni uporabi je hitra zaradi učinka prvega prehoda, zato je v zdravilo vključen zaviralec TPaze, tipiracilijev klorid. **PAKIRANJE*:** 20 filmsko obloženih tablet. **NAČIN PREDPISOVANJA IN IZDAJE ZDRAVILA:** Rp/Spec - Predpisovanje in izdaja zdravila je le na recept zdravnika specialista ustreznega področja medicine ali od njega pooblaščenega zdravnika. **Imetnik dovoljenja za promet:** Les Laboratoires Servier, 50, rue Carnot, 92284 Suresnes cedex, Francija. Številka dovoljenja za promet z zdravilom: EU/1/16/1096/001 (Lonsurf 15 mg/6,14 mg), EU/1/16/1096/004 (Lonsurf 20 mg/8,19 mg). **Datum zadnje revizije besedila: julij 2023. *Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila. Celoten povzetek glavnih značilnosti zdravila in podrobnejše informacije so na voljo pri: Servier Pharma d.o.o., Podmilščakova ulica 24, 1000 Ljubljana, www.servier.si.**

LON AD1 C1 2023-24. Samo za strokovno javnost. Datum priprave informacije: avgust 2023.

SERVIER
moved by you

Instructions for authors

The editorial policy

Radiology and Oncology is a multidisciplinary journal devoted to the publishing original and high-quality scientific papers and review articles, pertinent to oncologic imaging, interventional radiology, nuclear medicine, radiotherapy, clinical and experimental oncology, radiobiology, medical physics, and radiation protection. Papers on more general aspects of interest to the radiologists and oncologists are also published (no case reports).

The Editorial Board requires that the paper has not been published or submitted for publication elsewhere; the authors are responsible for all statements in their papers. Accepted cannot be published elsewhere without the written permission of the editors.

Submission of the manuscript

The manuscript written in English should be submitted to the journal via online submission system Editorial Manager available for this journal at: www.radioloncol.com.

In case of problems, please contact Sašo Trupej at saso.trupej@computing.si or the Editor of this journal at gsersa@onko-i.si

All articles are subjected to the editorial review and when the articles are appropriated they are reviewed by independent referees. In the cover letter, which must accompany the article, the authors are requested to suggest 3-4 researchers, competent to review their manuscript. However, please note that this will be treated only as a suggestion; the final selection of reviewers is exclusively the Editor's decision. The authors' names are revealed to the referees, but not vice versa.

Manuscripts which do not comply with the technical requirements stated herein will be returned to the authors for the correction before peer-review. The editorial board reserves the right to ask authors to make appropriate changes of the contents as well as grammatical and stylistic corrections when necessary. Page charges will be charged for manuscripts exceeding the recommended length, as well as additional editorial work and requests for printed reprints.

Articles are published printed and on-line as the open access:

(https://content.sciendo.com/raon).

All articles are subject to 1500 EUR + VAT publication fee. Exceptionally, waiver of payment may be negotiated with editorial office, at the time of article submission.

Manuscripts submitted under multiple authorship are reviewed on the assumption that all listed authors concur in the submission and are responsible for its content; they must have agreed to its publication and have given the corresponding author the authority to act on their behalf in all matters pertaining to publication. The corresponding author is responsible for informing the co-authors of the manuscript status throughout the submission, review, and production process.

Preparation of manuscripts

Radiology and Oncology will consider manuscripts prepared according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals by International Committee of Medical Journal Editors (www.icmje.org). The manuscript should be written in grammatically and stylistically correct language. Abbreviations should be avoided. If their use is necessary, they should be explained at the first time mentioned. The technical data should conform to the SI system. The manuscript, excluding the references, tables, figures and figure legends, must not exceed 5000 words, and the number of figures and tables is limited to 8. Organize the text so that it includes: Introduction, Materials and methods, Results and Discussion. Exceptionally, the results and discussion can be combined in a single section. Start each section on a new page, and number each page consecutively with Arabic numerals. For ease of review, manuscripts should be submitted as a single column, double-spaced text. The template for preparation of the manuscript is available in the editorial manager.

The Title page should include a concise and informative title, followed by the full name(s) of the author(s); the institutional affiliation of each author; the name and address of the corresponding author (including telephone, fax and E-mail), and an abbreviated title (not exceeding 60 characters). This should be followed by the abstract page, summarizing in less than 250 words the reasons for the study, experimental approach, the major findings (with specific data if possible), and the principal conclusions, and providing 3-6 key words for indexing purposes. Structured abstracts are required. Slovene authors are requested to provide title and the abstract in Slovene language in a separate file. The text of the research article should then proceed as follows:

Introduction should summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Materials and methods should provide enough information to enable experiments to be repeated. New methods should be described in details.

Results should be presented clearly and concisely without repeating the data in the figures and tables. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should discuss the results of the study in the light of previously published work.

Charts, Illustrations, Images and Tables

Charts, Illustrations, Images and Tables must be numbered and referred to in the text, with the appropriate location indicated. Charts, Illustrations and Images, provided electronically, should be of appropriate quality for good reproduction. Illustrations and charts must be vector image, created in CMYK color space, preferred font "Century Gothic", and saved as .AI, .EPS or .PDF format. Color charts, illustrations and Images are encouraged, and are published without additional charge. Image size must be 2.000 pixels on the longer side and saved as .JPG (maximum quality) format. In Images, mask the identities of the patients. Tables should be typed double-spaced, with a descriptive title and, if appropriate, units of numerical measurements included in the column heading. The files with the figures and tables can be uploaded as separate files.

References

References must be numbered in the order in which they appear in the text and their corresponding numbers quoted in the text. Authors are responsible for the accuracy of their references. References to the Abstracts and Letters to the Editor must be identified as such. Citation of papers in preparation or submitted for publication, unpublished observations, and personal communications should not be included in the reference list. If essential, such material may be incorporated in the appropriate place in the text. References follow the style of Index Medicus, DOI number (if exists) should be included.

All authors should be listed when their number does not exceed six; when there are seven or more authors, the first six listed are followed by "et al.". The following are some examples of references from articles, books and book chapters:

Dent RAG, Cole P. In vitro maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95. doi: 10.1038/bjc.1981.71

Chapman S, Nakielny R. *A guide to radiological procedures*. London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

Authorization for the use of human subjects or experimental animals

When reporting experiments on human subjects, authors should state whether the procedures followed the Helsinki Declaration. Patients have the right to privacy; therefore, the identifying information (patient's names, hospital unit numbers) should not be published unless it is essential. In such cases the patient's informed consent for publication is needed, and should appear as an appropriate statement in the article. Institutional approval and Clinical Trial registration number is required. Retrospective clinical studies must be approved by the accredited Institutional Review Board/Committee for Medical Ethics or other equivalent body. These statements should appear in the Materials and methods section.

The research using animal subjects should be conducted according to the EU Directive 2010/63/EU and following the Guidelines for the welfare and use of animals in cancer research (Br J Cancer 2010; 102: 1555 – 77). Authors must state the committee approving the experiments, and must confirm that all experiments were performed in accordance with relevant regulations.

These statements should appear in the Materials and methods section (or for contributions without this section, within the main text or in the captions of relevant figures or tables).

Transfer of copyright agreement

For the publication of accepted articles, authors are required to send the License to Publish to the publisher on the address of the editorial office. A properly completed License to Publish, signed by the Corresponding Author on behalf of all the authors, must be provided for each submitted manuscript.

Conflict of interest

When the manuscript is submitted for publication, the authors are expected to disclose any relationship that might pose real, apparent or potential conflict of interest with respect to the results reported in that manuscript. Potential conflicts of interest include not only financial relationships but also other, non-financial relationships. In the Acknowledgement section the source of funding support should be mentioned. The Editors will make effort to ensure that conflicts of interest will not compromise the evaluation process of the submitted manuscripts; potential editors and reviewers will exempt themselves from review process when such conflict of interest exists. The statement of disclosure must be in the Cover letter accompanying the manuscript or submitted on the form available on www.icmje.org/coi_disclosure.pdf

The use and explanation of AI and AI-assisted technologies in scientific writing

When authors use artificial intelligence (AI) and AI-assisted technologies in the writing process, they should consider that:

- These technologies should only be used to improve readability and language and to assist in the investigation of data. They should not replace researchers' primary tasks of explaining, the interpretation of data and drawing valid scientific conclusions.
- Apply the technology under human supervision and control, and carefully review and edit the result, because AI can produce authoritative-sounding results that may be incorrect, incomplete, or biased.
- Do not list AI or AI-assisted technologies as authors or co-authors, and do not cite AI as an author. Authorship carries with it responsibilities and tasks that only humans can perform.
- Disclose in your manuscript the use of AI and AI-enabled technologies in the writing process by following the instructions below. An appropriate statement should appear in the published paper. Please note that authors are ultimately responsible and accountable for the content of the paper.

Disclosure notes

Authors must disclose the use of AI and AI-assisted technologies in the writing process by adding a statement at the end of their manuscript in the main manuscript file before the bibliography, at the time of manuscript submission. The statement should be included in a new section titled 'Statement on the Use of AI and AI-Assisted Technologies in the Writing Process'.

Statement: during the preparation of this paper, the author(s) used [NAME TOOL / SERVICE] to create [REASON]. After using this tool/service, the author(s) have reviewed and edited the content as required and take full responsibility for the content of the publication.

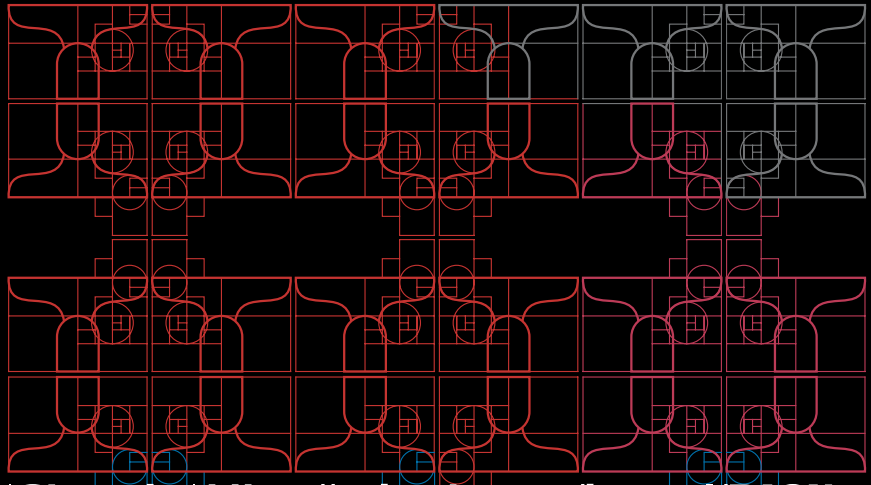
This statement does not apply to the use of basic tools to check grammar, spelling, references, etc. If there is nothing to disclose, it is not necessary to add a statement.

Page proofs

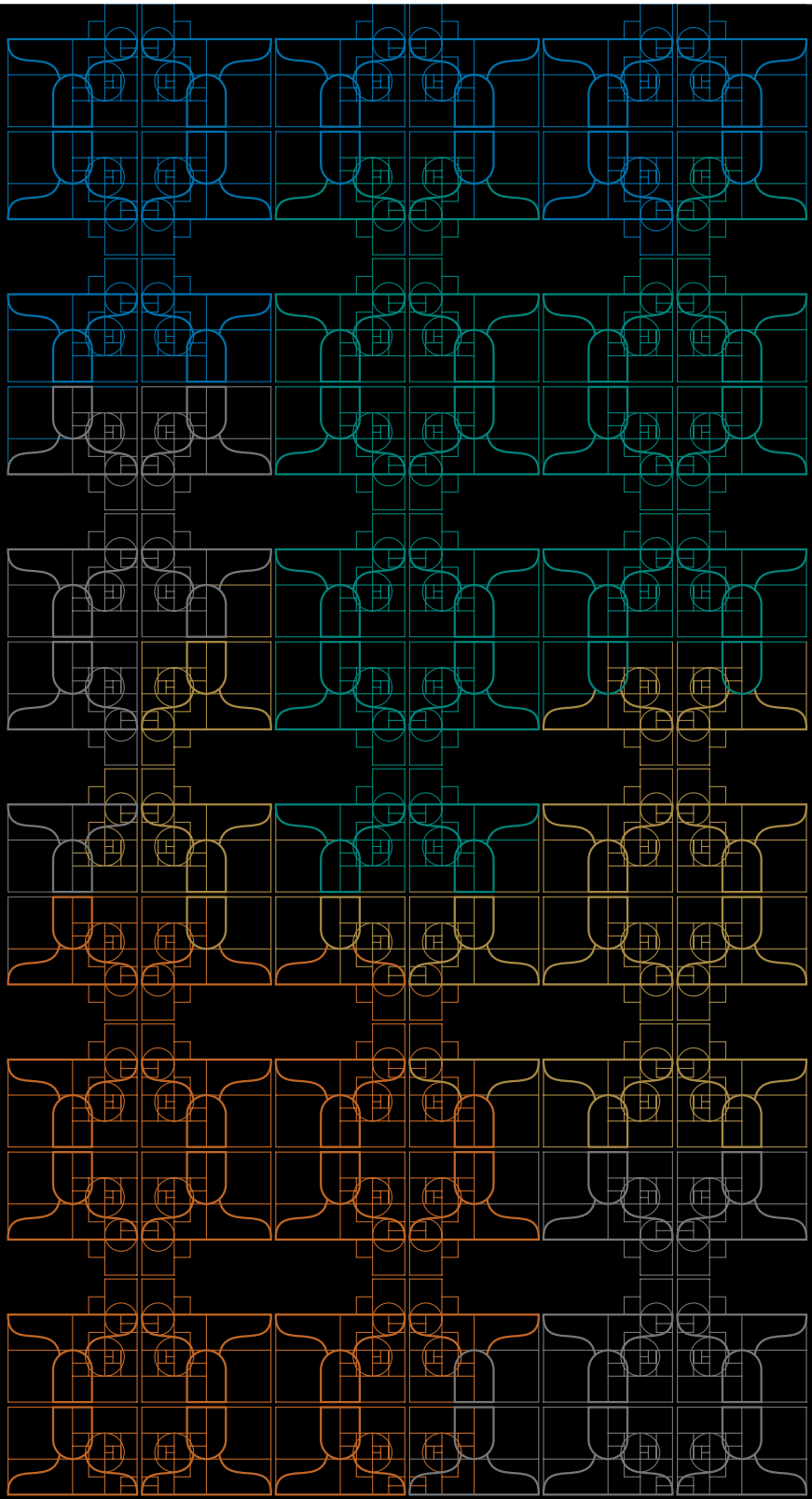
Page proofs will be sent by E-mail to the corresponding author. It is their responsibility to check the proofs carefully and return a list of essential corrections to the editorial office within three days of receipt. Only grammatical corrections are acceptable at that time.

Open access

Papers are published electronically as open access on https://content.sciendo.com/raon, also papers accepted for publication as E-ahead of print.



Radiology and Oncology | Ljubljana | Slovenia | https://sciendo.com/journal/RAON



ISSN 1318 - 2099