# RADIOLOGY AND ONCOLOGY

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# NOVO

# CABOMETYX®
## (kabozantinib) tablete
**60 mg | 40 mg | 20 mg**

- **PFS**[2]
- **OS**[2]
- **ORR**[2]

## CABOMETYX® pomembno izboljša PFS, OS in ORR v drugi liniji zdravljenja napredovalega karcinoma ledvičnih celic[1]

## RAZŠIRITEV INDIKACIJE:

**Sedaj tudi za zdravljenje napredovalega karcinoma ledvičnih celic (KLC) pri predhodno nezdravljnenih odraslih bolnikih s srednje ugodnim ali slabim prognostičnim obetom.[2]**

ORR: objektivna stopnja odziva; OS: celokupno preživetje; PFS: preživetje brez napredovanja bolezni


**Referenci:**
**1.** Choueiri TK, Escudier B, Powles T, *et al.* Cabozantinib versus everolimus in advanced renal cell carcinoma (METEOR): final results from a randomised, open-label, phase 3 trial. The Lancet Oncology. 2016;17(7):917-27.
**2.** Povzetek glavnih značilnosti zdravila Cabometyx.


## Skrajšan povzetek glavnih značilnosti zdravila

**CABOMETYX 20 mg filmsko obložene tablete**
**CABOMETYX 40 mg filmsko obložene tablete**
**CABOMETYX 60 mg filmsko obložene tablete**
(kabozantinib)

▼ 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.

**TERAPEVTSKE INDIKACIJE** Zdravljenje napredovalega karcinoma ledvičnih celic (KLC) pri predhodno nezdravljenih odraslih bolnikih s srednje ugodnim ali slabim prognostičnim obetom ter pri odraslih bolnikih po predhodnem zdravljenju, usmerjenem v vaskularni endotelijski rastni faktor (VEGF). V monoterapiji zdravljenje hepatocelularnega karcinoma (HCK) pri odraslih bolnikih, ki so se predhodno že zdravili s sorafenibom. **ODMERJANJE IN NAČIN UPORABE** Pri bolnikih s KLC in HKC je priporočeni odmerek 60 mg enkrat na dan. Zdravljenje je treba nadaljevati tako dolgo, dokler bolnik več nima kliničnih koristi od terapije ali do pojava nesprejemljive toksičnosti. Pri sumu na neželene reakcije na zdravilo bo morda treba zdravljenje začasno prekiniti in/ali zmanjšati odmerek. Če je treba odmerek zmanjšati, se priporoča zmanjšanje na 40 mg na dan in nato na 20 mg na dan. Prekinitev odmerka se priporoča pri obravnavi toksičnosti 3. ali višje stopnje po CTCAE (*common terminology criteria for adverse events*) ali nevzdržni toksičnosti 2. stopnje. Zmanjšanje odmerka se priporoča za dogodke, ki bi lahko čez čas postali resni ali nevzdržni. V primeru pojava neželenih učinkov 1. in 2. stopnje, ki jih bolnik prenaša in jih je možno enostavno obravnavati, prilagoditev odmerjanja običajno ni potrebna. Treba je uvesti podporno oskrbo. V primeru pojava neželenih učinkov 2. stopnje, ki jih bolnik ne prenaša in jih ni mogoče obravnavati z zmanjšanjem odmerka ali podporno oskrbo, je treba zdravljenje prekiniti, dokler neželeni učinki ne izzvenijo do ≤ 1. stopnje, uvesti podporno oskrbo in razmisliti o ponovni uvedbi zdravljenja z zmanjšanim odmerkom. V primeru pojava neželenih učinkov 3. stopnje je treba zdravljenje prekiniti, dokler neželeni učinki ne izzvenijo do ≤ 1. stopnje, uvesti podporno oskrbo in ponovno uvesti zdravljenje z zmanjšanim odmerkom. V primeru pojava neželenih učinkov 4. stopnje je treba zdravljenje prekiniti, uvesti ustrezno zdravniško oskrbo, in če neželeni učinki izzvenijo do ≤ 1. stopnje, ponovno uvesti zdravljenje z zmanjšanim odmerkom. Če neželeni učinki ne izzvenijo, je treba trajno prenehati z uporabo zdravila. Pri bolnikih z blago ali zmerno ledvično okvaro je treba kabozantinib uporabljati previdno. Uporaba se ne priporoča pri bolnikih s hudo ledvično okvaro. Pri bolnikih z blago okvaro jeter odmerka ni treba prilagajati. Pri bolnikih z zmerno okvaro jeter (Child Pugh B) priporočil za odmerjanje ni možno podati. Pri teh bolnikih je priporočljivo skrbno spremljanje celokupne varnosti. Pri bolnikih s hudo okvaro jeter (Child Pugh C) uporaba kabozantiniba ni priporočljiva. Način uporabe: Tablete je treba pogoltniti cele in jih ni dovoljeno drobiti. Bolnikom je treba naročiti, naj vsaj 2 uri pred uporabo zdravila in 1 uro po tem ničesar ne jedo. **KONTRAINDIKACIJE** Preobčutljivost na učinkovino ali katero koli pomožno snov. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI** Večina dogodkov se pojavi zgodaj v teku zdravljenja, zato mora zdravnik bolnika v prvih 8 tednih zdravljenja skrbno spremljati, da oceni, ali je treba odmerek prilagoditi. Dogodki, ki se običajno pojavijo zgodaj, vključujejo hipokalciemijo, hipokaliemijo, trombocitopenijo, hipertenzijo, sindrom palmarno-plantarne eritrodisestezije (PPES), proteinurijo in gastrointestinalne dogodke (bolečine v trebuhu, vnetje sluznice, zaprtje, driska, bruhanje). Pred uvedbo zdravljenja s kabozantinibom je priporočljivo izvesti preiskave delovanja jeter (ALT, AST in bilirubin), vrednosti skrbno spremljati med zdravljenjem in po potrebi prilagoditi odmerek. Bolnike je treba spremljati glede znakov in simptomov jetrne encefalopatije. Bolnike, ki imajo vnetno bolezen črevesja (npr. Crohnovo bolezen, ulcerozni kolitis, peritonitis, divertikulitis ali apendicitis), ki imajo tumorsko infiltracijo prebavil ali so imeli pred posegom na prebavilih zaplete (zlasti v povezavi z zapoznelim ali nepopolnim celjenjem), je treba pred uvedbo zdravljenja skrbno oceniti, nato pa natančno spremljati za pojav simptomov perforacij in fistul, vključno z abscesi in sepso. Trajna ali ponavljajoča se driska med zdravljenjem je lahko dejavnik tveganja za nastanek analne fistule. Uporabo kabozantiniba je treba pri bolnikih, pri katerih se pojavi gastrointestinalna perforacija ali fistula, ki je ni možno ustrezno obravnavati, prekiniti. Driska, navzea/bruhanje, zmanjšanje apetita in vnetje ustne sluznice/bolečina v ustni votlini so nekateri od najpogosteje poročanih neželenih učinkov na prebavila. Nemudoma je treba uvesti ustrezne medicinske ukrepe, vključno s podpornim zdravljenjem z antiemetiki, antidiaroiki ali antacidi, da se prepreči dehidracija, neravnovesje elektrolitov in izguba telesne mase. Če pomembni neželeni učinki na prebavila vztrajajo ali se ponavljajo, je treba presoditi o prekinitvi odmerjanja, zmanjšanju odmerka ali trajni ukinitvi zdravljenja s kabozantinibom. Kabozantinib je treba uporabljati previdno pri bolnikih, pri katerih obstaja tveganje za pojav venske trombembolije, vključno s pljučno embolijo, in arterijske trombembolije ali imajo te dogodke v anamnezi. Z uporabo je treba prenehati pri bolnikih, pri katerih se razvije akutni miokardni infarkt ali drugi klinično pomembni znaki zapletov trombembolije. Kabozantiniba se ne sme dajati bolnikom, ki hudo krvavijo, ali pri katerih obstaja tveganje za hudo krvavitev. Med zdravljenjem s kabozantinibom je treba spremljati vrednosti trombocitov in odmerek prilagoditi glede na resnost trombocitopenije. Zdravljenje s kabozantinibom je treba ustaviti vsaj 28 dni pred načrtovanim kirurškim posegom, vključno z zobozdravstvenim, če je mogoče. Kabozantinib je treba ukiniti pri bolnikih z zapleti s celjenjem rane, zaradi katerih je potrebna zdravniška pomoč. Pred uvedbo kabozantiniba je treba dobro obvladati krvni tlak. Med zdravljenjem je treba vse bolnike spremljati za pojav hipertenzije in jih po potrebi zdraviti s standardnimi antihipertenzivi. V primeru trdovratne hipertenzije, kljub uporabi antihipertenzivov, je treba odmerek kabozantiniba zmanjšati. Z uporabo je treba prenehati, če je hipertenzija resna ali trdovratna kljub zdravljenju z antihipertenzivi in zmanjšanemu odmerku kabozantiniba. V primeru hipertenzijske krize je treba zdravljenje prekiniti. Pri resni PPES je treba razmisliti o prekinitvi zdravljenja. Nadaljevanje zdravljenja naj se začne z nižjim odmerkom, ko se PPES umiri do 1. stopnje. V času zdravljenja je treba redno spremljati beljakovine v urinu. Pri bolnikih, pri katerih se razvije nefrotični sindrom, je treba z uporabo kabozantiniba prenehati. Pri uporabi kabozantiniba so opazili sindrom reverzibilne posteriorne levkoencefalopatije (RPLS), znan tudi kot sindrom posteriorne reverzibilne encefalopatije (PRES). Na ta sindrom je treba pomisliti pri vseh bolnikih s številnimi prisotnimi simptomi, vključno z epileptičnimi napadi, glavobolom, motnjami vida, zmedenostjo ali spremenjenim mentalnim delovanjem. Pri bolnikih z RPLS je treba zdravljenje prekiniti. Kabozantinib je treba uporabljati previdno pri bolnikih s podaljšanjem intervala QT v anamnezi, pri bolnikih, ki jemljejo antiaritmike, in pri bolnikih z relevantno obstoječo boleznijo srca, bradikardijo ali elektrolitskimi motnjami. Uporaba kabozantiniba je bila povezana z večjo pojavnostjo elektrolitskih nepravilnosti (vključno s hipokaliemijo, hiperkaliemijo, hipomagneziemijo, hipokalciemijo in hiponatriemijo), zato je priporočljivo spremljati biokemijske parametre in po potrebi uvesti ustrezno nadomestno zdravljenje v skladu s standardno klinično prakso. Bolniki z redko dedno intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze/galaktoze ne smejo jemati tega zdravila. Plodnost, nosečnost in dojenje: Ženskam v rodni dobi je treba svetovati, da v času zdravljenja s kabozantinibom ne smejo zanositi. Zanositev morajo preprečiti tudi ženske partnerice moških bolnikov, ki uporabljajo kabozantinib. Med zdravljenjem in še vsaj 4 mesece po končanju terapije morajo tako bolniki in bolnice kot tudi njihovi partnerji uporabljati zanesljiv način kontracepcije. Kabozantiniba se ne sme uporabljati med nosečnostjo, razen če zdravljenje ni nujno potrebno zaradi kliničnega stanja ženske. Matere med zdravljenjem s kabozantinibom in še 4 mesece po končanju terapije ne smejo dojiti. Zdravljenje s kabozantinibom lahko predstavlja tveganje za plodnost pri moških in ženskah. **INTERAKCIJE** Kabozantinib je substrat za CYP3A4. Pri sočasni uporabi močnih zaviralcev CYP3A4 (npr. ritonavirja, itrakonazola, eritromicina, klaritromicina, soka grenivke) je potrebna previdnost. Kronični sočasni uporabi močnih induktorjev CYP3A4 (npr. fenitoina, karbamazepina, rifampicina, fenobarbitala ali pripravkov zeliščnega izvora iz šentjanževke) se je treba izogibati. Razmisliti je treba o sočasni uporabi alternativnih zdravil, ki CYP3A4 ne inducirajo in ne zavirajo ali pa inducirajo in zavirajo le neznatno. Pri sočasni uporabi zaviralcev MRP2 (npr. ciklosporin, efavirenz, emtricitabin) je potrebna previdnost, saj lahko povzročijo povečanje koncentracij kabozantiniba v plazmi. Učinka kabozantiniba na farmakokinetiko kontraceptivnih steroidov niso preučili, vendar pa se priporoča dodatna kontracepcijska metoda (pregradna metoda). Zaradi visoke stopnje vezave kabozantiniba na plazemske beljakovine je možna interakcija z varfarinom v obliki izpodrivanja s plazemskih beljakovin, zato je treba spremljati vrednosti INR. Kabozantinib morda lahko poveča koncentracije sočasno uporabljenih substratov P-gp v plazmi. Osebe je treba opozoriti na uporabo substratov P-gp (npr. feksofenadina, aliskirena, ambrisentana, dabigatran eteksilata, digoksina, kolhicina, maraviroka, posakonazola, ranolazina, saksagliptina, sitagliptina, talinolola, tolvaptana) sočasno s kabozantinibom. **NEŽELENI UČINKI** Za popolno informacijo o neželenih učinkih, prosimo, preberite celoten povzetek glavnih značilnosti zdravila Cabometyx. Najpogostejši resni neželeni učinki zdravila v populaciji bolnikov s KLC so bili driska, hipertenzija, dehidracija, hiponatriemija, navzea, zmanjšanje apetita, embolija, utrujenost, hipomagneziemija in PPES. Najpogostejši neželeni učinki katere koli stopnje (ki so se pojavili pri vsaj 25 % bolnikov) v populaciji bolnikov s KLC so bili driska, hipertenzija, utrujenost, zvišanje vrednosti AST, zvišanje vrednosti ALT, navzea, zmanjšanje apetita, PPES, paragevzija, zmanjšanje števila trombocitov, stomatitis, anemija, bruhanje, zmanjšanje telesne mase, dispepsija in konstipacija. Najpogostejši resni neželeni učinki zdravila v populaciji bolnikov s HCK so bili jetrna encefalopatija, PPES, astenija in driska. Najpogostejši neželeni učinki katere koli stopnje (ki so se pojavili pri vsaj 25 % bolnikov) v populaciji bolnikov s HCK so bili driska, PPES, utrujenost, zmanjšanje apetita, hipertenzija in navzea. *Zelo pogosti (≥ 1/10):* anemija, hipotiroidizem, zmanjšan apetit, hipomagneziemija, hipokaliemija, paragevzija, glavobol, omotica, hipertenzija, krvavitev, disfonija, dispneja, kašelj, driska, navzea, bruhanje, stomatitis, konstipacija, bolečine v trebuhu, dispepsija, bolečina v zgornjem predelu trebuha, PPES, izpuščaj, bolečine v okončinah, utrujenost, vnetje sluznice, astenija, periferni edem, zmanjšanje telesne mase, zvišanje vrednosti ALT v serumu, zvišanje vrednosti AST. *Pogosti (≥ 1/100, < 1/10):* absces, trombocitopenija, nevtropenija, dehidracija, hipoalbuminemija, hipofosfatemija, hiponatriemija, hipokalciemija, hiperkaliemija, hiperbilirubinemija, hiperglikemija, hipoglikemija, periferna senzorična nevropatija, tinitus, venska tromboza, arterijska tromboza, pljučna embolija, gastrointestinalna perforacija, fistula, gastroezofagealna refluksna bolezen, hemoroidi, bolečina v ustni votlini, suha usta, jetrna encefalopatija, pruritus, alopecija, suha koža, akneiformni dermatitis, spremembe barve las oz. dlak, mišični krči, artralgija, proteinurija, zvišanje vrednosti ALP v krvi, GGT, kreatinina v krvi, amilaze, lipaze, holesterola v krvi, zmanjšanje števila belih krvnih celic. *Občasni (≥ 1/1.000, < 1/100):* limfopenija, konvulzije, pankreatitis, glosodinija, holestatični hepatitis, osteonekroza čeljusti, zvišanje vrednosti triglicerido v krvi, zapleti z ranami. *Neznana pogostnost (ni mogoče oceniti iz razpoložljivih podatkov):* možganska kap, miokardni infarkt. **Vrsta ovojnine in vsebina:** Plastenka vsebuje 30 filmsko obloženih tablet. **Režim izdaje:** Rp/Spec **Imetnik dovoljenja za promet z zdravilom:** Ipsen Pharma, 65 quai Georges Gorse, 92100 Boulogne-Billancourt, Francija **Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila!**
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# contents

*review*

# KRAS, NRAS, BRAF, HER2 and microsatellite instability in metastatic colorectal cancer - practical implications for the clinician

Vlad-Adrian Afrăsânie[1,2], Mihai Vasile Marinca[1,2], Teodora Alexa-Stratulat[1,2], Bogdan Gafton[1,2], Marius Păduraru[1,2], Anca Maria Adavidoaiei[1], Lucian Miron[1,2], Cristina Rusu[1]

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**Background.** Colorectal cancer is a successful model of genetic biomarker development in oncology. Currently, several predictive or prognostic genetic alterations have been identified and are used in clinical practice. The RAS gene family, which includes KRAS and NRAS act as predictors for anti-epithelial growth factor receptor treatment (anti-EGFR), and it has been suggested that NRAS mutations also play a role in prognosis: patients harboring NRAS alterations have a significantly shorter survival compared to those with wild type tumours. BRAF V600E mutations are rare and occur mostly in tumors located in the ascending colon in elderly female patients. BRAF is instrumental in establishing prognosis: survival is shorter by 10–16 months in BRAF-mutant patients, and BRAF may be a negative prognostic factor for patients who undergo hepatic or pulmonary metastasectomy. Moreover, this mutation is used as a negative predictive factor for anti-EGFR therapies. Two new biomarkers have recently been added to the metastatic colorectal cancer panel: HER2 and microsatellite instability. While HER2 is still being investigated in different prospective studies in order to validate its prognostic role, microsatellite instability already guides clinical decisions in substituted with advanced colorectal cancer.

**Conclusions.** There are current evidences that support using above mentioned genetic biomarkers to better identify the right medicine that is supposed to be used in the right patient. This approach contributes to a more individualized patient-oriented treatment in daily clinical practice.

Key words: metastatic colorectal cancer; KRAS; NRAS; BRAF; HER2; microsatellite instability

## Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common in women.[1] It is also one of the leading causes of death worldwide, accounting for 10% of all cancer deaths. Although screening, addressability and increased awareness have augmented the number of cases in the non-metastatic setting, approximately one in four individuals with CRC will be diagnosed in stage IV.[1,2] As such, a significant part of cancer research has focused on identifying novel therapies and therapeutic advances in the field of metastatic colorectal cancer (mCRC) over the last 20 years have significantly extended overall survival (OS) from 10 months to more than 20 months.[3] A large part of this improvement is due to the approval of new molecular therapies (such as Bevacizumab, Cetuximab, Panitumumab, Aflibercept and Regorafenib) that are given in combination with different classical or modern cytotoxic agents (including Oxaliplatin, Irinotecan Capecitabine or Trifluridine/Tipiracil).[4] Another cornerstone of improved patient management con-

sists of identifying markers and tumor molecular anomalies that predict treatment response and can discriminate between different types of prognosis in such patients. Additionally, because this improvement in survival has also been associated with substantial health care financial burden, appropriate selection of patients for specific treatments is of utmost importance. Currently, there are several biomarkers that help clinicians in making the optimal treatment decision: KRAS, NRAS, BRAF mutations, human epidermal growth factor receptor 2 (HER2) amplification and microsatellite instability (MSI) or mismatch repair (MMR), they all play a significant role in the process, facilitating selection of the right treatment for the right patient. The aim of this review is to provide clinicians with an update on the particular features of these biomarkers. The correlations between demographic, clinical, pathological and molecular characteristics of KRAS, NRAS, BRAF, HER2 and MSI and patient outcome will be presented, together with the role of these assessments in determining mCRC prognosis and treatment personalization.

This paper shows the impact of such biomarkers analyzing the results of clinical trials and their outcomes from the perspective of routine clinical practice.

## Methods

A search algorithm (Figure 1) based on a combination of the terms ''metastatic colorectal cancer'' AND "therapy" OR "treatment" AND "RAS" OR "KRAS" OR "NRAS" AND "BRAF" AND "HER2" AND "MSI" OR "MMR" was used for the search in PubMed and EMBASE, data was gathered from the beginning of the database PubMed and the search was updated until 30th of April 2019. All the studies which analyzed the biomarkers KRAS, NRAS, BRAF, HER2 and MSI, were considered eligible for inclusion in this review. Eligibility criteria for study selection included: 1) clinical studies/trials; and 2) reviews; 3) meta-analysis. The exclusion criteria were: a) articles not within the field of interest of this review: not discussing about clinical, pathological and molecular correlations, predictive factors, prognostic factors of the studied biomarkers; b) editorials, letters to the editor, commentaries, conference proceedings; c) case reports or small case series; d) articles not in English; e) studies not in humans.

Eligible studies reported on patients with metastatic colorectal cancer and included details on KRAS, NRAS, BRAF, HER2 and MSI biomarker status of the tumour, oncological outcomes and type of therapy implemented.

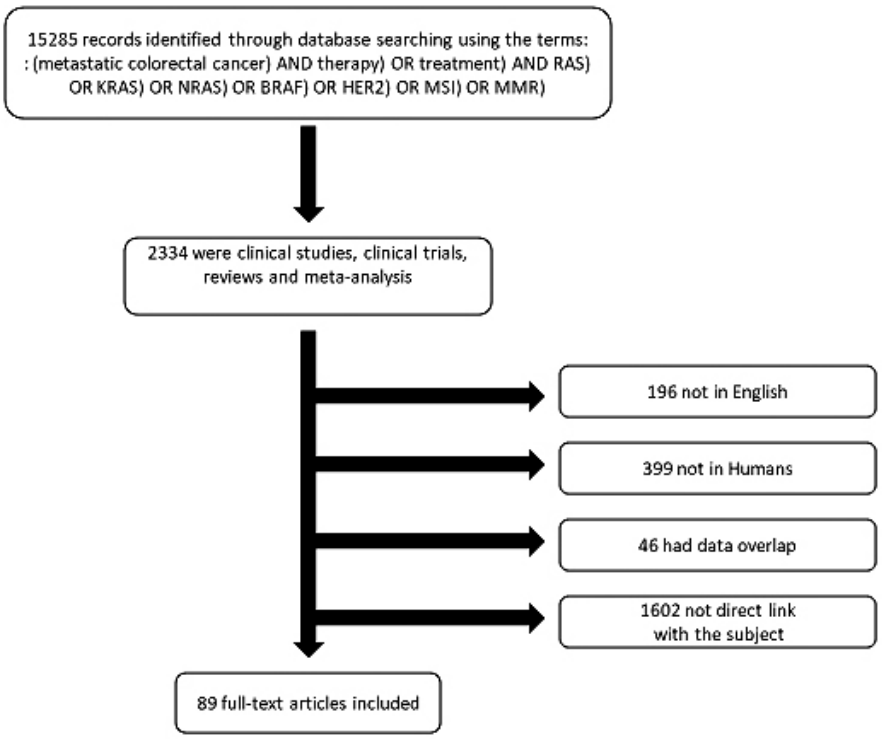


**FIGURE 1.** Flow chart of the search for the eligible studies.

### KRAS: an indispensable biomarker for anti-epidermal growth factor receptor treatment

RAS is a family of proteins expressed in all cell organs belonging to a class of protein called GTPase, and its role is to transmit signals within cells. These signals finally stimulate cell proliferation. RAS regulated signal pathways control processes like cell proliferation, cell differentiation, cell adhesion, apoptosis and cell migration. When they are mutated, the cell will have an increased potential of invasion and metastasis. The main members of the RAS family are KRAS and NRAS mutations.[5-7] These are point mutations in which a single nucleotide base is changed, inserted or deleted from a DNA sequence. Moreover, these are frequently somatic mutations (acquired during lifetime).[8] In the metastatic setting,

KRAS mutations occur in approximately 40% of the cases, especially in exon 2, codons 12 (70–80%) and 13 (15–20%). Different KRAS mutations are mainly located in exon 3, codons 59–61, and in exon 4, codons 117 and 146. In exon 2, mutations are common in codons 12: G12D, G12V and G12C; in exon 3, the affected codons are Q61H and Q61R, while in exon 4 the codons involved are A146T and A146V.[9]

The geographic distribution of RAS mutations is uneven. The distribution of KRAS mutation among clinical trials was 44.7% in Western European countries, 35.8% in Eastern European countries, while 19.5% of the patients were from the Middle East. Also, in one Middle Eastern study by Zekri *et al*., the percentage of mutations ranged from 13% to 45% depending on country.[10-15] Most of the data suggest that geographical distribution is not a significant factor in how such mutations are positioned in the codons. As for racial distribution, certain studies indicated that African-American patients have RAS tumours (68%) more often than Caucasians. It is unclear if such geographic and racial variations are due to genetic background or environmental and lifestyle differences.[16]

Concerning gender, data has been inconclusive. In a meta-analysis by Kafatos *et al.*, RAS mutations were distributed almost equally among men and women: 43.8% in men *vs.* 43.3% in women, P = 0.006.[17] In another study by Kwak *et al*., the rate of mutations was significantly higher in women than in men (46% *vs.* 34.4%, P = 0.03). Codon 12 mutations were more prevalent in women than in men (73.4% *vs.* 66.2%). In the same study, the occurrence of KRAS mutation subtypes did not appear to depend on gender (P = 0.592) and no significant differences were noticed regarding the codon 12 and codon 13 mutations in women versus in men (P = 0.166, P = 0.122).[18]

The full analysis of the RAS gene exons 2, 3 and 4 revealed that these mutations correlate with certain typical clinical, pathological and molecular features, depending on their exact location on exons and codons. For instance, the mutations of exon 2 and codon 12 are associated with the well/moderately differentiated adenocarcinoma subtype and the mucinous subtype.[19]

In terms of where tumours with KRAS mutations are located, findings are inconsistent: some studies have shown that the KRAS mutation does not correlate with the location of the tumour, while others have found that tumours with KRAS mutations occur more frequently in the caecum area. These studies are limited by the lack of clinical and histological data, as well as by their retrospective observational methodology.[18]

Epidermal growth factor receptor (EGFR) is considered a very important component of initiation and progression in colorectal cancer. It is a membrane-bound receptor tyrosine kinase and became a key target for monoclonal antibodies which bind on the extracellular domain of the receptor. The KRAS status is critical for the medical oncologists because it guides the treatment. KRAS mutations have been considered a predictive feature for resistance to EGFR biological therapy, as confirmed by the results of the CRYSTAL and OPUS trials.[20,21]

These indicated that adding Cetuximab to standard chemotherapy protocols such as FOLFOX (regimen of chemotherapy consisting of the next citotoxic agents: Oxaliplatin, 5-Fluorouracil and Folinic acid) and FOLFIRI (regimen of chemotherapy consisting of the next citotoxic agents: Irinotecan, 5-Fluorouracil and Folinic acid) has no benefit in patients with KRAS mutation. However, in patients with RAS wild-type (WT), the addition of Cetuximab resulted in improved prognosis.[22,23] The same conclusions were shown as well in clinical trials with high enrolment number like COIN, NORDIC or PRIME.[24-26] Some trials investigated potential correlations between KRAS mutation and Bevacizumab efficacy, but with negative results.[27] Another predictive element in relation to KRAS is the location of the tumour: left-side CRC tumours are more responsive to anti-EGFR treatment. This may be due to the more frequent activation of EGFR signalling in left-sided tumours compared to those on the right side.[28] In contrast with anti-EGFR treatment, the effectiveness of Bevacizumab is not dependent on tumour location.[29] Recent data indicates that different KRAS mutations exert different biological effects and their impact on EGFR resistance is not consistent throughout the mutational spectrum. Thus, not all tumours with KRAS mutation are resistant to EGFR-inhibiting treatment. This phenomenon is probably due to the heterogeneity of tumours. It should also be noted that patients with tumours on the right colon exhibit more frequent BRAF mutations, which result in relative resistance to anti-EGFR treatment.[28-30]

Available data is inconclusive regarding the prognostic role of KRAS. One study showed that patients with KRAS mutation who presented with stage IV CRC, synonymous with metastatic disease, had a higher mortality rate (34% versus 18.5%) and reduced OS (23.5 months versus 14 months) compared to patients without this muta-

tion. Also, the presence of the KRAS mutation was found to be an independent risk factor for reduced survival.[8] The mutation status of KRAS has not been associated significantly with OS in the case of patients receiving best supportive care (BSC) in a randomized phase III trial comparing Cetuximab with BSC.[33] One explanation for such inconsistent results is that different mutations in different codons may lead to variable tumour activity of the KRAS protein.[34]

## NRAS: the little brother of KRAS

Patients with NRAS mutation form a distinct mCRC subgroup from a clinical and molecular standpoint. 3–5% of CRC show a mutation in exons 2, 3 and 4 of the NRAS gene, but the impact of these genetic changes is less studied compared to KRAS and BRAF mutations due to the low number of patients.[34]

One study by Schiripa *et al*. found the NRAS mutation in 6% of the 785 included patients. Available literature indicates that the clinical and pathological features of patients with KRAS and NRAS mutation are similar. In another study, tumours with NRAS mutation were identified on the proximal colon in 33% of the cases, on the distal colon in 36% and at the rectal level in 31% of the cases. KRAS and NRAS mutant tumours exhibit similar metastasis patterns, namely their dissemination is frequently hepatic, pulmonary and peritoneal. Nevertheless, there are individual differences: low incidence of mucinous histology in patients with NRAS mutation (4% versus 26%, P = 0.012) and less frequent lung metastasis (30% versus 35%).[34]

Patients with NRAS mutation respond poorly to anti-EGFR treatment. De Roock *et al*. evaluated the role of the NRAS mutation in a cohort of patients treated with Cetuximab and chemotherapy. Only one of the 13 patients responded to treatment. A phase II trial that included patients with NRAS mutation found them non-responsive to anti-EGFR treatment. Similar results were obtained in a phase III trial testing Panitumumab against BSC: none of the patients with NRAS mutation responded to the treatment.[35] Similar results were found in another study: only one of the 37 patients responded with an objective response rate of 2.7%. Given the ineffectiveness of anti-EGFR treatment in patients with NRAS mutation, the European Drug Agency does not recommend the use of anti-EGFR drugs in such patients.[34]

In Schiripa's study, patients with NRAS mutation had a lower OS rate compared to WT patients – 25.6 months *vs*. 42.7 months. There were no differences in the survival of patients with NRAS and those with KRAS mutations. OS depended on the position of the mutation on the exons. In mCRC patients with NRAS mutation, it was significantly shorter in exon 3 compared to mCRC RAS WT patients (HR 2.85; 95% CI 1.87–4.36, P < 0.01) and to patients with NRAS mutation in exon 2 (HR 2.0; 95% CI 1.04–4.0, P = 0.039).[34,36]

## BRAF: rare, but important

BRAF plays a role in MAP-kinase (MAPK) pathway activation and contributes to cellular growth, proliferation and differentiation, as well as to other key cellular processes such as migration, apoptosis and cellular survival. Approximately 90% of BRAF mutations occur at the level of T1799 transversion in exon 15, which leads to the substitution of valine for glutamic acid (V600E).[37] This substitution regulates phosphorylation, increasing BRAF activity by approximately ten times compared to WT.[38]

CRC patients with BRAF mutation are a small and unique group that make up 8%–12% of all the patients suffering from CRC.[39-44] Concerning the epidemiology of the BRAF mutation, several studies have revealed similar rates of occurrence across the world, with only minor variations between regions.[45-48] The BRAF mutation has been reported in multiple studies in association with various clinical and pathological parameters in mCRC patients. It is more common in women older than 70, and for tumours located in the right colon.[49] BRAF mutations are less frequent if the left colon is affected (4%) and in rectal cancers (2%).[44] In terms of aggressiveness, approximately 60% of the BRAF mutant tumours are poorly differentiated, and only 36% of them are well or moderately differentiated. Histologically, the mucinous subtype is more frequently associated with BRAF mutant cancers (22–67%).[46-49] Unlike most colorectal cancers, tumours with BRAF mutations metastasize more frequently in the peritoneum and less commonly in the lungs and liver.[50]

The relationship between BRAF mutations and certain molecular tumour characteristics has been investigated. The BRAF and KRAS mutations are mutually exclusive. The BRAF mutation coexists with the PIK3CA mutation in 13% of the patients and with the PTEN mutation in 22% of the patients.[51-53]

BRAF V600 mutations are significantly more common in patients with MSI high (38.9%) than in those with MSI-low (9.3%; OR = 8.18; 95% CI = 5.08–

13.17).[51] Patients with sporadic CRC and MSI-high carry the BRAF mutation in 91% of the cases.[54,55] Therefore, BRAF mutation testing is also useful in identifying patients with Lynch syndrome. Patients with MSI-high and the absence of BRAF mutation should undergo genetic polymerase chain reaction testing to confirm Lynch syndrome.[56,57]

BRAF mutations are considered a biomarker for negative prognosis in mCRC. Several trials such as COIN, PRIME, CRYSTAL and OPUS, as well as a meta-analysis of 21 trials with patients in the metastatic stage, have found reduced survival and shorter progression-free survival (PFS) in mCRC patients with BRAF mutation. Thus, regardless of the approach to treatment, median survival is generally reported as 10–16 months shorter in CRC patients with BRAF mutation than in those without it.[53,58] In recent years, the role of the BRAF mutation in patients undergoing metastasectomy has been discussed. In a retrospective study of 309 patients whose secondary hepatic lesions were surgically removed, recurrence-free survival was 5.7 months for those with BRAF mutation compared to 11 and 14.4 months for RAS mutant and RAS WT without BRAF mutation, respectively (P = 0.003).[59] Renaud *et al*. evaluated the pulmonary metastasectomies retrospectively in 180 patients with BRAF mutation, KRAS mutant and WT CRC. Patients with CRC and BRAF mutation had a lower survival rate following surgery compared to those with KRAS mutant tumours or WT (15, 55 and 98 months, respectively, P < 0.0001).[60]

Several post-hoc analyses of phase III randomized trials have assessed the predictive role of the BRAF V600E mutation concerning the effectiveness of anti-EGFR therapies. The results of these retrospective studies did not reach statistical significance and were insufficient for a definitive conclusion about the potential use of BRAF V600E as a biomarker for determining primary resistance to anti-EGFR agents in CRC.[61-65] Therefore, the predictive role of the BRAF mutation for anti-EGFR agents after two meta-analyses is still unclear.[66,67] Pietrantonio *et al.* concluded that the BRAF mutations could be a negative predictive factor for anti-EGFR agents, thus supporting the meta-analysis conducted by Yuan *et al*.[68] However, another meta-analysis by Rowland *et al*. of 7 randomized controlled trials looking at OS and PFS concluded that the evidence is insufficient in order to justify the exclusion of anti-EGFR agents in the case of patients with BRAF mutation.[53,67,68]

It has been suggested that BRAF-mutant patients might benefit more from an intensive chemotherapy regimen, such as the FOLFOXIRI (regimen of chemotherapy consisting of the next citotoxic agents: Oxaliplatin, Irinotecan, 5-Fluorouracil and Folinic acid) and Bevacizumab protocol. Loupakis *et al*. obtained encouraging results in their study of 15 patients with BRAF mutation in a validation cohort with a median OS of 24.1 months and median PFS of 11.8 months.[69] Although the results were confirmed in the phase 3 trial TRIBE, this strategy was not embraced by all oncologists due to the high toxicity profile in which FOLFOXIRI-Bevacizumab was compared with FOLFIRI-Bevacizumab as the first line of treatment in mCRC. Recently, the FDA has approved the combined use of Encorafenib (a small molecule which blocks BRAF by acting as a competitive RAF kinase inhibitor), Binimetinib (an inhibitor of the mitogen-activating kinase) and Cetuximab for the treatment of mCRC patients with BRAF V600E mutation who underwent one or two lines of chemotherapy for their metastatic disease based on the results of the BEACON trial, which found a 62% OS rate 1 year after the analysis. The median PFS for the patients treated with this triple combination was of 8 months (95% CI = 5.6–9.3) regardless of whether or not they had previously benefitted from one or two lines of treatment. The overall response rate was 48%, and in the case of patients who had previously undergone a single line of treatment it was 62%.[70]

Non-V600 BRAF mutations are a special and infrequent category (they occur in 2% of mCRC patients). Certain differences between patients with V600 and those with non-V600 BRAF mutations were noticed: the latter were found in younger patients (58 versus 68), mostly male (65% versus 46%), with well-differentiated tumours located less often in the right colon and which more frequently suffer concurrent RAS mutations. Survival was also much longer in this category of patients compared to mCRC patients with V600E BRAF mutation or RAS WT (60.7 months versus 11.4 and 43 months, respectively, P < 0.001). Non-V600 BRAF mutations define a distinct molecular subtype of mCRC with excellent prognosis.[53]

## HER2: the stranger on the shore

HER2 oncogene is a member of the tyrosine kinase family similar to EGFR, HER-1, HER-3 and HER-4. HER-2 is located on chromosome 17 and codes a transmembrane protein of 185 kD which is activated through ligand binding. HER-2 activation initiates the signal pathways, including MAPK and PI3K/AKT, which are essential for cellular prolif-

eration and differentiation. Meanwhile, the family of receptors is located on the normal cells; multiple studies have shown that they are overexpressed in multiple malignant tumors, including colorectal cancer.[71] HER2 amplification is a relevant genetic alteration in mCRC. This fact was documented in the HERACLES and MyPathway clinical trials. This biomarker can be screened at diagnosis and has a prevalence of approximately 5% in patients with KRAS WT mCRC.[72] Seo *et al*. investigated different correlations between different clinicopathological variables and the HER2 status. Overexpression of HER2 was not associated with gender and microsatellite status but was correlated with an aggressive tumoural behaviour which includes profound invasion, lymphatic metastases, distant metastases, perineural invasion and distal colon location with the highest incidence in the rectum.[73-75]

HER2 can be a predictive factor for anti-EGFR therapies. Two retrospective clinical series supports the idea that HER2 signalling activation could determine cetuximab resistance.[76-79] Raghav *et al*. analysed the impact of HER2 amplification and the efficacy of monoclonal antibodies in RAS and BRAF WT mCRC in a cohort of 99 patients, which included 99 patients. 37 of 99 patients had HER2 amplification identified with next-generation sequencing. Median PFS with anti-EGFR treatment was significantly shorter for the patients with HER2 amplification compared to those without amplification (2.9 months versus 8.1 months, $P < 0.0001$).[72] Yonesaka *et al*., evaluated the clinical impact of de novo HER2 amplification in 233 patients treated with cetuximab.[78] Median PFS and OS were reduced in patients with amplified HER versus unamplified HER2, 3 months versus 5 months and OS was 10.2 months versus 30.5 months. ($P < 0.0013$).[80] In the HERACLES-A clinical trial, which included only patients with HER2 positive mCRC patients who received previously Panitumumab and Cetuximab had resistance to Trastuzumab. However, these data are retrospective, and they must be carefully taken into consideration because they have to be validated in prospective clinical trials. HERACLES-A clinical trial opened new therapeutic perspectives in mCRC with the use of HER2 dual blockade with Lapatinib and Trastuzumab in patients with KRAS WT in exon 2 (codons 12 and 13) pretreated with four lines or more of chemotherapy and with resistance to Cetuximab and Panitumumab. The objective response rate was 35%, clinical benefit was 70%, median PFS was 5,5 months, and the safety profile was agreeable.[72] The early trials proposed a negative prognostic impact of HER2 overexpression, but more recent trials did not confirm this fact.[80,81] Li's meta-analysis indicated that HER2 overexpression probably has a minor impact on OS in patients with mCRC. The prognostic role of HER2 in mCRC remains uncertain due to few clinical trials which analyzed this problem.[82]

## Microsatellite instability: the new player which brings hope

Microsatellites are repetitive sequences of coding, and non-coding DNA.[83] MSI is the result of the inability of MMR gene to repeat the DNA errors appeared during replication. Insertions and deletions represent somatic mutations in these repetitive sequences of DNA, and they determine genomic instability. MMR genes inactivation is the result of MLH1 promoter hypermethylation or the germinal mutations of MLH1, MSH2, MSH6 and PMS2.[84] Germinal anomalies of MSI represent the molecular basis of Lynch syndrome.[85] MSI is detected in approximately 15% of patients with mCRC; only 3% of cases are associated with Lynch syndrome and the other 12% are caused by sporadic hypermethylation of the MLH1 gene. The MSI prevalence is similar across different populations: 12% of Afro-Americans are positive, 12% of Hispanics are positive, and 12% of Caucasians are positive. CRC tumours are more frequently positive for MSI in stage II and III than in stage IV. Sporadic MSI positive tumours are located proximal, appear more frequent in older women, are poorly differentiated, have mucinous histology and have pronounced lymphocytic infiltration.[86]

Mismatch deficiency causes multiple somatic mutations that can produce multiple immunogenic neoantigens and antigens, which will increase the response to checkpoint inhibitors. Le *et al*. in a phase II study have shown that patients with deficient mismatch CRC were treated with anti-PD-1 pembrolizumab and they had an objective response rate of 62% compared with MSI-L tumours. Nivolumab monotherapy and the nivolumab ipilimumab combination in patients with pretreated MSI-H mCRC was studied. After a median follow-up of 21 months, patients treated with nivolumab had a response rate of 34%. The responses were durable; 64% of patients had at least a response for a year. After one year, 44% of patients did not progress, and 73% were alive. After a median follow-up of 13.4 months, the patients who received the combination of nivolumab and ipilimumab had a response rate of 55%, 71% did not have progres-

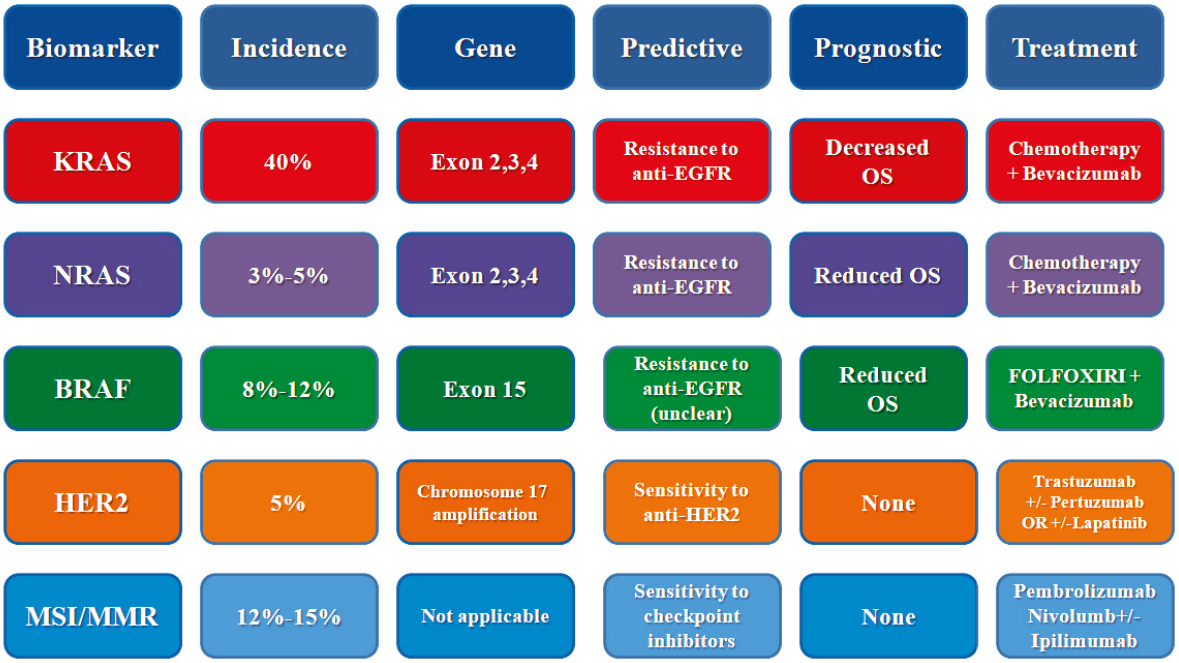


**FIGURE 2.** Summary features of biomarkers in stage IV colorectal cancer

EGFR = epithelial growth factor receptor; FOLFOXIRI = regimen of chemotherapy consisting of 5-fluorouracil, folinic acid, oxaliplatin and irinotecan; MMR = mismatch repair; MSI = microsatellite instability; OS = overall survival; WT = wild type

sion, and 85% were alive. The discovery of MSI in CRC widened the molecular landscape and created the premises for a new type of systemic treatment: immunotherapy. MSI-H became a predictive biomarker for immunotherapy in stage IV currently CRC.[87]

MSI represents a favourable prognostic factor in stage II and III of disease, but this is not applicable for metastatic disease. In a study of 2439 patients with mCRC, no significant survival differences were observed between patients with MSI-H and MSI-L tumours (3.82 versus 2.95 years; P = 0,76).[88,89]

## Conclusions

Biomarker testing in mCRC patients has become a routine in clinical practice. While the predictive role of KRAS and NRAS is well-known and widely used in treatment selection, other features of these biomarkers are only now being investigated in prospective studies. NRAS-mutated tumors are associated with reduced OS and resistance to anti-EGFR treatment. Similarly, the presence of a BRAF mutation in mCRC seems to also predict resistance to anti-EGFR treatment and identifies a very poor-prognosis subgroup of patients. More recently, HER2 overexpression has been linked to sensitivity to anti-Her2 treatment in mCRC patients and MSI/MMR status has been shown to predict tumor response to checkpoint inhibitors. All these information help identify personalized treatments for cancer patients, thus increasing overall survival and significantly decreasing drug-related toxicity.

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*review*

# Heterotopic ossification: radiological and pathological review

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**Background.** Heterotopic Ossification (HO) is a common condition referring to ectopic bone formation in soft tissues. It has two major etiologies, acquired (more common) and genetic. The acquired form is closely related to tissue trauma. The exact pathogenesis of this disease remains unclear; however, there is ongoing research in prophylactic and therapeutic treatments that is promising.

**Conclusions.** Due to HO potential to cause disability, it is so important to differentiate it from other causes in order to establish the best possible management.

Key words: heterotopic ossification; radiology; pathology

## Introduction

Heterotopic Ossification (HO), also known as paraosteoarthopathy, myositis ossificans, and heterotopic calcification[1] among others, is a commonly occurring condition that refers to ectopic bone formation in soft tissues. HO can be subdivided into two major types: acquired and genetic, with acquired being the most predominate. Acquired HO is closely related to tissue trauma and can be seen after joint surgery, musculoskeletal trauma, central nervous system injury, and even burns.[2] HO develops in up to 44% of patients undergoing hip arthroscopy or replacement, 10-20% of those with CNS injury, and 4% of those with burns covering greater than 30% of body surface.[3-10] Many cases of HO lead an indolent course, however severe cases can cause inflammation, pain, immobility and functional impairment.[11] Due to its potential to cause disability, it is imperative to be able to distinguish HO from other etiologies including tumoral calcinosis, osteosarcoma, or dystrophic calcification to provide adequate treatment.

### Pathophysiology

Acquired HO can be broadly categorized in to three etiologic subtypes: neurogenic from central nervous system injury, orthopedic covering fractures, fixations, joint replacements, *etc.*, and trauma related to burns and high velocity impacts.[3] The formation of HO is tied to the underlying inflammatory process, which can even be demonstrated in genetic cases of HO where patients report prodromal symptoms of pain, swelling, and erythema prior to ectopic bone formation.[12] Trauma-induced HO is also correlated with the severity of the trauma, infection, total burn coverage[13] and cytokine concentration in affected tissues.[3,14] As a result, the most frequently used prophylactic medications are nonsteroidal anti-inflammatory drugs.[15] However, the underlying mechanisms for HO formation are still not clear. The Literature suggests multiple cellular origins for the formation of HO, pointing to muscle satellite cells[16], smooth muscle cells[17], and even endothelial cells.[18] Although the exact cellular origin is debated, it is commonly accepted to

be multipotent cells in the local tissue. The requirements necessary for HO formation include having an inducing agent, an osteogenic precursor, and a permissive environment for osteogenesis[19,20] which when met leads to proliferation and formation of bone.[21] Bidner *et al.* have proposed that failure to regulate the immune system or inflammatory response lead to the release of inciting agents that lead to HO.[19,22] Further investigations by Salisbury *et al.* and Kan *et al.* have implicated bone morphogenic protein type 2 (BMP-2) as a pro-inflammatory agent by stimulating release of substance p and calcitonin gene-related peptide from sensory nerves.[23,24] Further investigations could support BMP's role in HO formation and lead to formulation of targeted therapies.[3,21] Other suggested contributory factors include prostaglandin (specifically PGE-2), tissue hypoxia, and an imbalance between parathyroid hormone and calcitonin.[25] A review performed by Cholok *et al.* showed multiple potential contributory cell lineages with likely varying signalling pathways, highlighting the current lack of understanding in HO formation.[3] All in all, the precise mechanisms of HO formation remain vague and need further investigation.

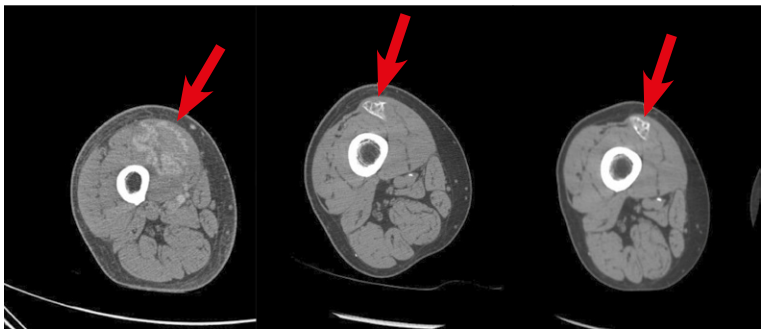

**FIGURE 1.** Progression of Heterotopic Ossification from presentation (left), 4 months (middle), and 8 months (right). Axial CT with contrast depicts initial hyperemia with increasing calcification at the site of injury with eventual outer cortical and inner cancellous bone formation.

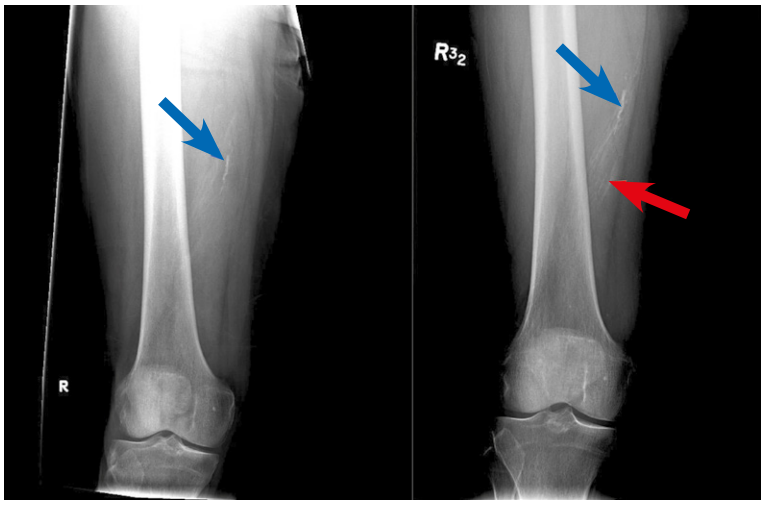


**FIGURE 2.** AP X-rays show previous vascular calcifications (Left-blue arrow) with no apparent masses at the site of injury at presentation. At 4 months follow up, there is increased calcifications noted (blue arrow) with expansion to the adjacent soft tissue area (red arrow) both are consistent with Heterotopic Ossification.

## Clinical presentation and diagnosis

Patients presenting with HO typically complain of inflammatory symptoms including pain, swelling, erythema, and warmth along with joint immobility, which appear anytime from 3 to 12 weeks after the precipitating event.[11,25-28] The most common sites of occurrence, in a decreasing order, are the hips, knees, shoulders, and elbows.[25,27] The gold standard method for diagnosing HO is through imaging studies, mainly radiography and computerized tomography (CT).[3] The downfall to these types of imaging is that they are not able to detect calcifications for at least 6 weeks after the inciting trauma.[25,29] Three-phase bone scintigraphy is the most sensitive method for detecting HO, with the earliest detection being 2.5 weeks post trauma.[25,30] It is also effective in monitoring HO progression and determining the appropriate time to stage surgical intervention.[25,26,30] Activity on bone scans usually peaks a few months after the inciting event and returns to baseline by 12 months.[25]

Early screening methods used before imaging studies include serum alkaline phosphate levels and 24-hour urinary PGE2. Alkaline phosphate levels can increase two weeks after trauma, reaching 3.5 times baseline by 10 weeks, and then returning to baseline by 18 weeks. A rapid increase in 24-hour PGE2 urinary secretion has also been shown to suggest HO and would indicate further imaging studies.[31,32]

Upon suspicion of HO on imaging, it has been suggested to perform a biopsy to confirm the diagnosis; however, current recommendations are to follow up with imaging studies in four weeks, which together with the history of trauma can confirm the diagnosis.[33]

## Imaging and classification

A soft tissue mass is the earliest finding of HO on imaging, it is often depicted as a peripheral zone of mineralization in acquired cases.[33] With time, these outer regions can mature in to a peripheral cortex with a well-defined cancellous bone inte-

rior detectable by CT (Figures 1-2 and 3).[29,33] Radiography (Figure 4) and CT scan remain the gold standard for diagnosis due to their ability to detect immature bone formation and the relatively cheap cost.[3,29] In the acute phase of HO, there is increased tissue vascularization and density, which can be detected on Magnetic Resonance (MR).[34] This region appears isointense or hyperintense to muscle on T1-weighted images and hyperintense on T2 weighted images with pronounced surrounding inflammation.[34,35] As the rim of calcification forms, signal void begins to appear on the periphery on all sequences.[35,36] During this maturing phase, MR imaging results in non-specific findings and heterogenous signal that mimics many other pathologic processes.[29,37] Once mature, HO presents as cancellous fat that is hyperintense on T1 and T2 weighted images outlined by the hypointense cortical bone[29] and this can be considered diagnostic. Therefore, when MR detects a mature HO, no further imaging is necessary. On the other hand, early MRI has a great advantage in excluding other differential diagnosis possibilities, as we can observe the "striate pattern" and "checkerboard-like pattern" appearance in T2-WI and contrast-enhanced MRI images[38] or it can be detected by displacing the fascial planes, especially at the periphery of the lesion.[39] Recognizing these MRI patterns in HO could be very beneficial in the early phases as the condition is commonly misdiagnosed for an osteomyelitis or even a malignancy, mostly sarcomas.[40-42]

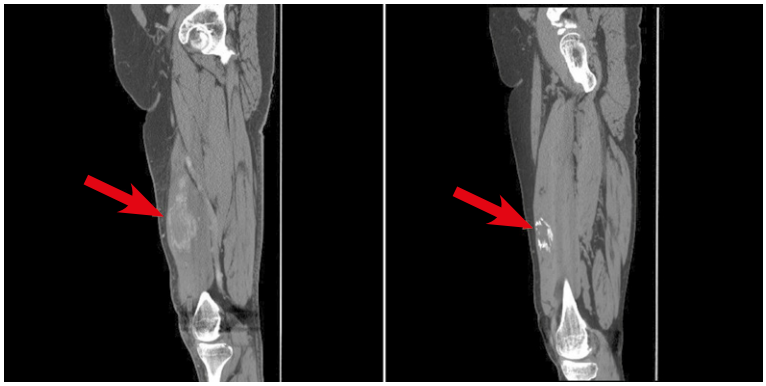

**FIGURE 3.** Heterotopic Ossification shown with initial hyperemia without calcification at presentation (left- red arrow) with increasing organized calcification seen after 4 months on Non-contrast CT (Right-red arrow).

Ultrasonography (US) is proved to be a sensitive imaging modality for soft tissues lesions and calcifications.[43,44] It is also safe, of low-cost, and easy to perform and repeat.[45] US has the great advantage of bedside application as well, which could be more feasible for bed-ridden patients.[45,46] Qing Wang *et al.* discussed a new concept for monitoring the trauma-induced HO. The study gives a guidance to the orthopedist to modify the treatment and make an individualized rehabilitation program. They have shown that the grey-scale values are different during the different phases of HO maturation, and so US allows for a quantitative assessment during the rehabilitation of HO.[47]

Staging of HO is commonly done using the Brooker classification (Table 1), which was initially developed using anteroposterior radiographs of the hip.[9] There has been some criticism of this classification as anteroposterior radiographs cannot distinguish between bridging or overlapping calcifications.[48] To simplify and reduce variability, Della Valle *et al.* (Table 2) created a modified classification using only three distinct grades.[49] However, a third and more comprehensive classification was established by Schmidt and Hackenbroch (Ta-

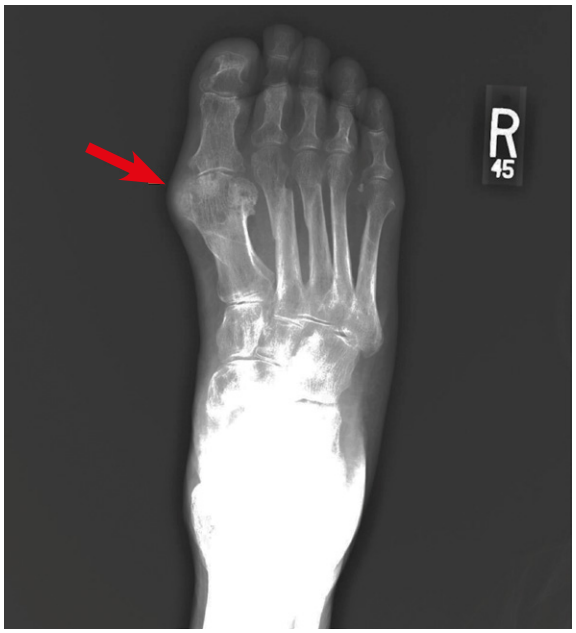


**FIGURE 4.** Severe gout presenting on the first metatarsophalangeal joint. AP X-ray of the right foot shows a medial pararticular calcified soft tissue mass at the level of the first metatarsophalangeal joint (red arrow), resulting in adjacent intraosseous erosions with sclerotic borders.

**TABLE 1.** Brooker classification of heterotopic ossification[9]

| | |
|---|---|
| **Class 1** | Islands of bone within the soft tissues over the hip |
| **Class2** | Bone spurs from the pelvis or proximal end of the femur, leaving at least one centimeter between opposing bone surfaces. |
| **Class 3** | Bone spurs from the pelvis or proximal end of the femur, reducing the space between opposing bone surfaces to less than one centimeter. |
| **Class 4** | Apparent bone ankylosis of the hip |

TABLE 2. Della Valle classification of heterotopic ossification[49]

| | |
|---|---|
| Class 1 | Absence of HO or islands measuring <1 cm in length |
| Class 2 | Islands >1 cm or spurs leaving at least 1 cm between femur and pelvis |
| Class 3 | Spurs leaving <1 cm between opposing surfaces or bony ankylosis |

TABLE 3. Schmidt and Hackenbroch classification of heterotopic ossification[50]

| | |
|---|---|
| Region 1 | Heterotopic ossifications strictly below tip of greater trochanter |
| Region 2 | Heterotopic ossifications below and above tip of greater trochanter |
| Region 3 | Heterotopic ossifications strictly above tip of greater trochanter |
| Grade A | Single or multiple heterotopic ossifications < 10 mm in maximal extent without contact with pelvis or femur |
| Grade B | Heterotopic ossifications > 10 mm without contact with pelvis but with possible contact with femur; no bridging from femur to proximal part of greater trochanter, with no evidence of ankylosis |
| Grade C | Ankylosis by means of firm bridging from femur to pelvis |

ble 3) with the goal of classifying HO while considering ossification within the region of surgical approach.[50] From these classifications, an important distinction for reporting and assessing severity is determining whether the space -between two opposing bone surfaces- is greater than or less than one centimeter.[9,49,50]

## Differential diagnosis

Many pathologies can imitate HO clinically or radiographically. It is vital to understand the similarities and differences of these mimetics when considering the diagnosis of HO. A few differentials that should be considered are briefly discussed below.

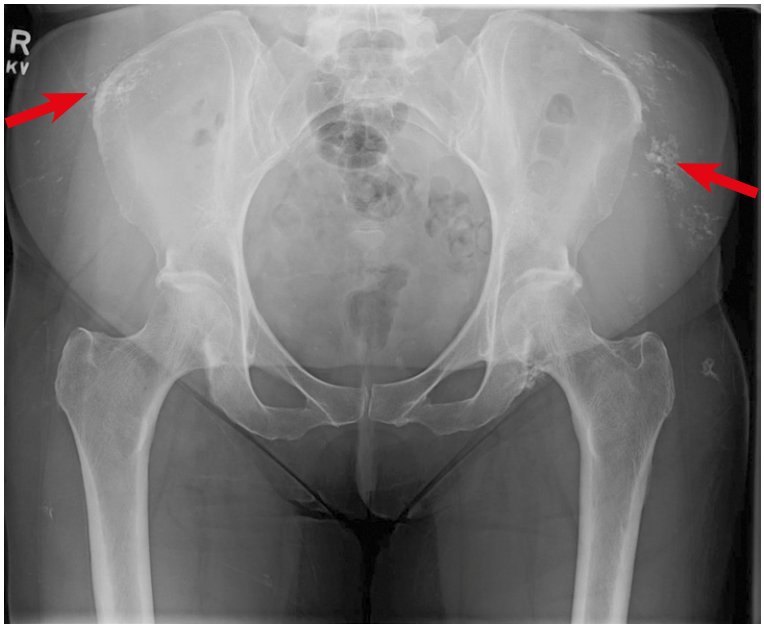


FIGURE 5. Dystrophic calcifications secondary to dermatomyositis are seen in the peripheral soft tissue (2 red arrows). They appear as hazy ill-defined opacities on plain film.

## Dystrophic calcification

Dystrophic calcification (DC) is the calcification that occurs in soft tissue post inflammation and damage. The mechanism is thought to be either disruption of cell membranes during cellular stress allowing calcium to enter and subsequently be concentrated in the mitochondria or by creating an acidic environment in the tissue that lacks calcification inhibitors.[51]

It is well documented to occur in cases of collagen vascular diseases like dermatomyositis (Figure 5), systemic lupus erythematosus, and scleroderma[52], but has also been identified in other disease processes.[51] On plain film, DC appears as amorphous calcification with a hazy ill-defined appearance that can increase in density over time.[53] CT will similarly show peripheral amorphous hyperdensities, with MRI showing hypointense signals in T1 and T2 weighted images (Figure 6).[54]

The distinguishing difference between DC and HO is organization. DC and HO are virtually indistinguishable on plain films, CT, or MRI early in the disease process as mineralization occurs. HO will begin to organize and ossify over the course of months into lamellar bone while DC will remain as amorphous, non-ossified calcifications.[55]

## Chondrocalcinosis

Chondrocalcinosis is calcification within fibrous or cartilaginous structures and is frequently associated with calcium pyrophosphatase disease (CPPD).[56] In cases of CPPD, there is usually acute, painful inflammation of a joint, often the knee, where calcium phosphate crystals are deposited.[57] Microcrystals can then impregnate cartilage causing arthritic symptoms, which can range from mild to severe with joint destruction.[57] On plain films, this appears as a dense line within hyaline cartilage that runs parallel to the articular surface.[56] CT has excellent sensitivity and specificity for detecting chondrocalcinosis and can better visualize the linear hyperintense calcifications (Figure 7).[56] There is often a concurrent degenerative joint disease with joint space narrowing and large osteophyte formation.[58] The linear deposition contrasts with HO, which presents as a peripheral circumferential calcific mass on both plain films and CT with minimal intra-articular involvement. MRI has little utility in

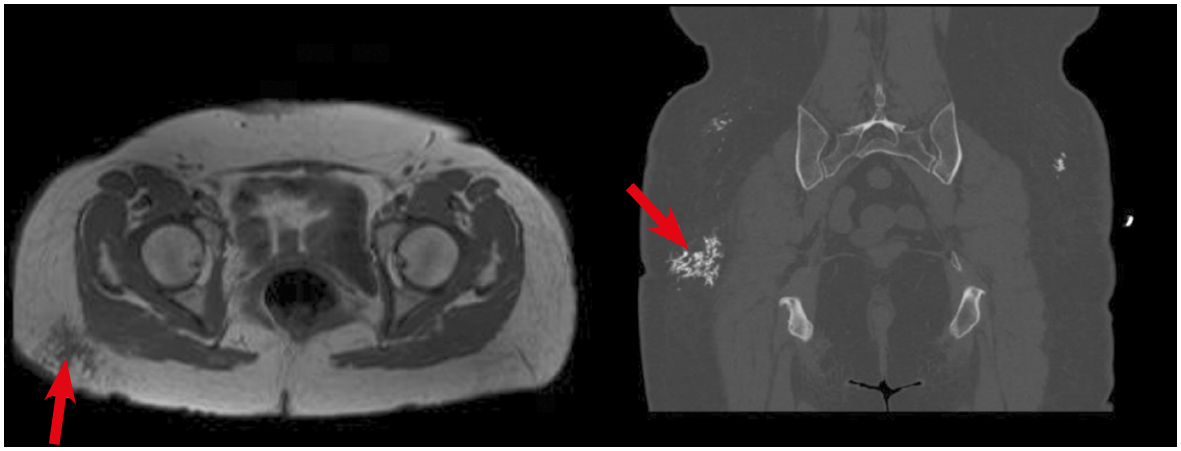

**FIGURE 6.** T1 weighted non-contrast MRI (left-red arrow) of dystrophic calcifications show hypointense signal in patchy patterns. These appear as calicified hazy patches on CT (right-red arrow).

diagnosing Chondrocalcinosis, as the calcifications are not well visualized in tissues.[56]

## Tumoral calcinosis

Tumoral calcinosis (TC) refers to a syndrome characterized by calcium salt deposition in peri-articular soft tissue.[59] A major component of TC is hyperphosphatemia secondary to genetically acquired decrease in phosphate secretion[59-61] or chronic renal failure and resulting hyperparathyroidism.[59] Patients present with joint pain, swelling, or immobility most commonly in the hip, elbow, shoulder, foot, or wrist.[59,62-64] Unlike HO, TC lesions grow slowly over the course of several years.[65] Plain radiographs, ultrasound and CT scan, all can be used for diagnosis and would show fluid filled, lobulated, cystic calcifications in peri-articular tissue.[66]

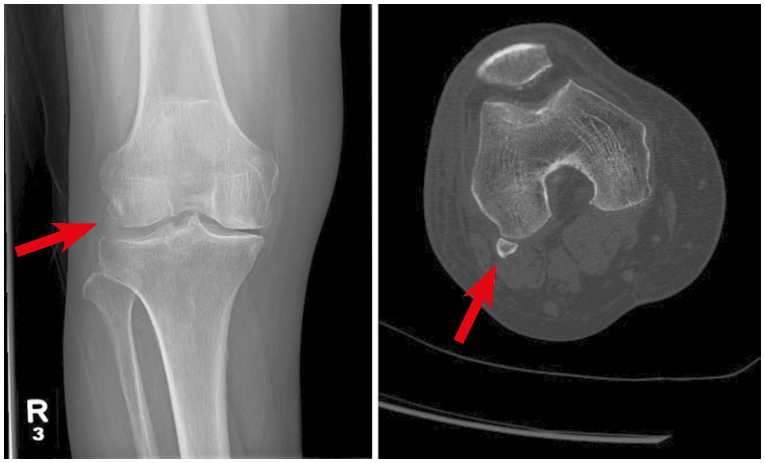


**FIGURE 7.** Calcium Pyrophosphate Deposition disease can lead to calcification of intra-articular cartilage. There is opacification of the lateral joint space on plain film (left-red arrow) and a more clearly defined mineralization seen near the lateral condyle on CT (right-red arrow).

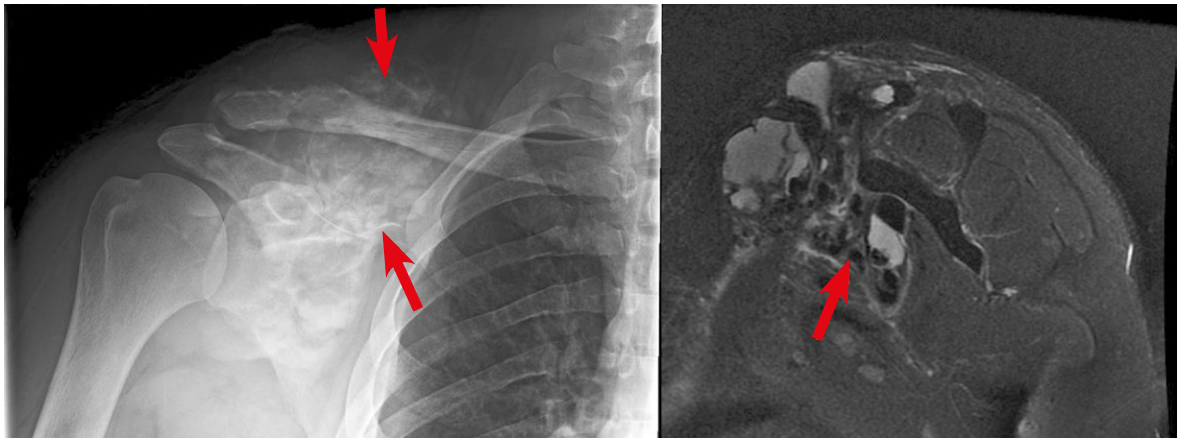

**FIGURE 8.** X-ray of the (left- 2 red arrows) shoulder show opacified cystic, lobulated peri-articular lesions in Tumoral Calcinosis. Coronal MRI T2 sequencing (right-red arrow) reveals hypointense lesions with septal enhancement, hyperintense fluid filled cavities and fluid –fluid levels consistent with sedimentation.

T1 and T2 weighted MRI show a hypointense lesion with septal enhancement (Figure 8). HO is not cystic in nature and lacks the lobulated pattern on both CT and MRI. HO also presents with hyperintense signal centrally with a hypointense cortical shell on MRI. Management of TC is clinically determined based on the symptoms and the size of calcinosis with surgical or needle decompression being most common interventions.[66]

## Avulsion fracture

An avulsion fracture (AF) is the separation of a bone fragment at the site of tendon attachment, often occurring after a traumatic injury. Patients with this injury typically have a definite history of trauma accompanied by pain, swelling, and loss of joint function.[67] Findings on imaging can be seen immediately post trauma, depicting sharply delineated bone fragments (Figure 9). Large avulsed fragments can appear identical to matured HO, therefore having a clinical history is important. In addition, HO will not be visible on a plain film until weeks after the inciting trauma and will not mature into cortical bone for many months.[25] CT of avulsion fractures helps delineate fracture sites and show displaced hyperdense cortical bone.[67] HO can be distinguished from AF on CT, showing a ring of hyperdense cortical bone with a hypodense interior.[29,33] MRI may be useful in detecting local tissue damage seen in avulsion fragments; however, findings are consistent with inflammation and are non-specific.[67]

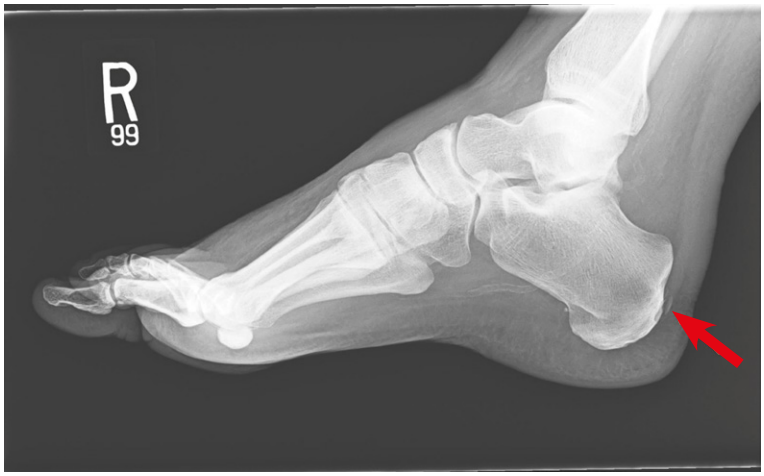


**FIGURE 9.** An avulsed piece of bone is seen on the posterior aspect of the calcaneus secondary to trauma (red arrow).

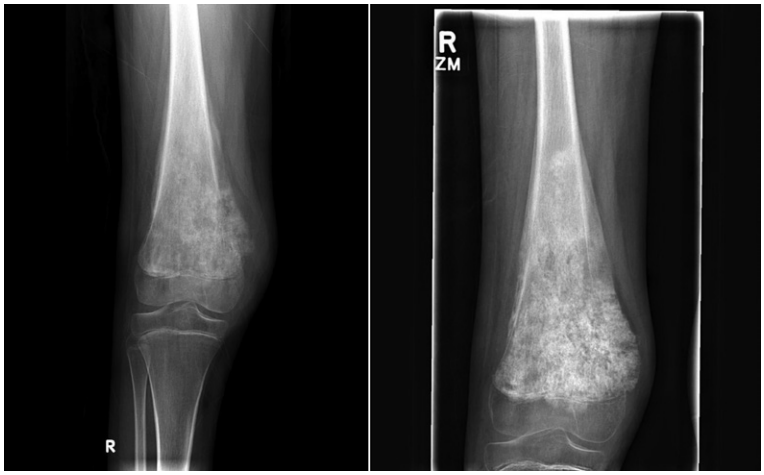


**FIGURE 10.** The "sunburst" appearance with cloudlike density of untreated Osteosarcoma is observed in the distal femur (left-red arrow). After chemotherapy, the lesion ossifies and becomes increasingly opaque on plain film (right-red arrow), consistent with positive therapeutic response).

## Primary osteosarcoma

Osteosarcomas (OS) are the most common primary bone tumor, developing from uninhibited osteoid production by malignant mesenchymal cells.[68] Patients present with localized pain and swelling, which then proceeds to joint immobility. This type of tumor is commonly seen in the metaphysis of long bones, most commonly the distal femur, proximal tibia, and proximal humerus; in a descending fashion.[68,69] On plain radiographs, it can present as osteoblastic, osteolytic, or with mixed appearances, and have patchy calcifications from the newly developing bone in the surrounding soft tissue.[68] The imaging appearance is commonly described as a "sunburst" appearance or as having cloudlike density (Figure 10).[70] CT scan is highly sensitive to calcification and is useful in showing the amorphous osteoid formation in OS, which can help distinguish it from organized circumferential osteoid formation in HO. MRI of OS shows heterogenous signal intensities on T1- and T2-weighted images due to a mixture of amorphous osteoid, hemorrhage, and necrosis.[70,71] Radiographs can be correlated with a low signal intensity on T1-wieghted imaging and hyperintensity on STIR imaging indicating mineralized matrix deposition with small periosteal reaction. Other findings include cortical bone destruction and marrow invasion not typically seen with HO.[71]

## Tophaceous gout

Gout is a type of inflammatory arthritis caused by the deposition of monosodium urate crystals in joints and surrounding tissue.[72] Clinically, this con-

dition presents with an acute onset pain and swelling at the site of deposition, typically the feet and knees but can also be seen elsewhere.[72] Early radiographic studies can often be negative, however in chronic gout patients, punched erosions with well-defined sclerotic borders can form extra marginally, articularly, or para-articularly with preservation of the joint space.[72,73] In severe cases, extreme bone destruction can occur with large periarticular lesions, joint space widening, and concurring osteoarthritis.[72-74] Tophi on CT are seen as discrete masses with a higher intensity than adjacent soft tissue.[75] CT is also useful in defining well-demarcated erosions with overhanging osteophytes seen in gout.[75] MRI is only beneficial in identifying soft tissue abnormalities around affected joints rather than tophi themselves, leading to low specificity and utility.[75] When seen, tophi appear with decreased signal intensity on T1 weighted images and heterogeneous signal on T2.[75] HO can be distinguished from tophaceous gout on x-ray and CT by lack of intraosseous erosions, peripheral calcifications in the soft tissue, and formation of cortical bone. MRI is not useful in distinguishing between the two unless the HO is mature, when complete lamellar bone is seen.

## Calcific tendonitis

Calcific Tendonitis refers to the condition of calcium deposition in tendons.[76] This is clinically depicted by chronic pain with activity, tenderness, swelling, and joint immobility that is commonly localized to the rotator cuff tendons.[76,77] The etiology remains unknown; however, severity has been associated with endocrine diseases.[78] Pathology can be noted by standard AP radiograph with internal and external rotation views showing dense homogenous calcification typically noted proximal to the greater tubercle (Figure 11).[79] Ultrasound, used to evaluate a rotator cuff injury, can show a hyperechoic lesions with reproducible pain in palpation during the procedure.[80] Calcific tendonitis can also be viewed with susceptibility-weighted imaging, which presents as a hyperintense lesion at tendon insertion site with occasional central hypointensity. It lacks the well-defined shape of HO and the hyperintense core seen on T1 weighted images.[81]

## Fibrodysplasia Ossificans Progressiva

Fibrodysplasia Ossificans Progessiva is an extremely rare genetic form of HO in which patients

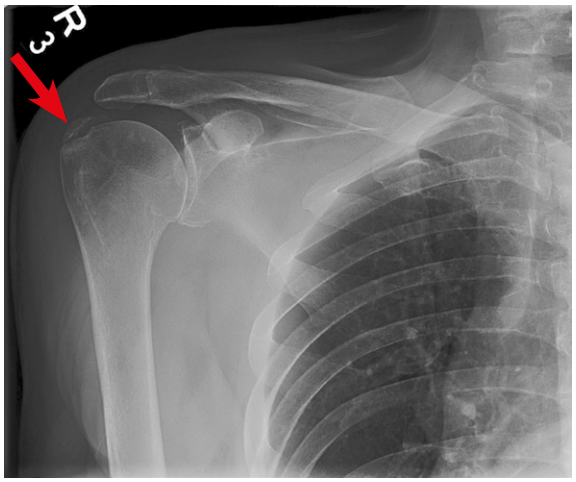


**FIGURE 11.** Opaque linear coarse calcification along the expected location of the supraspinatus tendon insertion onto the greater tubercle of the humerus (red arrow), consistent with Calcific Tendonitis.

repair mechanism ossifies the fibrous tissue at the trauma site, leaving the patient permanently frozen secondary to minor trauma.[82] Patients initially present with characteristic malformations of the large toes at birth with painful soft tissue swelling and ectopic bone formation within the first decade of life.[82-84] Laboratory changes include increased serum alkaline phosphatase and urinary basic fibroblast growth factor during acute episodes. Radiographic imaging shows extensive heterotopic bone formation diffusely with no specific pattern and ankylosis of adjacent joints with heterotopic bone formation.[82] MRI can show heterotopic bone formation with underlying edema and subtle soft tissue changes indicating pre-osseous lesions, noted as hyperintense lesion on fat suppressed T2 imaging.[85] CT imaging can be used for volumetric analysis of ossification that is unattainable via radiographs or MRI, showing the extent of joint ankylosis with 3D rendering and assessment of severity via Lederson grading scale.[85] This condition can be distinguished from traumatic HO by early onset and severe disseminated ossification.

## Treatment

Treatment for HO is divided into prophylaxis for high-risk patients and management of already formed HO. Due to the large variability in etiology and underlying mechanisms for HO and individualized patient risk factors, there is little agreement on appropriate treatment regimens. Commonly used prophylaxis includes NSAIDs, localized low

dose radiation, or a combination there-of with the most popular being NSAID alone.[86,87] Prophylactic NSAIDs have shown to reduce the occurrence of ectopic bone formation when given peri-operatively compared to placebo, but at the expense of medication side effects such as gastrointestinal ulcers, bleeding, and delayed bone healing.[88,89] Although there is a decrease in HO formation, NSAIDs had no effect on pain or physical function compared to placebo.[88,90,91] NSAIDs target pro-inflammatory prostaglandins, which have been shown to be integral to osteogenesis and are thought to have some effect by suppressing the migration and proliferation of mesenchymal cells.[21,92,93] The NSAID of choice is the non-selective cox inhibitor indomethacin.[94] Cox-2 specific inhibitors have been suggested to reduce side effects associated with nonselective cox inhibitors; however, their cardiovascular side effects and lack of safety with routine use limit their use.[15]

Coventry *et al.* first established radiation therapy (RT) as an effective treatment in 1981, and further studies by Childs *et al.* and Chao *et al.* confirmed its benefits.[95-97] In the retrospective cohort study by Childs *et al.* covering 263 patients whom experienced traumatic acetabular fractures, 5.3% of patients receiving RT also developed ectopic bone formation compared to 60% of patients without any treatment. The drawbacks to RT include potential side effects such as carcinogenesis, bone disunion, and oligospermia as well as the higher cost.[15] Strauss *et al.* determined that the total cost of RT was approximately 45 times higher than that of NSAIDs.[98] The high cost of RT limits its utility, especially considering it has not been shown to be more effective than NSAID therapy.[99] Other therapies currently under development and clinical testing include BMP antagonists, selective ALK receptor inhibitors, Noggin protein delivery, and retinoic acid.[21]

Surgical management currently remains the only effective treatment for a preformed ectopic bone. Indications for surgery include symptomatic disabilities and radiographic evidence showing the cessation of bone growth.[3] Surgery should not be performed until 12 to 18 months after HO formation to allow maturation of the lesion and patient's tissue has had time to recover to decrease intraoperative complications and HO reoccurrence.[28,100] Although efficacious, surgery inherently causes tissue trauma, which can simulate the same inflammatory conditions for HO formation and is therefore complicated by high reoccurrence rates.[101]

## Conclusions

Heterotopic ossification is a commonly seen condition occurring secondary to trauma and may cause mild to severe disability. The exact pathogenesis of this disease remains unclear; however, there is an ongoing promising research to develop prophylactic and therapeutic treatments that is promising. Distinguishing HO from other mimics help clinicians better manage the disease and improve patient care.

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*review*

# Multigene expression signatures in early hormone receptor positive HER 2 negative breast cancer

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**Background.** The standard treatment of hormone receptor positive, HER2 negative early breast cancer (BC) is surgery followed by adjuvant systemic therapy either with endocrine therapy alone or with the addition of chemotherapy followed by endocrine therapy. Adjuvant systemic therapy reduces the risk of recurrence and death from BC. Whether an individual patient will benefit from adjuvant chemotherapy is an important clinical decision. Decisions that rely solely on clinical-pathological factors can often lead to overtreatment. Multigene signatures represent an important progress in optimal selection of high risk patients that might benefit from the addition of chemotherapy to adjuvant endocrine therapy.

**Conclusions.** Several signatures are already commercially available and also accepted by international guidelines. Oncotype DX and MammaPrint have been most extensively validated and supported by level IA evidence.

Key words: hormone receptor positive HER-2 negative early breast cancer; adjuvant systemic therapy; multigene signatures

## Introduction

Breast cancer (BC) is the most common cancer in women in Slovenia and worldwide. More than 1300 women in Slovenia were diagnosed with breast cancer in 2015.[1] Approximately two thirds of BC are hormone receptor positive.[2] The standard treatment of hormone receptor positive, HER2 negative (HR+HER2-) early BC is surgery followed by adjuvant systemic therapy either with endocrine therapy alone or with the addition of chemotherapy followed by endocrine therapy. Adjuvant systemic therapy reduces the risk of recurrence and death from BC by approximately one third.[3,4] Whether an individual patient will benefit from adjuvant chemotherapy is an important decision. Classical clinical-pathological parameters (tumor size, nodal status, histological grade, proliferation index, age, hormone receptor status and menopausal status) are helpful in defining the risk of recurrence. However, these parameters do not take into account an individual biology of a tumor and substantial number of patients with early BC are thus over-treated and exposed to toxic effects of chemotherapy without any benefit.[5] Several multigene expression signatures have been developed to better prognosticate disease outcome.

Several of these signatures are commercially available and accepted by international guidelines, including the Oncotype DX recurrence score (Genomic Health), PAM50 Prosigna risk of recurrence (NanoString), Breast Cancer Index (BCI) (bioTheranostics), EndoPredict (MyriadGenetics), and MammaPrint (Agendia BV). Oncotype DX

and MammaPrint have been most extensively validated, including in prospective randomized trials, TAILOR x and MINDACT and are therefore most commonly used. They are commercially available; however they are not reimbursed in Slovenia.[6-9] Here, we focused on Oncotype DX and MammaPrint as other assays are much less frequently used in routine clinical practice.

## Oncotype DX

Oncotype DX is performed on RNA extracted from formalin-fixed paraffin-embedded tumor tissue using quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) and contains 5 reference genes and 16 cancer-related genes. The recurrence score (RS) is the result of mathematical formula of the weighted expression of each gene. The cut-off points are divided into 3 categories: low, intermediate and high risk.[10,11]

Its prognostic value was first evaluated on archived tissue from HR+HER2- lymph node negative patients from NSABP B-14 study and was confirmed later on in other studies.[12,13] Paik *et al*., demonstrated its ability to predict chemotherapy sensitivity in lymph node negative HR+HER2- early BC patients. Patients with high RS had benefited from chemotherapy, with the 10-year metastasis rate being decreased by 27.6% for those patients who received adjuvant chemotherapy. In contrast, there was no benefit of adding chemotherapy to patients with low RS.[10-13] The evidence is less strong for patients with lymph node positive disease. Five studies are relevant in this context: South West Oncology Group study (SWOGS8814), TransATAC, West German Cancer Group (WSG) PlanB study and two population based registries.[10,14-18] The results of these studies consistently show that a considerable percent of patients have a low-risk genomic signature despite positive nodal status and thus nodal positivity should not uniformly lead to decision of adding adjuvant chemotherapy to endocrine therapy (Table 1).

SWOG S8814 study data represents the strongest evidence available thus far that Oncotype DX predicts chemotherapy benefit in lymph node positive patients. The study was prospectively planned to examine this association and was applied to a randomised phase 3 trial with an endocrine therapy alone or in combination with chemotherapy. The test for interaction of chemotherapy with RS was significant. The study found significant improvement in disease free survival (DFS) when chemotherapy was added to endocrine therapy in patients with high genomic risk (RS ≥ 31) (HR: 0.59, p = 0.003) and no improvement in DFS for adding chemotherapy to endocrine therapy for patients with low RS (< 18).[10]

Until the results of TAILORx (Trial Assigning IndividuaLized Options for Treatment) study which aimed to answer whether chemotherapy would reduce the risk for recurrence in intermediate risk group this was unclear. In TAILORx study different cut-offs were used as initially set.[11,12] This study was designed to test whether chemotherapy is beneficial for women with intermediate RS (RS 11–25). 10253 women with HR+HER2-, node negative BC who met the criteria for consideration of adjuvant chemotherapy (tumor size 11-50 mm, or more than 5 mm with additional pathological unfavourable characteristics such as intermediate/high nuclear grade and presence of lymphovascular invasion) were enrolled. Women were assigned to one of four treatment groups on the basis of RS. Those with a RS ≤ 10 were assigned to receive endocrine therapy only, and women with RS ≥ 26 were assigned to receive chemotherapy plus endocrine therapy. Women with intermediate score of 11 to 25 were randomized to receive either endocrine therapy alone or in combination with chemotherapy. The study found no improve-

**TABLE 1.** Recurrence score (RS) distribution among studies that validated Oncotype DX in node positive breast cancer (N = 9055)

| Study | RS low (%) | RS intermediate (%) | RS high (%) |
|---|---|---|---|
| SWOG S8814 | 40 | 28 | 32 |
| TransATAC | 52 | 31 | 17 |
| SEER | 57 | 36 | 7 |
| Clait | 53 | 36 | 10 |
| PlanB | 19 | 63 | 19 |

First four of the studies used standard cut-offs (RS < 18, 18–30, ≥ 31), the PlanB study used non-standard cut-offs (RS < 12, 12–25, > 25), the same as TAILORx, RxPONDER study.

**TABLE 2.** Estimated survival rates according to recurrence score (RS) and treatment assigned in the intention to treat population (TAILORx trial)

| | 9-year DFS (%) | 9-year OS (%) |
|---|---|---|
| Low risk; RS ≤ 10, N = 1619 (16.7%) endocrine therapy | 84 | 93.7 |
| Intermediate risk; RS 11–25, N = 3399 (34.9%) endocrine therapy | 83.3 | 93.9 |
| Intermediate risk; RS 11–25, N = 3312 (34%) chemotherapy and endocrine therapy | 84.3 | 93.8 |
| High risk; RS ≥ 26, N = 1389 (14.4%) chemotherapy and endocrine therapy | 75.7 | 89.3 |

DFS = disease free survival; ITT = intention to treat; N = number; OS = overall survival; RS = recurrence score

ment in DFS when chemotherapy was added to endocrine therapy in intermediate risk group (HR for DFS for endocrine vs endocrine and chemotherapy: 1.08; 95 CI, 0.94–1.24, p = 0.26).[13] Estimated survival rate according to risk group are depicted in Table 2.

Exploratory analysis was conducted to search for any subgroups who might derive some benefit from chemotherapy in the intermediate risk group. An interaction between age and RS was found (p = 0.004), with some benefit of chemotherapy in younger patient population (< 50 years) with RS 16 to 25. In this group of patients there were 2% fewer distant recurrences when chemotherapy was added for RS 16–20, and 7% fewer for RS 21–25.[13] This information should be discussed with individual patients who fit in either category. The results of TAILORx suggest that Oncotype DX may identify up to 85% of women with HR+HER2- early BC older than 50 years with RS ≤ 25 and 40% of younger women (≤ 50 years ) with a RS ≤ 15 who can safely be spared adjuvant chemotherapy.[13]

We conclude that for patients with HR+HER2- lymph node negative BC patients older than 50 years RS 25 or more should be considered a cut-off point for adjuvant chemotherapy recommendation, whereas younger patients (less than 50 years) should be informed about the modest benefit of adding adjuvant chemotherapy at lower cut-off point (RS 16). For lymph node positive patients, the cut-off is less clear. Results from the published studies suggest that patients with HR+HER2- lymph node positive BC and RS < 18 do not benefit from adjuvant chemotherapy and for patients with RS ≥ 31 chemotherapy should be considered.[10,16-18] The results of the ongoing prospective trial RxPONDER (Treatment for Positive Node, Endocrine Responsive Breast Cancer) will give us further insight into RS cut-off point for chemotherapy benefit in lymph node positive BC.[19]

## MammaPrint

MammaPrint was developed by the Netherlands Cancer Institute group using DNA microarray analysis of gene expression arrays on frozen tissue from 78 primary BC tumors.[20] The gene expression panel contains 70 genes correlated with evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, limitless replicative potential, tissue invasion and metastasis and sustained angiogenesis.[21] A mathematical model is used to calculate score that stratifies patients into low- and high risk group.[20,22]

The first retrospective validation of MammaPrint was performed by van de Vijver and colleagues, on a consecutive series of 295 BC tumors (lymph node positive and negative). MammaPrint accurately distinguished a good-prognosis group which had a 10-year overall survival of 95% from a poor-prognosis group which had a 10-year overall survival of 55% (p ≤ 0,001).[22,23] However, there was one major disadvantage for the implementation of MammaPrint and this was the requirement for good quality RNA from fresh frozen tissue specimen. Improvements in RNA processing have enabled microarray diagnostics for formalin-fixed, paraffin-embedded (FFPE) tissue. Later on, MammaPrint was successfully translated to FFPE on 580 tumor samples.[24]

RASTER trial was the first prospective phase 3 trial assessing MammaPrint. This study confirmed the feasibility of collecting good quality fresh frozen tissue for analysis and confirmed prognostic value of MammaPrint in lymph node negative T1-T3 BC for distant recurrence and also compared it with Adjuvant!Online (AOL).[25,26] Other studies further investigated MammaPrint in patients with lymph node positive BC. In the study of Mook *et al.*, the prognostic value of MammaPrint was demonstrated to be superior to classical clinical-pathological factors in patients with 1–3 positive lymph nodes for predicting breast cancer specific survival (BCSS).[27]

Prospective, randomized, phase 3, MINDACT study (Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy) was performed to test the clinical utility of the addition of the MammaPrint to standard clinical–pathological criteria in selecting patients for adjuvant chemotherapy.[28-30] The study enrolled 6693 women with T1-T3 operable tumors, lymph node negative (app 80%) and positive (one to three positive lymph nodes). It was performed using fresh frozen tissue. MammaPrint was used to determine genomic risk and AOL version 8.0 was used to determine clinical risk. Low clinical risk was defined by low grade and tumor size <= 3cm, intermediate grade and tumor size <= 2cm, and high grade and tumor size <= 1cm, in lymph node negative patients, whereas only low grade and tumor size <= 2cm were considered low clinical risk in lymph node positive patients (Table 3). The patients were divided into four main groups, according to their clinical and genomic risk. Women at low clinical and genomic risk did not receive chemotherapy, whereas those at high clinical and genomic risk did receive such therapy. Patients

**TABLE 3.** Definition of high clinical risk tumors in MINDACT trial according to lymph node status

| Lymph node negative (N = 2114, 64%) | Lymph node positive (N = 1214, 36%) |
|---|---|
| G1, tumor size > 3 cm | G1, tumor size >2 cm |
| G2, tumor size > 2 cm | G2, any size |
| G3, tumor size > 1 cm | G3, any size |

**TABLE 4.** Distribution of risk groups according to clinical and genomic prediction and treatment assigned in MINDACT trial (N = 6693)

| Risk groups | Percentage N (%) | Treatment regimen |
|---|---|---|
| **Low clinical and low genomic** | 2745 (41.0) | no chemotherapy |
| **Low clinical and high genomic** | 592 (8.8) | randomization: chemotherapy vs no |
| **High clinical and low genomic** | 1550 (23.2) | randomization: chemotherapy vs no |
| **High clinical and high genomic** | 1806 (27.0) | chemotherapy |

**TABLE 5.** Estimated survival rates according to risk groups and treatment assigned in the intention-to-treat population

| | 5-year DFS (%) | 5-year OS (%) |
|---|---|---|
| **c-low/g-low** | 92.8 | 98.4 |
| **c-high/g-low: chemotherapy vs no chemotherapy** | 92.9 vs. 90.1 | 98.4 vs. 97.0 |
| **c-low/g-high: chemotherapy vs no chemotherapy** | 92.1 vs. 90.1 | 97.1 vs. 97.8 |
| **c-high/g-high** | 85.3 | 94.7 |

c-low/high = clinical low/high risk; g-low/high = genomic low risk/high

with discordant results were randomized to receive or not receive adjuvant chemotherapy (Table 4).[28-30]

Among patients at low clinical and high genomic risk, those who were randomized on the basis of genomic risk and therefore received chemotherapy had similar outcomes compared to those who were randomized to no chemotherapy on the basis of clinical risk.[28-30] Therefore, we can conclude that there is no use for MammaPrint risk assessment in patients with clinically low risk disease. Among patients at high clinical and low genomic risk, those who underwent randomization on the basis of clinical risk and received chemotherapy the DFS rate was 2.8 percentage points higher, and OS rate was 1.4 percentage points higher compared to those without chemotherapy. The study was not powered to assess the statistical significance of these differences or to exclude the benefit of chemotherapy.[28-30] But the results implicate that chemotherapy could be avoided in patients with high clinical and low genomic risk at a cost of the above mentioned differences and this should be discussed with a patient (Table 5). The use of MammaPrint in clinical high risk group would lead to a reduction in the use of adjuvant chemotherapy in 46.2% of patients.[28-30] In addition to this, ultra-low threshold was identified, which defines patients with indolent disease behaviour whose long-term risk of death from breast cancer is extremely low after surgery alone without any systemic therapy.[31]

## Other multigene signatures

Other prognostic multigene signatures have also been validated in clinical trials and some are recommended by international guidelines as well.

- **EndoPredict:** It is RNA-based and uses reverse transcriptase polymerase chain reaction of 12 genes to calculate prognostic score. It was validated retrospectively using prospectively collected data and tumor tissue from two Austrian Breast Cancer Study Group trials (ABCSG-6 and ABCSG-8). EndoPredict calculates a risk score, which can be used together with tumor size and nodal status for the calculation of a risk score (EPclin). Its applications include prediction of distant recurrence at 5 and 10 years in each individual patient and may add to decision about extended endocrine therapy.[32,33]
- **Predictor Analysis of Microarray 50 (PAM50):** PAM50 risk of recurrence score is a 50 gene test that uses microarray and quantitative reverse transcription polymerase chain reaction to provide a risk of recurrence score (ROR) that takes into account the PAM50 profile and clinical features of the patient, such as tumor size and proliferation score. ROR is used for prediction of individual risk of distant recurrence at 10 years. It was validated in lymph node negative as well as positive patients from ABCSG-8 and ATAC trial. The relationship between 10-year risk of distant recurrence and the ROR score differs markedly between node-negative and lymph-node positive patients (10- year risk of distant recurrence in low risk lymph node negative group was 4.9%, while in lymph node positive group (1–2 positive lymph nodes) 12.3%). Prosigna assay results are reported as ROR score from 0 to 100 in two ways, node-negative cancers are classified as low (0–40), intermediate (41–60), or high

(61–100) risk and node-positive cancers are classified as low (0–40) or high (41–100) risk.[34,35]

- **Breast Cancer Index (BCI):** The BCI is a score calculated according to 2-gene group expression, the 2-gene ratio HOXB13:IL17BR (H:I ratio) and the expression of 5 proliferation genes known as molecular grade index (MGI score). The TransATAC and the Stockholm trials in which patients received adjuvant endocrine therapy, provided the clinical validation. In postmenopausal patients with HR+HER2-, lymph node negative BC it might serve as a predictive test for the likelihood of benefit from extended adjuvant endocrine therapy.[36-38] This test has no FDA approval.

## Discussion

Prognosis of patients with early BC has improved significantly in the last two decades mostly due to effective adjuvant systemic treatment.[3,4] However, about two-thirds of patients with lymph node-negative BC are cured by loco-regional treatment already and they represent more than 50% of early BC patients.[39] Additionally 25–30% of patients with 1 to 3 positive lymph nodes remain free of distant metastases without adjuvant chemotherapy.[40] Therefore, these patients might safely be spared from toxic effects of chemotherapy. Based solely on traditional clinical-pathological characteristics it is not possible to reliably identify the high risk patients that would potentially benefit from adjuvant chemotherapy. Multigene signatures represent an important progress in optimal selection of these patients.[41] Their clinical utility for risk prediction was confirmed in different clinical studies. Oncotype DX and MammaPrint are the most extensively studied among them.

Oncotype DX and MammaPrint, both of them have demonstrated efficacy for evaluation of recurrence risk in women with stage I and II BC with up to 3 positive lymph nodes.[13,29] But from the published studies and clinical use, we can draw out some differences. MammaPrint provides a binary result for prognosis as low- and high-risk, whereas Oncotype DX provides also intermediate risk, which keeps clinicians in uncertainty. TAILORx study prospectively addressed this issue and provides strong evidence that chemotherapy is of limited benefit in this patient subgroup. Nevertheless, there are some patients (younger than 50 with RS 16-25) in the intermediate risk group that might derive some benefit from adjuvant chemotherapy. There were also some crucial differences in the inclusion criteria for the two studies testing the utility of MammaPrint and Oncotype DX.[13,29] According to these studies MammaPrint can be applied to a wider variety of patients, namely those with any ER status, largely as a result of gene selection the signature includes (mostly estrogen signalling genes in Oncotype DX), but this is of limited clinical utility.[28] While MammaPrint was validated also on lymph node positive BC patients (1-3positive lymph nodes), the evidence for the use of Oncotype DX in these patients population is weaker.[9] We are awaiting the results of RxPONDER trial, which will provide further information on this topic.[19] On the other hand, Oncotype DX is the only multigene signature that has both, prognostic and predictive value for chemotherapy sensitivity. The idea that prediction of treatment benefit can be concluded from prognosis is flawed and a statistical test for an interaction between a biomarker and treatment is necessary to determine biomarkers' predictive utility.[5,42,43] The findings from NSABP-B20, TAILORx, SWOG 8814 trials have confirmed a clear interaction between chemotherapy benefit and Oncotype DX result.[10,12,13]

One of the most important benefits of genomic testing is the selection of patient in which treatment with adjuvant chemotherapy can be safely omitted. However, the added value of multigene signatures for de-escalation of chemotherapy to no chemotherapy in daily clinical practice is still unclear. Eighty-five percent of older (>50 years) and 40% of younger patients in TAILORx trial and 46% of clinical high risk patients in MINDACT trial could be spared the addition of adjuvant chemotherapy. However, these numbers cannot be compared directly because the design and inclusion criteria for these two studies were different. The utility of multigene signatures was considered in all patients with tumors greater than 1 cm (or 5 mm and adverse characteristics) in TAILORx, while MammaPrint use was meaningful only in clinical high risk patients. Also the number of patients classified as low genomic risk varied significantly between the two tests; Oncotype DX identified only about 17% of patients as low genomic risk, and 69% as intermediate, whereas MammaPrint identified 64% in the whole population and 46% in clinical high risk population as low genomic risk.[13,30] If MINDACT criteria for definition for high clinical risk were applied to TAILORx population, 3.5% of patients with genomic low risk (low RS), 17.4% in intermediate RS and only 7.9% in high RS fit criteria for clinical high risk.

Some information on de-escalation of chemotherapy prescription by the use of multigene signa-

tures might be drawn from large studies performed on real-life patients cohorts. Use of Oncotype DX and MammaPrint was evaluated on 476,128 women from the National Cancer Database. Multigene signature use was associated with a significant decrease in rate of chemotherapy administration (24.6 vs. 37.2%). Chemotherapy was administered to a higher percentage of patients undergoing MammaPrint compared to Oncotype DX ( 41.3% vs. 23.4%, $p < 0.001$).[44]

Retrospective analysis that matched Oncotype DX results with SEER registry clinical data for over 40,000 node negative HR+HER-2-patients did not show lower chemotherapy use in real-life patients who had Oncotype DX performed compared to those without (22.7% vs. 22%), although Oncotype DX was prognostic for five-year breast-cancer-specific mortality.[45] Also in some other retrospective population-based cohorts, the use of multigene signatures did not lead to a reduction of chemotherapy use.[46-48]

Currently there is no data on which test provides the best prognostic information. In a systematic review which included 22 studies for Oncotype DX, 4 for MammaPrint, and 1 for both Prosigna and EndoPredict. The hypothetical application of chemotherapy for the same patient, with and without the results of the multigene test was analysed. A decrease in chemotherapy use for all tests was confirmed. When the results were pooled per assay, the decrease in chemotherapy to no chemotherapy was 45.7% for Oncotype DX and 32.2% for MammaPrint.[49]

Direct comparison of 6 multigene signatures (including Oncotype DX, EndoPredict, BCI, PAM50, Clinical Treatment Score (CTS) and 4-marker immunohistochemical score (IHC4) for prediction of distant recurrence in addition to clinical information was performed in the population of TransATAC trial. MammaPrint was not included in this study. All signatures provided similar prognostic information during the first 5 years of follow-up for lymph node negative patients, but PAM50, BCI, and EndoPredict were significantly more prognostic during 5–10 years, which may indicate they have molecular components that are more specifically prognostic for late recurrence, such as ER-signalling pathway. For women with 1 to 3 positive nodes, the independent prognostic strength of all of them was weaker.[50] The prospective OPTIMA trial compared performance of Oncotype DX, MammaPrint, PAM50 and IHC4 for evaluation of individual patient risk. Among these signatures a marked disagreement when applied to the same patient was found in the majority (60.6%) of tumors. From a biological perspective, it is entirely predictable that tests that measure different genes give dissimilar results. However, the proportions of patients identified as low, intermediate, or high risk were broadly similar irrespective of which test was used (low/intermediate risk: 82.1% for Oncotype DX, 72.0% for IHC4, 65.6% for Prosigna and 61.4% for MammaPrint).[51] No patient outcome data were available at the time of analysis and therefore we cannot draw any conclusion about the comparison on clinical utility of these tests. The performance of multiple gene signatures in one patient is not feasible in clinical practice.

Based on this, multigene expression signatures are endorsed as validated decision making tool in early BC by different international guidelines. However, there are differences regarding credibility of different multigene signatures given the number and quality of studies differ considerably among them (Table 6). St Gallen recommendation support the use of multigene signatures, however the recommendation is broad and does not support specific assay. The St Gallen Panel does not uniformly endorse the use of multigene signatures in node positive cases, although the panel agrees that they offer additional prognostic information in these patients. The same is true for ESMO guidelines which support multigene signature use (except for BCI) and are not specific as to the lymph node status.[2,8] All multigene signatures are recommended for use in HR+HER2- lymph node negative or positive BC by the European Group on Tumor Markers (EGTM), except for BCI.[6] Oncotype DX is the only multigene signature assigned with NCCN category of preference as preferred in lymph node negative patients (category 1 evidence) and is the only signature with predictive value, MammaPrint has category 1 recommendation as prognostic for lymph node negative and positive patients.[9]

**TABLE 6.** Recommendations for the use of multigene signatures in ER-positive, HER-negative breast cancer patients by different expert panels

| TEST | ASCO | NCCN | ESMO* | St Gallen Group* | EGTM |
|---|---|---|---|---|---|
| **Oncotype DX** | Ln - , strong | Ln -, 1<br>Ln +, 2A | IB | Yes | Ln +/- |
| **MammaPrint** | Ln -, strong<br>Ln +, moderate | Ln -/+, 1 | IB | Yes | Ln +/- |
| **PAM50** | Ln -, moderate | Ln -/+, 2A | IB | Yes | Ln +/- |
| **EndoPredict** | Ln -, moderate | Ln -/+, 2A | IB | Yes | Ln +/- |
| **BCI** | Ln -, moderate | Ln NR, 2A | no | Yes | Ln - |

Ln = lymph nodes; NR = not reported

ASCO guidelines strongly recommend the use of Oncotype DX and MammaPrint in lymph node negative patients and MammaPrint is the only multigene signature endorsed by ASCO guidelines for lymph node positive patients (Table 6).[7]

At the time being we do not know which of the multigene signature has the most accurate prognostic value. However, Oncotype DX and MammaPrint have currently the most extensive level of evidence and are most widely used. The decision to choose one of them is in most cases based on individual oncologist experiences. Nevertheless, price and accessibility might be also important since in many European countries as well in Slovenia the test is still not covered by the insurance companies. Future studies and data from national and institutional patient's registries will help us to more optimally guide the use of appropriate multigene signatures and subgroups for testing and give us information on long-term outcome in order to determine the place of these assays in daily clinical practice.

## Conclusions

Multigene signature assays provide prognostic information that augments the one from clinical-pathologic features and reflects tumor biology. Decisions that rely solely on clinical-pathological factors may often lead to overtreatment and in these cases the information provided by multigene signatures may reduce the use of unnecessary adjuvant chemotherapy without increasing the risk of relapse. In contemporary management of HR+HER2- early BC clinical decisions regarding adjuvant systemic therapy should be made after considering both genomic results and clinical-pathological features. However, risk stratification according to clinical-pathological features still remains crucial and multigene signature assays should be used mostly for cases where clinical-pathological parameters do not clearly imply or oppose the benefit of chemotherapy.

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*research article*

# Relationship between sex hormones levels and $^{18}$F-FDG uptake by the ovaries in premenopausal woman

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**Background.** The study was conducted to evaluate the effect of sex hormones on F-18 fluorodeoxyglucose ($^{18}$F-FDG) uptake by normal ovaries.

**Patients and methods.** A total of 197 premenopausal women were included in this study. Based on $^{18}$F-FDG positron emission tomography/computed tomography (PET/CT) images obtained from these subjects, the association of ovarian $^{18}$F-FDG uptake with levels of sex hormones, including estradiol, progesterone, testosterone, follicle-stimulating hormone, and luteinizing hormone was investigated. We also analysed the relationship between the menstrual cycle and ovarian $^{18}$F-FDG uptake.

**Results.** The highest ovarian $^{18}$F-FDG uptake occurred at 2 weeks after the onset of menstruation (median maximum standardized uptake value [SUVmax] = 3.40, median mean SUV [SUVmean] = 2.20), and the lowest ovarian $^{18}$F-FDG uptake was observed during the first week of the menstrual cycle (median SUVmax = 1.60, median SUVmean = 1.20). Ovarian $^{18}$F-FDG uptake was weakly positively correlated with progesterone levels (*rho* = 0.28, $p < 0.001$ for SUVmax, *rho* = 0.30, $p < 0.001$ for SUVmean), and this pattern was consistently observed in subjects in the follicular-phase group (*rho* = 0.29, $p = 0.003$ for both SUVmax and SUVmean) but not in subjects in the luteal-phase group.

**Conclusions.** Based on PET images, ovarian glucose metabolism in premenopausal women tended to increase slightly with increasing progesterone concentration.

Key words: ovary; fluorodeoxyglucose F18; positron-emission tomography; gonadal steroid hormones

## Introduction

$^{18}$F-FDG PET, which utilizes a radiolabeled analogue of glucose, is widely used clinically in patients with cancer to evaluate staging and the therapeutic response.[1] However, $^{18}$F-FDG uptake may be high not only in malignant lesions but also in normal tissues.[2-4] High uptake of $^{18}$F-FDG in the pelvic region of women, particularly by the ovaries, can be confusing for nuclear-medicine physicians interpreting PET images.

The ovary is a major reproductive organ characterized by cyclic changes in sex hormones, which are believed to affect the degree of $^{18}$F-FDG uptake by the ovary. Ovarian $^{18}$F-FDG uptake is typically negligible in postmenopausal women[5,6]; if incidental ovarian $^{18}$F-FDG uptake is found on PET, the possibility of a malignant lesion is sufficiently high to recommend further evaluation by magnetic resonance imaging (MRI) or ultrasonography.

However, in premenopausal women, ovarian $^{18}$F-FDG uptake occurs even under normal or be-

nign conditions such as a follicular ovarian cyst or hemorrhagic corpora lutea.[7] Therefore, in daily practice, nuclear-medicine physicians do not routinely recommend further radiographic evaluation in the event of ovarian $^{18}$F-FDG uptake. This makes it difficult to draw conclusions about ovarian $^{18}$F-FDG uptake in premenopausal women. We investigated physiological ovarian $^{18}$F-FDG uptake, since previous studies on ovarian $^{18}$F-FDG uptake in premenopausal women are limited and most focused on the degree of change in $^{18}$F-FDG uptake during the menstrual cycle.[5,6,8,9] Furthermore, how sex hormones affect this uptake has not been clearly determined.

The purpose of this study was to investigate the relationship between sex hormones levels and $^{18}$F-FDG uptake by ovaries in premenopausal woman.

## Patients and methods

### Patients

This study included 197 premenopausal women (median age, 44 years) who were diagnosed with breast cancer between March 2015 and July 2017 and underwent $^{18}$F-FDG PET/CT to determine pretreatment status, as well as sex-hormone assays (including estradiol, progesterone, testosterone, follicle-stimulating hormone [FSH] and luteinizing hormone [LH]). Hormone assays were performed on the same day as PET. The date of the last normal menstrual period (LNMP) of all patients was also recorded and used to divide the women into two groups based on their menstrual phase on the day of the tests: a follicular-phase group (days 1–13) and a luteal-phase group (days 14–31). We also noted how many weeks had elapsed since the date of onset of menstruation. Patients were excluded if they had a history of ovarian disease, sex hormone therapy or an irregular menstrual cycle of more than 31 days.[10]

The clinical design of this retrospective study was approved by the Institutional Review Board of Ajou University (AJIRB-MED-OBS-18-354). The need for informed consent was waived.

### $^{18}$F-FDG PET/CT protocol

After fasting for at least 6 hours, patients were administered 5 MBq/kg $^{18}$F-FDG intravenously. The blood glucose level at the time of the $^{18}$F-FDG injection was < 8.3 mmol/L in all patients. Patients were instructed to rest comfortably for 60 min, and to urinate before the scan. Whole-body PET/CT images were obtained with a Discovery ST 8 slice CT scanner or Discovery STE 16 slice CT scanner (GE Healthcare, Milwaukee, WI, USA). Seven or eight frames (3 min/frame) of PET emission data were acquired in three-dimensional mode after a noncontrast CT scan from the base of the skull to the upper thigh (120 kV, 30–100 mA in the AutomA mode; section width = 3.75 mm). Emission PET images were reconstructed using an iterative method (ordered-subsets expectation maximization with two iterations and 20 subsets; field of view = 600 mm, slice thickness = 3.27 mm) and attenuation was corrected with noncontrast CT.

### Image analysis

A nuclear-medicine specialist with 13 years of PET experience, blinded to the clinical data, reviewed the $^{18}$F-FDG PET/CT images on an AW workstation (version 4.4; General Electric Healthcare, Chicago, IL, USA). The volume of interest (VOI) was placed on the ovarian area showing higher $^{18}$F-FDG uptake than the background activity on the PET image. If there was no PET uptake, the VOI was drawn on the right and left ovarian areas on the CT image. The SUVmax and SUVmean were calculated from these VOIs based on injected dose and body weight. The higher values of the right and left ovarian SUVs were used for the statistical analysis.

### Statistical analysis

All statistical analyses were done using MedCalc Statistical Software (ver. 18.5; MedCalc Software bvba, Ostend, Belgium). First, we calculated the required sample size. A significance (a) level of 5% and statistical power (1 − b) of 80 % were considered acceptable for the purposes of the study. A sample size of 145 patients was required to attain an appropriate confidence range; thus, the obtained sample size (N = 197 patients) was sufficient for the statistical analysis.

The Kolmogorov–Smirnov test was used to assess whether the data were normally distributed. None of the data followed a normal distribution, so they are presented as medians and interquartile range (IQR). The Mann–Whitney test was used to compare groups distinguished based on the menstrual phase. The Kruskal–Wallis test was used to compare ovarian $^{18}$F-FDG uptake among groups distinguished based on weeks since onset of menstruation. If the Kruskal–Wallis test was significant, a post-hoc analysis was performed for pairwise

**TABLE 1.** Patient characteristics

| | Menstrual phase on test day | | Total | p-value |
|---|---|---|---|---|
| | Follicular phase | Luteal phase | | |
| Number of patients, n (%) | 100 (50.8) | 97 (49.2) | 197 (100) | |
| Age, years | 45 (42–47) | 44 (38–47) | 44 (40–47) | 0.150 |
| Sex hormone levels | | | | |
| Estradiol, pg/ml | 125 (76–176) | 150 (84–182) | 126 (81–179) | 0.292 |
| Progesterone, ng/ml | 1.62 (1.26–2.53) | 12.30 (3.85–18.95) | 2.71 (1.42–12.11) | < 0.001* |
| Testosterone, ng/ml | 0.34 (0.21–0.52) | 0.37 (0.23–0.51) | 0.36 (0.23–0.51) | 0.342 |
| FSH, mIU/ml | 5.7 (3.9–8.1) | 2.9 (1.7–4.1) | 3.7 (2.4–6.1) | < 0.001* |
| LH, mIU/ml | 5.9 (2.9–8.6) | 3.6 (2.5–6.6) | 4.6 (2.7–7.8) | 0.105 |
| SUVmax of ovary | 1.70 (0–2.70) | 2.10 (1.50–3.63) | 2.00 (0–3.23) | 0.030* |
| SUVmean of ovary | 1.30 (0-1.85) | 1.60 (0.9–2.40) | 1.40 (0–2.10) | 0.015* |

All continuous variables are shown as medians (interquartile range). *p-value < 0.05

FSH = follicular-stimulating hormone; LH = luteinizing hormone; SUVmax = maximum standardized uptake value; SUVmean = mean standardized uptake value

comparisons of the subgroups. Spearman's rank coefficient correlation test was used to examine the correlations between the ovary SUVs and levels of sex hormones. Correlations were classified as very weak ($|rho| < 0.20$), weak ($|rho| = 0.20–0.39$), moderate ($|rho| = 0.40–0.59$), strong ($|rho| = 0.60–0.79$), or very strong ($|rho| \geq 0.80$).[11] All P values < 0.05 were considered significant.

## Results

Of all patients, 100 (50.8%) underwent $^{18}$F-FDG PET in the follicular phase and 97 (49.2%) in the luteal phase. Progesterone levels were significantly higher in the luteal-phase group than in the follicular-phase group (12.30 vs. 1.62 nmol/L, $p < 0.001$) and FSH was significantly higher in the follicular-phase group than in the luteal-phase group (5.7 vs. 2.9 mIU/mL, $p < 0.001$). Age, and the levels of all other sex hormones measured (estradiol, testosterone, and LH), did not differ significantly between the two groups. The median SUVmax and SUVmean values of the ovaries in all subjects were 2.00 and 1.40, respectively, and these values were significantly higher in the luteal-phase group than in the follicular-phase group (2.10 vs. 1.70, $p = 0.030$ for SUVmax, 1.60 vs. 1.30, $p = 0.015$ for SUVmean). The patient characteristics are summarized in detail in Table 1.

In total, 71, 45, 53, and 28 subjects were tested at 1, 2, 3 and 4 weeks after the onset of menstruation, respectively, and the degree of $^{18}$F-FDG uptake by the ovaries differed significantly among these groups ($p < 0.001$ for both SUVmax and SUVmean). Post-hoc analysis showed that the group with the highest $^{18}$F-FDG uptake was the group tested 2 weeks after the onset of menstruation (median SUVmax = 3.40 [IQR 2.00–4.93], median SUVmean = 2.20 [IQR 1.50–3.13]), and SUV values in this group were significantly higher than those in all other groups. The lowest levels of ovarian $^{18}$F-FDG uptake were observed in the group tested at 1 week after the onset of menstruation; the SUV values (median SUVmax = 1.60 [IQR 0–2.18], median SUVmean = 1.20 [IQR 0–1.50]) of this group were significantly lower than those of the other groups, except those tested at 4 weeks after the onset of menstruation. $^{18}$F-FDG uptake by the ovaries in the group tested at 3 weeks after the onset of menstruation (median SUVmax = 2.00 [IQR 0.68–3.33], median SUVmean = 1.40 [IQR 0.45 –2.23]) differed significantly from that in the other groups, except that in the group tested at 4 weeks after the onset of menstruation (median SUVmax = 1.80 [IQR 1.50–2.30], median SUVmean = 1.45 [IQR 1.15–1.70]). Figure 1 shows the ovarian $^{18}$F-FDG uptake values by group.

The results for the entire cohort showed that the ovarian SUVmax and SUVmean values were weakly positively correlated with progesterone levels (*rho* = 0.28, $p < 0.001$ for SUVmax, *rho* = 0.30, $p < 0.001$ for SUVmean) (Figure 2). Levels of the other sex hormones (estradiol, testosterone, FSH, and LH) were not correlated with ovarian $^{18}$F-FDG uptake. The ovarian SUVmean was very weakly correlated with age (*rho* = 0.15, $p = 0.035$).

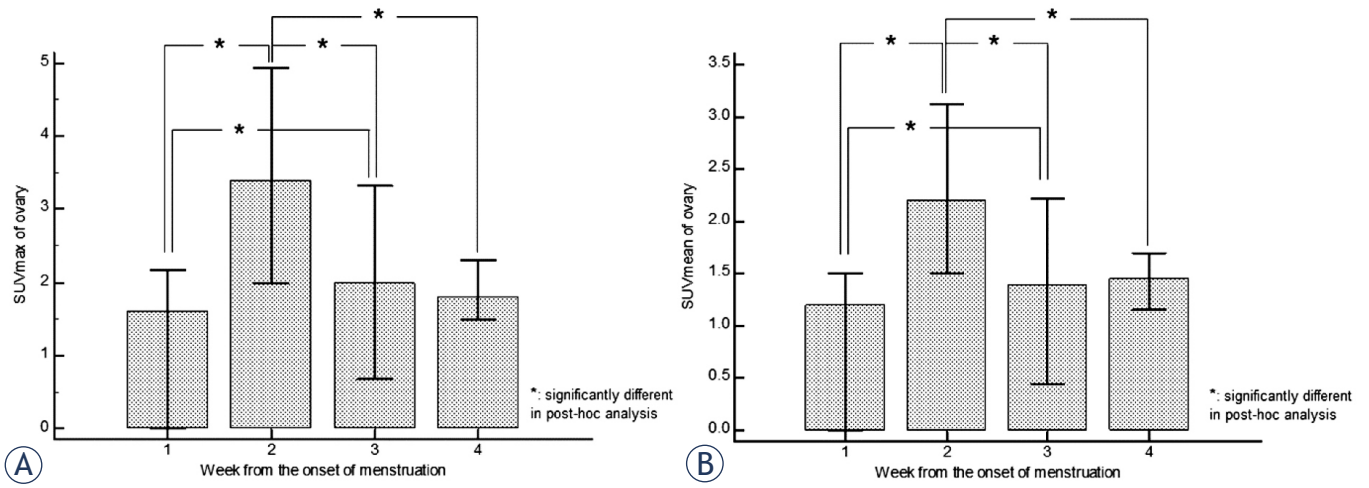


**FIGURE 1.** Ovarian $^{18}$F-FDG uptake according to the number of weeks since the onset of menstruation. **(A)** The ovarian maximum standardized uptake value (SUVmax) was highest at 2 weeks after the onset of menstruation and lowest during the first week after menstruation. The ovarian SUVmax at weeks 3 and 4 was between that for the first and second weeks. **(B)** Comparisons of ovarian SUVmean showed a trend similar to that of SUVmax.

Analysis of the phase groups revealed that when progesterone levels were high, ovarian $^{18}$F-FDG uptake was slightly higher (*rho* = 0.29, $p$ = 0.003 for both SUVmax and SUVmean) in the follicular-phase group. In the luteal-phase group, no correlation was found between sex-hormone levels and ovarian $^{18}$F-FDG uptake. The data and statistical analysis results are shown in detail in Table 2.

## Discussion

Ovarian $^{18}$F-FDG often presents a challenge to nuclear-medicine physicians interpreting $^{18}$F-FDG PET/CT images. Ovarian $^{18}$F-FDG uptake can occur depending on the phase of the menstrual cycle, particularly in premenopausal women[6, 9], but a clear mechanism for this remains unclear. We postulated that sex hormones are associated with ovarian $^{18}$F-FDG uptake and aimed to evaluate this relationship.

Our study found that progesterone was weakly positively correlated with ovarian $^{18}$F-FDG uptake in all subjects in the follicular-phase group, but none of the sex hormones was correlated with ovarian $^{18}$F-FDG uptake in the luteal-phase group. Progesterone acts on the endometrium and plays a major role in preparing the uterus for implantation

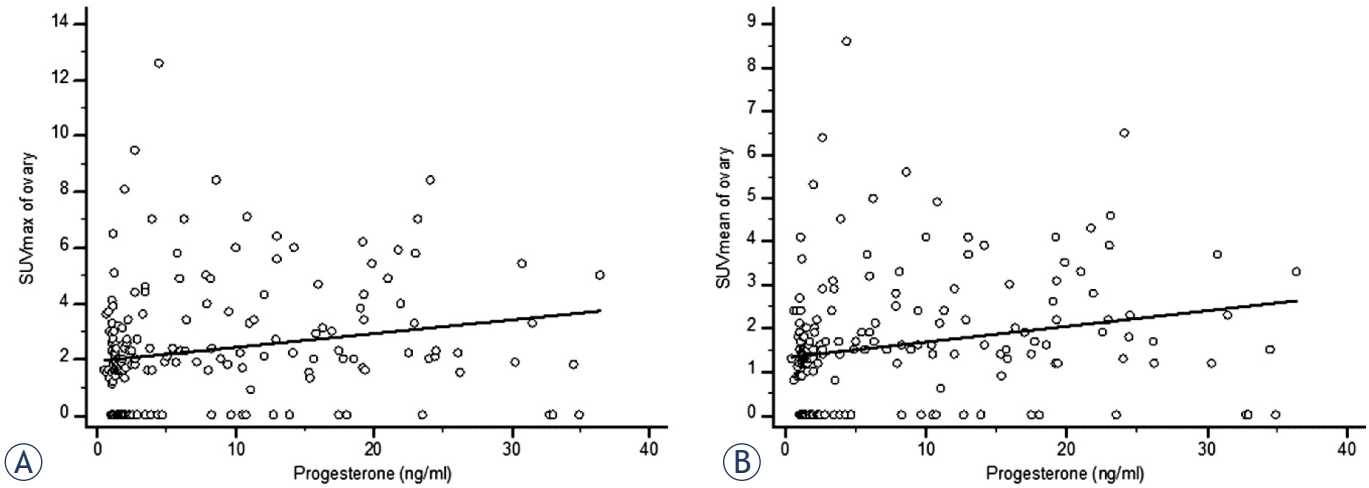


**FIGURE 2.** Scatter diagrams of the relationship between progesterone level and ovarian $^{18}$F-FDG uptake in all subjects. **(A)** Progesterone levels were weakly correlated with ovarian SUVmax ($p$ < 0.001, *rho* = 0.28). **(B)** A weakly positive correlation was also detected between progesterone level and ovarian SUVmean ($p$ < 0.001, *rho* = 0.30).

**TABLE 2.** Correlation between ovarian $^{18}$F-FDG uptake and sex hormone levels

| Parameters | | Follicular phase (n = 100) | | Luteal phase (n = 97) | | Total (N = 197) | |
|---|---|---|---|---|---|---|---|
| | | Spearman's rho (95% CI) | p-value | Spearman's rho (95% CI) | p-value | Spearman's rho (95% CI) | p-value |
| Age | Correlation with SUVmax of ovary | −0.15 (−0.34–0.04) | 0.125 | −0.06 (−0.26–0.13) | 0.500 | −0.14(−0.27–0.01) | 0.051 |
| Estradiol | | 0.12 (−0.07–0.30) | 0.235 | −0.03 (−0.23–0.16) | 0.721 | 0.05 (−0.08–0.19) | 0.426 |
| Progesterone | | 0.29 (0.09–0.45) | 0.003* | 0.13 (−0.07–0.32) | 0.131 | 0.28 (0.14–0.40) | <0.001* |
| Testosterone | | −0.08 (−0.27–0.12) | 0.428 | 0.13 (−0.07–0.32) | 0.129 | 0.03 (−0.11–0.17) | 0.669 |
| FSH | | -0.18 (−0.37 to −0.01) | 0.082 | 0.18 (−0.10–0.43) | 0.200 | −0.10 (−0.16 to −0.04) | 0.174 |
| LH | | 0.07 (−0.24–0.38) | 0.657 | −0.01 (−0.28–0.27) | 0.973 | −0.02 (−0.24–0.18) | 0.792 |
| Age | Correlation with SUVmean of ovary | −0.14 (−0.32–0.05) | 0.164 | −0.10 (−0.29–0.09) | 0.318 | −0.15 (−0.28–0.01) | 0.035* |
| Estradiol | | 0.09 (−0.09–0.29) | 0.324 | −0.06 (−0.26–0.13) | 0.532 | 0.03 (−0.10–0.17) | 0.601 |
| Progesterone | | 0.29 (0.09–0.46) | 0.003* | 0.16 (−0.04–0.35) | 0.161 | 0.30 (0.17–0.42) | <0.001* |
| Testosterone | | −0.13 (-0.31–0.06) | 0.197 | 0.13 (−0.06–0.32) | 0.193 | 0.01 (−0.12–0.15) | 0.840 |
| FSH | | −0.16 (−0.33–0.07) | 0.122 | 0.20 (−0.08–0.45) | 0.154 | −0.13 (−0.18 to −0.05) | 0.116 |
| LH | | −0.67 (−0.39–0.27) | 0.700 | 0.01 (−0.27–0.29) | 0.954 | −0.04 (−0.25–0.17) | 0.690 |

*p < 0.05

CI = confidence interval; FSH = follicle-stimulating hormone; LH = luteinizing hormone; SUVmax = maximum standardized uptake value; SUVmean = mean standardized uptake value

and pregnancy from the late proliferative phase to the luteal phase.[12, 13] The finding that progesterone levels correlated with ovarian glucose metabolism in the follicular phase may be explained as follows. During most of the follicular phase, progesterone is secreted from the adrenal cortex; this secretion is regulated by unknown elements in the ovary. Progesterone secretion switches from the adrenal cortex to the ovaries before ovulation.[14] In other words, progesterone secretion during the follicular phase is the result of a complex interaction between the adrenal cortex and the ovaries, which may account for the correlation between progesterone levels and ovarian glucose metabolism during this period. We acknowledge that this hypothesis is speculative and further research will be needed for confirmation. Estradiol and FSH, which play important roles in follicular growth, and LH, which plays a key role in ovulation[15], were not correlated with ovarian $^{18}$F-FDG uptake; this has rarely been reported, so interpreting this finding is difficult. The relationship between the ovaries and sex-hormone levels is very complex and therefore difficult to elucidate. This study is significant in that it is the first to reveal a relationship between ovarian $^{18}$F-FDG uptake and sex hormone levels, but more research will be needed to clarify the mechanism of their relationship.

Another noteworthy result of this study was that ovarian $^{18}$F-FDG uptake was very weakly negatively correlated with age. With aging, the ovaries gradually decrease in function.[16] Our results show that changes in ovarian metabolism due to aging are reflected in $^{18}$F-FDG uptake.

Lerman *et al.* reported that $^{18}$F-FDG uptake was detectable in normal ovaries during the ovulatory phase in premenopausal women.[5] Nishizawa *et al.* reported increased ovarian $^{18}$F-FDG uptake from the late follicular phase to the early luteal phase.[8] Kim *et al.*, demonstrated that increased ovarian $^{18}$F-FDG uptake occurs mainly 10–25 days after the onset of menstruation, which corresponds to the late follicular, ovulatory, and early to mid-luteal phases.[9] Our results do not differ from those of previous studies. In our study, ovarian $^{18}$F-FDG uptake was highest at 2 weeks after the onset of menstruation, when the follicle is actively proliferating, implying $^{18}$F-FDG uptake by the pre-ovulatory follicle. In addition, ovarian $^{18}$F-FDG uptake was higher during the luteal phase than during the follicular phase, which may have been due to increased $^{18}$F-FDG uptake by the corpora lutea in this phase. A possible reason for the increased $^{18}$F-FDG uptake by the pre-ovulatory follicle and corpus luteum is as follows. Active growth of pre-ovulatory follicles has been associated with increased metabolic demand and glucose-transporter-3 expression, which is regulated by interleukin-1β during the peri-ovulatory phase, leading to increased $^{18}$F-FDG uptake.[17] Angiogenesis and the cytokine-mediated inflammatory reaction have been suggested as being involved in $^{18}$F-FDG uptake by the

corpus luteum, because the mechanism of corpus luteal formation is similar to those of wound healing and tumour formation.[18] In our study, the lowest $^{18}$F-FDG uptake observed was at 1 week after the onset of menstruation, which may have been due to lower $^{18}$F-FDG uptake in small follicles before growth.[9]

This study included only subjects with regular menstrual cycles, and excluded those who had a menstrual cycle exceeding 31 days. Patients with irregular menstrual cycles were excluded from the study because of a concern that their sex hormones would not be in balance. Most previous reports included subjects with irregular menstrual cycles[5,8,9], and the results of those studies are likely to have been biased accordingly. Therefore, a strength of the present study was that it excluded such subjects.

In this study, progesterone was higher in the luteal-phase group than in the follicular-phase group, while FSH levels were significantly higher in the follicular-phase group than in the luteal-phase group. Changes in sex hormones during the menstrual cycle have been well documented, and the high progesterone levels observed during the luteal phase and elevated FSH levels observed during the follicular phase are consistent with previous data.[19] The results demonstrate that the self-reported LNMP data of our subjects accorded with the actual levels of the sex hormones, thus demonstrating the accuracy and objectivity of this study.

The limitations of this study were as follows. First, the ovarian status of the subjects was evaluated using non-contrast enhanced CT, as part of the PET/CT examination. The ovary can be examined more precisely by contrast-enhanced CT, MRI, or ultrasonography, but this study was retrospective, so it was not possible to perform these radiological examinations in all patients. However, the ovaries of the subjects showed no pathological lesions on non-enhanced CT. Future studies will require more detailed radiological examinations to validate our results. Second, this study did not report results regarding uterine $^{18}$F-FDG uptake, although numerous studies have reported $^{18}$F-FDG uptake by the endometrium as well as the ovaries.[5,6,8,20] However, in our study, many subjects had small or large uterine myomas (51/197; 25.8%,), so it was difficult to determine normal uterine $^{18}$F-FDG uptake. Myomas are associated with varying levels of $^{18}$F-FDG uptake, so we judged that assessing physiological uterine $^{18}$F-FDG uptake would be problematic. Finally, this study included patients with breast cancer, but the design might have been more appropriate for healthy volunteers; unfortunately, we were constrained to retrospective use of $^{18}$F-FDG PET/CT data in patients with breast cancer, albeit that we attempted to evaluate physiological ovarian uptake by omitting patients who had a previous history of ovarian disease or hormone therapy.

## Conclusions

Ovarian $^{18}$F-FDG uptake in premenopausal women was positively correlated with progesterone levels. This correlation was detected during the follicular phase of the menstrual cycle, while the other sex hormones measured (estradiol, testosterone, FSH, and LH) were not associated with ovarian $^{18}$F-FDG uptake.

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*research article*

# Local recurrence of soft tissue sarcoma: a radiomic analysis

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**Background.** To perform a radiomics analysis in local recurrence (LR) surveillance of limb soft tissue sarcoma (STS)

**Patients and methods.** This is a sub-study of a prospective multicenter study with Institutional Review Board approval supported by ESSR (European Society of Musculoskeletal Radiology). radiomics analysis was done on fast spin echo axial T1w, T2w fat saturated and post-contrast T1w (T1wGd) 1.5T MRI images of consecutively recruited patients between March 2016 and September 2018.

**Results.** N = 11 adult patients (6 men and 5 women; mean age 57.8 ± 17.8) underwent MRI to exclude STS LR: a total of 33 follow-up events were evaluated. A total of 198 data-sets per patients of both pathological and normal tissue were analyzed. Four radiomics features were significantly correlated to tumor size ($p < 0.02$) and four radiomics features were correlated with grading ($p < 0.05$). ROC analysis showed an AUC between 0.71 (95%CI: 0.55–0.87) for T1w and 0.96 (95%CI: 0.87–1.00) for post-contrast T1w.

**Conclusions.** radiomics features allow to differentiate normal tissue from pathological tissue in MRI surveillance of local recurrence of STS. radiomics in STS evaluation is useful not only for detection purposes but also for lesion characterization.

Key words: sarcoma; recurrence; magnetic resonance imaging; ROC curve

## Introduction

radiomics is an advanced quantitative image features analysis defined as the conversion of clinical images to higher dimensional data and the subsequent mining of these data for improved decision support in research and clinical practice.[1] The majority of clinically available medical images can potentially be evaluated with radiomics analysis. In this perspective, images of computed tomography, Magnetic Resonance Imaging (MRI), ultrasound, mammography or digital breast tomosynthesis and Fluorodesoxyglucose Positron-emission tomography-computed tomography (FDG PET/CT) include more data than what is visible on human eyes.[1-3] Indeed, mathematical algorithms of radiomics examine hundreds of quantitative images considering medical images as data sources. The extracted imaging data could be the product of the mechanisms occurring at a genetic and molecular level linked to the genotypic and phenotypic characteristics of the tissue.[4-7] Among its applications, radiomics has been evaluated to differentiate normal and pathological tissue.[1-3,5] Hence, we thought about the potential use of radiomics in local recurrence surveillance of soft tissue sarcoma. The incidence of local recurrence (LR) of STS is about 6,5%–25% and is associated with poor outcome for patients.[7] According to the American College of Radiology (ACR) Appropriateness Criteria guidelines, MRI is the most appropriate imaging test for LR surveillance of malignant or aggressive musculoskeletal soft-tissue tumors.[8] However, data informing the appropriate use of MRI in the

surveillance setting are conflicting.[5,8,9] Indeed, MRI can differentiate local recurrence from post-surgical seroma, hematoma, inflammation and scarring, but some post-operative changes in the surgical bed can be similar to those of recurrence with conventional T1-weighted, T2- weighted, and post-contrast sequences posing diagnostic dilemmas especially when sarcoma recurrence has low signal intensity on fluid-sensitive images.[1,8-11] We hypothesized that radiomics analysis of MRI of patients undergoing follow-up for STS allows to differentiate normal tissue from pathological tissue of LR. Therefore, the aim of our study was to perform a radiomics analysis of MRI of patients undergoing local surveillance for Soft-tissue sarcoma recurrence.

**TABLE 1.** MRI Parameters

| Manufacturer | Siemens Healthcare, Erlangen, Germany |
|---|---|
| **T1-weighted MR imaging** | |
| Repetition time / echo time (TR/TE) | 500/8 |
| Acquisition voxel size ($mm^3$) | 0.6x0.7x3.0 |
| **T2-weighted MR imaging*** | |
| Repetition time / echo time (TR/TE) | 6200/110 |
| Acquisition voxel size ($mm^3$) | 0.6x0.7x3.0 |
| **T1-weighted MR imaging* with Gadolinium** | |
| Repetition time / echo time (TR/TE) | 5/3 |
| Acquisition voxel size ($mm^3$) | 0.6x0.7x3.0 |

* T2-weighted MR imaging and T1- weighted MR imaging with Gadolinium are acquired with fat-saturation

## Patients and methods

This is an exploratory study of an ongoing Italian prospective (blind) multicenter study with institutional review board approval (blind). Written informed consent was obtained from participants. This study is endorsed by ESSR (European Society of Musculoskeletal Radiology). Prospective recruitment of patients, as per protocol, includes MRI and US with commercially available equipment. MRI parameters of sequences included in the radiomics analysis are reported in Table 1.

### Patients

All consecutive MRI Images of follow-up events acquired between March 2016 to September 2018 were included. A follow-up event was considered a complete MRI assessment to exclude STT LR. Inclusion criteria were: patients included were 18 years and older operated on for localized soft tissue sarcomas of the limb. Exclusion criteria were patients unable to understand or execute written informed consent, unable or unwilling to agree to follow-up during observation period and patients with metastatic disease.

### Experimental design

This study focused on finding if some radiomics features could discriminate on MRI patients who had confirmed significant disease at histology (LR) from those who did not. To ensure unbiased assessment, all MRI annotations were performed blinded to the biopsy findings. Only cases with matched locations (surgery, biopsy, reports, and radiologist's delineations) were considered for inclusion in this study.

Two data-sets were created: pathological and control.

Each data set includes MRI images each with distinctive Regions of interest (ROIs). ROIs were positioned by two researchers (blind and blind; R1 and R2 respectively) expert in quantitative image analysis (8 and 4 years of experience) blindly one from each other. R1 and R2 ROIs data were used separately for intra- and inter-observer agreement estimation while the mean value was used for other estimations. Discrepancies higher that 15% between R1 ROI and R2 ROI were handled with arbitration.

Regions of interest including all the visible tumor or suspicious area represented the pathological data-set. Regions of interest in the same slices of tissue with no imaging evidence of recurrence according radiological assessment represented the control data-set. ROIs of control-data set were positioned not before 20 mm to the tumor to exclude inclusion of tumoral tissue. We did not include cancer cases with recurrences detected only with US. Image analysis was done per-lesion and not per-patient (Figure 1).

### Radiomics analysis

Radiomics analysis was performed on all MRI images included in pathological and control data-set within manually selected ROIs (Figure 1) as previously done for other 3D radiological techniques.[2] From each image, we extracted 104 image features using an open-source software platform for

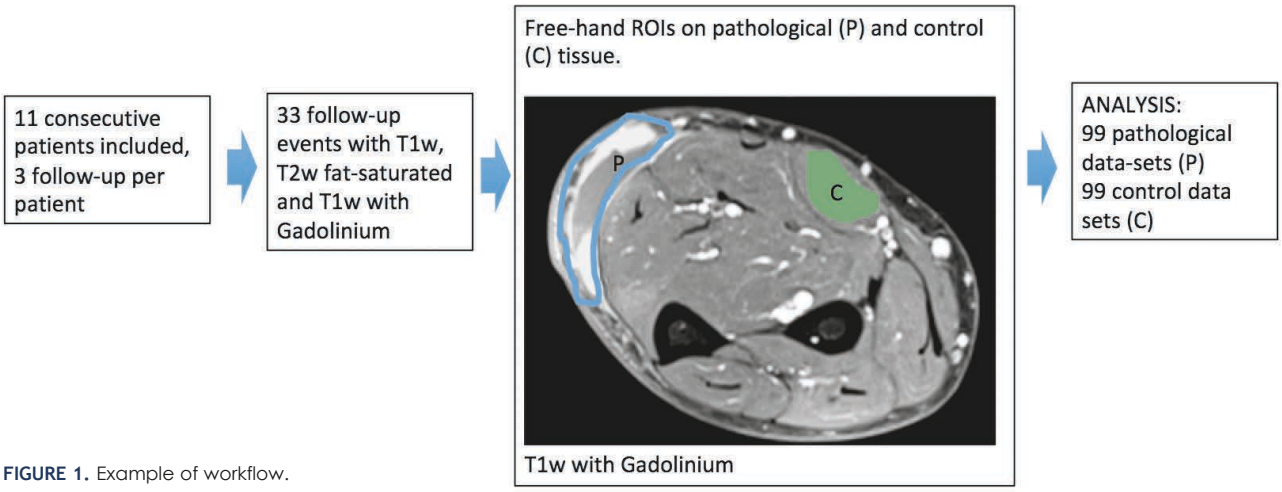


**FIGURE 1.** Example of workflow.

medical image informatics, image processing, and three-dimensional visualization (3D Slicer 4.7; www.slicer.org) built over two decades through support from the National Institutes of Health and a worldwide developer community. 3D-Slicer can be employed for quantitative image feature extraction and image data mining research in large patient cohorts.[6]

### Preprocessing

All patients were examined by using a 1.5-Tesla equipment (Magnetom Avanto, Siemens Heathcare) with a standard protocol. For each patient, T1-weighted (T1w), Gd-enhanced T1-weighted (T1WGd) with 0.1-mmol/kg doses of gadoteric acid and T2-weighted with fat saturation (T2w fs) volume images were resampled to an uniform pixel spacing of 0.5 x 0.5 x 3mm. Then they were cropped to the lesion region of interest as delineated by R1 and R2. Inhomogeneity correction was applied to T1w and T2w fat saturated images to account for the presence of bias field artifacts. Next, T1w and T2w fs images were corrected for inherent acquisition-to acquisition signal intensity variations (non standardness) using scale-based standardization. This procedure was applied to mitigate the inherent drift phenomenon that accompanies MRI intensities as previously done in literature.[12]

### Statistical analysis

**A)** Comparison of radiomics features of normal and pathological tissue on MRI was done with non-parametric tests (Mann-Whitney U test for unpaired data with 1000 bootstraps samples to compare patients and controls) considering a p value of 0.05 as statistically significant. **B)** Accuracy was measured using receiver operating characteristic (ROC) analyses to estimate the area under the curve (AUC) and by estimating thresholds for sensitivity and specificity for the radiomics features that significantly differed between patients and controls, considering the mean value, to avoid over-fitting. Ninety-five percent confidence intervals (95%CIs) were calculated. Using statistical software, p values below 0.05 were considered statistically significant. **C)** Correlation analysis and univariate linear regression were performed to determine the association between the radiomics features and the corresponding clinical and prognostic parameters. Bonferroni correction was used to adjust for multiple comparisons. **D)** Mean time for single patient radiomics analysis was also calculated. Reading time was estimated with a commercially available stopwatch including the time to download images, perform image adjustment and analysis and finally data collection in the database. Statistical tests were done using statistical software (STATA MP, StataCorp, 4905 Lakeway Dr, College Station, TX, USA and MedCalc). **E)** Intra-observer agreement was estimated: for research purposes Cronbach's alpha was considered acceptable if between 0.7 and 0.8.[13]

## Results

Intra-observer agreement resulted to be 0.62 (95% CI: 0.52–0.67) for single measurements and 0.75 (95% CI: 0.69–0.80) for the average measure and was deemed acceptable for the purpose of the

study. There were no discrepancies higher that 15% between R1 ROI and R2 ROI requesting arbitration.

N = 11 adult patients (6 men and 5 women) with suspicious STS LR were included for a total of 33 follow-up events on MRI. A total of 198 data-sets per patients of both pathological and normal tissue were analyzed (99 pathological and 99 control data-sets). Characteristics of the 19 pathological findings in 33 follow-up events are reported in Table 2. N = 3 patients had multiple lesions.

After feature number reduction to avoid overfitting, Mann-Whitney U test identified n = 7, n = 13 and n = 12 features able to differentiate pathological tissue from normal tissue on T1w MRI images, T2w fat saturated MRI images and T1wGd respectively ($p < 0.001$). Table 3 shows feature domain according to different MRI sequences.

Some radiomics features were significantly correlated to tumor size: 4 features (Compactness, MajorAxis, Flatness, Mean) with $r = 0.75$ with $p < 0.02$, on T1w MRI images, 2 features (MajorAxis, RootMeanSquared) on T2w fat saturated MRI images with $r = 0.65$ with $p < 0.01$, and 2 features (MajorAxis, Maximum) on T1wGd with $r = 0.65$ with $p < 0.01$. Four radiomics features (Sum entropy, difference entropy, energy, lmc2) were correlated with grading (4 features with $r = 0.74$ and $p < 0.05$) on T1w MRI images and none on T2w fat saturated MRI images and T1wGd respectively.

**TABLE 2.** Distribution of the extremity soft tissue sarcoma patients' clinical characteristics in 19 pathological findings of 11 patients in 33 follow-up events. N = 3 patients had multiple lesions

| Clinical Characteristic | |
|---|---|
| Age (years) | 57.8 ± 17.8 |
| Tumor size (mm) | 26,2 ± 16.9 |
| **Grade (%)** | |
| G1 | 4 (21) |
| G2 | 6 (37) |
| G3 | 8 (42) |
| Unassigned | 1 (5) |
| **Depth (%)** | |
| Superficial | 6 (32) |
| Deep | 13 (68) |
| **Location (%)** | |
| Upper extremity | 5 (26) |
| Lower extremity | 14 (74) |
| **Histology (%)** | |
| Pleomorphic liposarcoma | 6 (33) |
| Myxofibrosarcoma | 5 (27) |
| Myxoid liposarcoma | 2 (10) |
| Leiomyosarcoma | 2 (10) |
| Nerve sheath tumors | 2 (10) |
| Synovial sarcoma | 2 (10) |

**TABLE 3.** Feature domain according to different MRI sequences

| Feature | Description | Significance | T1-weighted MR imaging | T2-weighted MR imaging* | T1-weighted MR imaging with Gadolinium |
|---|---|---|---|---|---|
| Shape domain | descriptors of the three-dimensional size and shape of the ROI. | These features are independent from the gray level intensity distribution in the ROI and are therefore only calculated on the non-derived image and mask | 1 | 1 | 2 |
| First order | Mean, standard deviation, median, and range; first-order differentials computed using Sobel operators | Localize hypo- and hyperintense regions; gradients detect edges and quantify region boundaries | 1 | 1 | 1 |
| Gray level co-occurrence matrix (GLCM) | Localization of regions with significant intensity changes; gradients detect edges and quantify region boundaries | Localizes regions based on underlying heterogeneity of voxel intensities | 3 | 7 | 6 |
| Gray level run lenght matrix (glrlm) | quantifies gray level runs, which are defined as the length in number of pixels, of consecutive pixels that have the same gray level value. | In a gray level run length matrix the element describes the number of runs with gray level and length occur in the image (ROI) along angle | 2 | 3 | 6 |
| Gray level size zone matrix (glszm) domain | It is an advanced statistical matrix used for texture characterization. It estimates bivariate conditional probability density function of the image distribution values | represent the count of how many times a given size of given grey level occur | 0 | 0 | 0 |

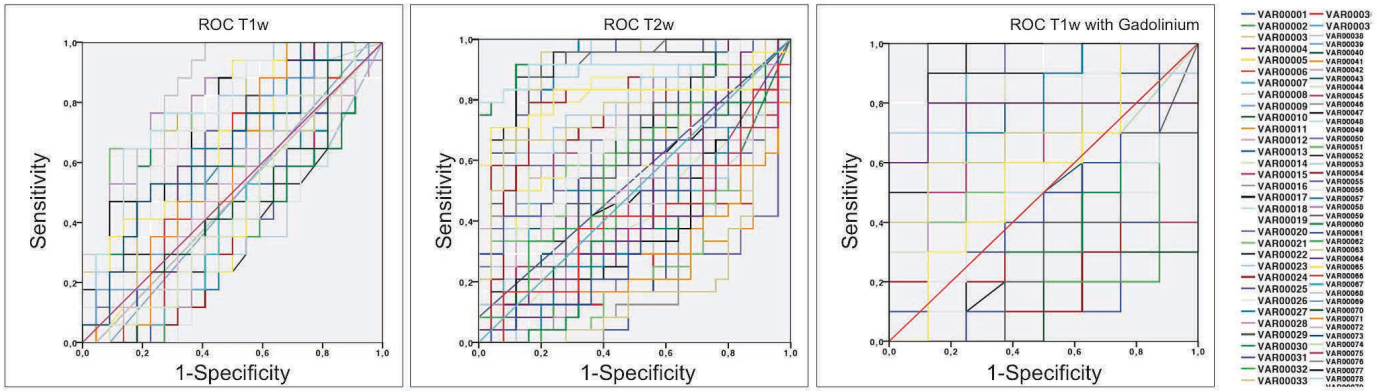


**FIGURE 2.** Examples of AUCs with a reduced number of features on T1w, T2w fat saturated with fat-saturation, and T1w post-Gadolinium showing a better performance for T2w fat saturated with fat-saturation and T1w post-Gadolinium ($p < 0.05$). Features from 1 to 26 belong to the shape domain; features (VAR00..) from 27 to 45 belong to the first order domain; features from 46 to 72 belong to the glcm (gray-level co-occurrence matrix) domain; features from 73 to 88 belong to gray level Run Lenght Matrix (glrlm) domain; features from 88 to 104 belong to the gray level size zone matrix (glszm) domain.

**TABLE 4.** ROC results according to different MRI sequences of the selected features.* T2-weighted MR imaging and T1-weighted MR imaging with Gadolinium are acquired with fat-saturation. Areas under the curve for differentiation of normal and pathological tissue (LR) had $p < 0.05$

| Sequence | Minimum AUC | 95%CI | Maximum AUC | 95%CI |
|---|---|---|---|---|
| T1-weighted MR imaging | 0.71 | 0.54–0.88 | 0.84 | 0.77–0.96 |
| T2-weighted MR imaging* | 0.81 | 0.67–0.95 | 0.91 | 0.83–1.00 |
| T1-weighted MR imaging* with Gadolinium | 0.87 | 0.69–1.00 | 0.96 | 0.87–1.00 |

ROC analysis performed on T1w images, T2w fat saturated images and T1wGd showed an AUC between 0.71 (95%CI: 0.55–0.87) and 0.96 (95%CI: 0.87–1.00). Detailed results are reported in Table 4 and Figure 2. T2w fat saturated with fat-saturation and T1w post-Gadolinium showed a better performance than T1w images ($p < 0.05$).

Mean time to perform radiomics analysis was at least 5 h per patient including the creation of a data set of 19 patients with suspicious cancer visible at MRI, therefore the total time to perform analysis, excluding the time to recruit and select patients was at least 100 h.

## Discussion

In this study we performed a radiomics analysis on MRI images of patients suspected of having STS LR belonging to an ongoing prospective trial. The aim was to investigate whether radiomics features derived from standard clinical MRI sequences could be used to differentiate normal tissue from cancerous tissue of LR. In clinical practice, differentiating normal from pathological tissue on MRI in patients suspected of having LR is relevant especially when LR are not nodules, but plaque-like "tails" of tumor on MRI, especially for both undifferentiated pleomorphic sarcoma and myxofibrosarcoma.[5,11] Despite the relatively low number of patients included in this study, we had a high percentage of pleomorphic sarcoma/liposarcoma and/or myxofibrosarcoma accounting for 11/19 of the lesions evaluated. The number of lesions evaluated in this study is similar to the number of pleomorphic sarcoma/liposarcoma and/or myxofibrosarcoma evaluted in the study by Corino *et al.*[14] In the study by Corino *et al.*[14], a radiomics approach with first order features performed on 13 pleomorphic sarcoma/liposarcoma and/or myxofibrosarcoma resulted in good accuracy and AUC. In the present study, the number of pleomorphic sarcoma/liposarcoma and/or myxofibrosarcomas evaluated could be considered relatively high considering that patients underwent MRI evaluation for LR and not of a primary tumor. MRI could be challenging in these patients and the use of radiomics could enhance the role of MRI in STS local surveillance. Chou *et al.*[15] reported MRI sensitivity for LR detection ranging from 69%without contrast medium to 90% with contrast-enhanced sequences. AUCs of this study, especially for T1w post-gadolinium and T2w fat saturated are better that 90%. As reported by Fayad L. *et al.*[16], postoperative inflammation and fibrosis in the surgical bed may share many of the same characteristics as tumor on conventional MR images. Indeed they can occasionally appear mass-like. Only in the absence of abnormal T2-weighted signal intensity the presence of recurrent tumor could be excluded. Very rarely, a sarcoma recurrence

may be of low signal intensity on T2-weighted images and a T1-weighted study shows architectural distortions due to the tumoral presence. We believe that in these difficult radiological cases, radiomics may help even expert Radiologists in the detection of LR. A preliminary report showed that using a radiomics approach to characterize musculoskeletal tumors, machine learning performs even better than expert radiologists[17] (Chhabra A, personal communication). Indeed, the main result of this study is that few radiomics features can differentiate LR from normal tissue on T1w MRI images, T2w fat saturated MRI images and T1w post-gadolinium sequences. These features belonged to different classes. The majority of them belonged to the gray level co-occurrence matrix (GLCM) domain possibly reflecting underlying heterogeneity of voxel intensities. Although it is known that in soft-tissue tumors fluid-sensitive images should have a more favorable contrast between tumor and surrounding because skeletal muscle is of intermediate signal intensity, radiomics features were able to differentiate LR from normal tissues even on T1w sequences. This data reflects the great value of this application for STT surveillance. In addition, a very good AUC can be obtained with only few features for every MRI sequence. We acknowledge that, according to standard clinical practice, T2w fat saturated and post-contrast T1w sequences had the best AUCs. After feature reduction to avoid decrease the performance[18] we found that a set of few radiomics features had an AUC generally better than data reported for conventional MRI. Indeed, radiomics based AUC for T1-weighted MR imaging with Gadolinium reached a best result of 0.96. These data support the design of future studies using of radiomics for STS surveillance. In addition, in spite of the small number of patients, some radiomics features correlated with tumor size and grading. These data support the hypothesis that few radiomics features could reflect tumoral biology and aggressiveness.[14] We acknowledge that mean time to perform radiomics analysis was long and intra-observer agreement resulted acceptable for the purpose of the study. In addition, a different selection of the ROI could lead to a different measurement of the features even with good intra- and inter-observer agreement. Further improvements are needed to accelerate the workflow in clinical practice and to keep ROIs as stable as possible although we did not have any cases with discrepancies higher that 15%.

This study has several limitations. First, radiomics features were not extracted on ADC maps, that have been demonstrated to assess tumor cellularity even when different scanners are used.[14] However, this is not a multicentric study and the MRI image sample was included from only one center. Second, the study population was relatively small. However, considering the incidence of LR in patients who underwent surgery for STT (reported rates of LR range from 6.5% to approximately 25%)[5], the number of patients and images evaluated seem to be sufficient for the purpose of the study. The presence of a relatively high number of pleomorphic sarcoma and myxofibrosarcoma increases the clinical significance of the study because they are tumors difficult to be evaluated on MRI. Selection biases are excluded due to consecutive patient enrollment in the present study. Moreover, three MRI sequences per lesion were analyzed increasing data robustness. Finally, we also acknowledge that future software developments will reduce the necessity of freehand ROIs positioning.[19] We also believe that further research will be critical to fully unveil the potential or radiomics in STT evaluation, indeed, it has been studied that radiomics features extracted from MR images are independently associated with survival when accounting for age and tumor grade and helps in differential diagnosis.[20,21]

In conclusion, radiomics features allow to detect LR on MRI images in STS local surveillance. radiomics in STS evaluation is useful not only for detection purposes but also for lesion biological characterization.

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*research article*

# Dusp6 inhibits epithelial-mesenchymal transition in endometrial adenocarcinoma via ERK signaling pathway

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**Background.** Endometrial adenocarcinoma (EAC) is one of the most commonly diagnosed gynaecological malignancies among female population of the developed countries. DUSP6 is a negative regulator of ERK signaling, which is a molecular switch involved in MAPK signaling during the progress of malignancies. DUSP6 was previously found to inhibit tumorigenesis and EMT-associated properties in several cancers, however, its exact role in EAC remains unclear.

**Methods.** The level of DUSP6, (E-cad) and (N-cad) in EAC cancerous tissues and respective adjacent non-cancerous tissues were examined by western-blot or immunohistochemistry. The cell growth, invasion and migration abilities were measured in Ishikawa 3H12 endometrial cancer cell lines with overexpressed or knock down DUSP6. Protein levels of EMT-associated markers E-cadherin, N-cadherin and Vimentin were also determined. The impacts of DUSP6 on ERK signaling was assessed by detection of ERK and p-ERK.

**Results.** Down-regulation of DUSP6 was observed in EAC compared with the normal controls. The overexpression of DUSP6 significantly attenuated tumor cell growth, invasion, migration abilities and inhibited EMT-associated markers, while knock down of DUSP6 showed opposite trends. Overexpression of DUSP6 also down-regulated p-ERK and the knock down of DUSP6 inversely up-regulated p-ERK level.

**Conclusions.** DUSP6 inhibited cell growth, invasion and migration abilities in Ishikawa 3H12 cells as well as attenuating EMT-associated properties. This tumor suppressive effect of DUSP6 in EAC is achieved by inhibiting ERK signaling pathway.

Key words: DUSP6; ERK; EMT; endometrial adenocarcinoma

## Introduction

Endometrial cancer (EC) is one of the most commonly diagnosed gynaecological malignancy among female population in the developed countries.[1] Over 300.000 new cases of endometrial cancer were diagnosed in 2012 around the world according to the data of the World Cancer Research Fund (WCRF)[2] and an increasing number of patients has been noted in recent years.[3] Endometrial adenocarcinoma (EAC) frequently occurs in pre- and post-menopausal women, which initiate from the occurrence of endometrial hyperplasia. Current treatments for EAC include surgery, radiotherapy, chemotherapy and hormone therapy for early-stage diseases.[5] However, therapeutic efficiency of above treatments was extremely limited, hence searching for potential therapeutic targets in EAC become urgent.

Tumor suppressive role of dual-specificity phosphatase 6 (DUSP6) in cancers is suggested to act through ERK/MAPK signaling pathway. Intracellular ERK/MAPK signaling pathway is involved in a variety of cancer transformation as well as their tumorigenesis such as breast cancer[10], lung cancer[11], ovarian cancer[12], pancreatic cancer.[13] MAP kinases phosphatases (MKPs) that belong to the DUSP family are involved in the ERK/MAPK

signaling cascade. The DUSP family mediates the activity of MAPK signaling pathway through dephosphorylating both of tyrosine and threonine residues on their targets.[14] Specifically, DUSP6 negatively regulates ERK1/2 by dephosphorylating the tyrosine and threonine residues.[15,16] Evidence demonstrates tumor suppressive roles of DUSP6 in different cancers. For example, the loss of DUSP6 was frequently observed in primary ovarian cancer and proved to induce chemoresistance in ovarian cancer.[12] ERK1/2 has been previously shown to induce EMT process in esophageal squamous cell carcinoma and nasopharyngeal carcinoma.[17] In addition, the tumorigenesis potential of ERK pathway has been reported in mounting studies regarding endometrial cancer recently.[18-20] It is noteworthy that ERK pathway in human Ishikawa endometrial cancer was reported to induce cell migration and invasion via regulating EMT-associated factors such as MMP family. By the negative regulation of the ERK pathway, the underlying mechanisms of DUSP6 on EMT progress involved in EAC need further investigation.

Evidence suggests the role of DUSP6 in suppressing the cell invasion and EMT-associated properties in several carcinomas such as esophageal squamous cell carcinoma previously[21], however, few studies reveal the impacts of DUSP6 on EMT process via regulating ERK pathway in endometrial adenocarcinoma. Therefore, the present study aims to investigate the potential functions of DUSP6 on EMT-associated properties in endometrial adenocarcinoma via mediating the ERK signaling with human Ishikawa endometrial cancer cell lines. Both overexpression and knockdown of DUSP6 were constructed to evaluate the functions on regulating the expressions of EMT- markers, cell growth, invasion and migration abilities. The protein levels of ERK and phosphorylated ERK were also analyzed to determine the regulatory role of DUSP6 on the ERK signaling. Our results suggest DUSP6 inhibits EMT process in endometrial adenocarcinoma *in vitro* via suppressing the ERK signaling pathway.

## Materials and methods

### Tissue samples, cell culture

Tumor and the corresponding adjacent tumor tissues (more than 5 cm from the tumor site without cancer cell infiltrations) were obtained from 35 endometrial adenocarcinoma patients from 2016 to 2018 in the Department of Obstetrics and Gynecology, Shandong Provincial Hospital. The study was authorized by the Ethics Committee of Shandong University, China. Written consents were obtained from all patients. Additionally, Ishikawa 3H12 (Purchased from ATCC, USA) high-differentiated endometrial adenocarcinoma cell line was used in *in vitro* study. The cells were cultured in DMEM supplemented with 5% charcoal-stripped fetal bovine serum (FBS), 100μ/ml of penicillin and 100μ/ml of streptomycin at 37°C in a humidified environment with 5% $CO_2$ in air.

### Constructs of cell transfection

Short hairpin RNA (shRNA) plasmids which targets the coding region of DUSP6; or by transfecting cells with non-target shRNA control vector were constructed by GenePharma, Shanghai and cloned into pGPU/GFP/Neo. The constructs were transfected into Ishikawa cells by Lipofectamine 3000 according to the manufacturer's protocol. cDNA of DUSP6 was obtained and synthetized by GenePharma, Shanghai and cloned into pcDNA3.1 plasmid. Ishikawa 3H12 cells were transiently transfected with either pcDNA3.1-Dusp6 plasmid or empty vector (EV) using Lipofectamine 3000 according to the manufacturer's protocol. Whole cell lysates were collected at the indicated time points after transfection to verify the expression level of DUSP6.

### MTT assay

Cell viability was evaluated with MTT (Qiagen, German) assays according to the manufacturer's instructions. Transfected Ishikawa 3H12 cells were seeded at a density of 6.000 cells per well in 96-well plates in RPMI 1640 containing 10% FBS overnight and then maintained in 0.5% FBS media for 96h before testing. The absorbance was measured at a wavelength of 490 nm on a Synergy Multi-Mode Microplate Reader (Biotek, USA). Each assay was performed on five replicate wells.

### Immunohistochemistry analysis

Fresh endometrial adenocarcinoma tumor tissue samples were washed using phosphate buffered saline (PBS) and fixed using formalin. After dehydration and paraffin-embedding, samples were cut and mounted onto glass slides. Formalin-fixed sections were deparaffinized and rehydrated and incubated with 0.6% hydrogen peroxide in methanol. Then antigen retrieval was performed and the

sections were blocked and incubated overnight with rabbit anti-human DUSP6/E-cad/N-cad primary antibody (1:100, Abcam, USA) at 4°C. HRP-conjugated goat anti-rabbit IgG secondary antibody was deployed afterwards. DAB was used as chromogenic agent. Same sections incubated with non-immune serum instead of primary antibody were used as a negative control. The experiments were performed in duplicate. The intensity of the staining of DUSP6/E-cad/N-cad was scored by a clinical pathologist who was blinded with any knowledge of the pathological data.

### Wound healing assay

To evaluate the migration of cells, Ishikawa 3H12 cells overexpressed or knockdown of DUSP6 were cultured until confluent beforehand. Then the cell monolayer was scratched with a pipette tip (Scar bar: 1mm) and incubated for 24 h, the relative wound areas were calculated through: migrated distance of DUSP6 expression or knockdown clones/ migrated distance of vector-alone stable clones x 100.

### Transwell Matrigel assay

A micropore chamber assay was used to assess the invasion ability of Ishikawa 3H12 cells that with the overexpression/knockdown of DUSP6. Matrigel was diluted by pre-cold serum free DMEM medium at 1: 5 before use. A total number of $3x10^4$ cells were seeded into the top chamber of a 24-well Matrigel-coated micropore polycarbonate membrane filter with 8μm pores (Becton Dickinson Labware, Franklin Lakes, NJ) for invasion assays. The DMEM containing 10% FBS was added to the bottom chamber as a chemoattractant. The cells were then incubated at 37°C for 22 h. The members were washed and fixed followed by stained by crystal violet. The cells migrated through the membranes were counted under the inverted light microscope at 10x magnification. All tests were performed in triplicate.

### RNA extraction and RT-qPCR

To verify the expression of DUSP6 in transfected cell, total RNA was isolated with TRIzol reagent (Life Technologies, USA) and converted to first-strand cDNA using M-MLV Reverse Transcriptase (USB, Cleveland, OH). The mRNA level was analyzed with quantitative real-time PCR assay. Specific primers were synthesized as the following sequences show: DUSP (forward) 5′-GAACTGTGGTGTCTTGGTACATT-3′ and (reverse) 5′-GTTCATCGACAGATTGAGCTTCT-3′.[22] The GAPDH gene was used as an internal control and was amplified from the same cDNA samples for 25 cycles. The cDNA of a normal endometrial cell line was used as a positive control.

### Western blot analysis

The whole cell lysates were extracted using RIPA buffer with protein inhibitors cocktail (Complete Mini, Roche Diagnostics, Switzerland). The concentration of total cellular protein was measured using BCA assay kit (Pierce, IL, USA) according to manufacturer's instructions. 30 μg of proteins from each sample were electrophoresed to separate on 10% sodium dodecysulfate-polyacryl-amide gels (SDS-PAGE) and then transferred to polyvinylidene difluoride membrane (PVDF, CA, USA). The PVDF membranes were blocked with 5% non-fat dry milk for 1 h at room temperature and incubated at 4°C overnight with the primary antibodies against: DUSP6, E-cadherin, N-cadherin, Vimentin, ERK, p-ERK, GAPDH (1:1000, Cell Signaling, USA). The membranes were then washed and incubated with the secondary antibody against rabbit at 37°C for 1 h and protein bands were detected by the chemiluminescence method.

### Statistical analysis

Data are presented as means ±SD from three independent experiments. All statistical analyses were performed using SPSS 13.0 (SPSS Inc, USA). For comparisons between groups, one-way analysis of variance was performed. Western blot analysis was quantified by Image J. P values <0.05 were considered as statistically significant.

## Results

### Down-regulation of DUSP6 and E-cadherin and up-regulation of process-cadherin in endometrial adenocarcinoma

By determining the expressions of DUSP6, E-cadherin and N-cadherin in both endometrial adenocarcinoma cancerous and the corresponding adjacent non-tumor tissues using western blot and immunohistochemistry analysis, down-regulation of DUSP6 (Figure 1A) and E-cadherin (Figure 1B) were obeserved in cancerous specimens compared

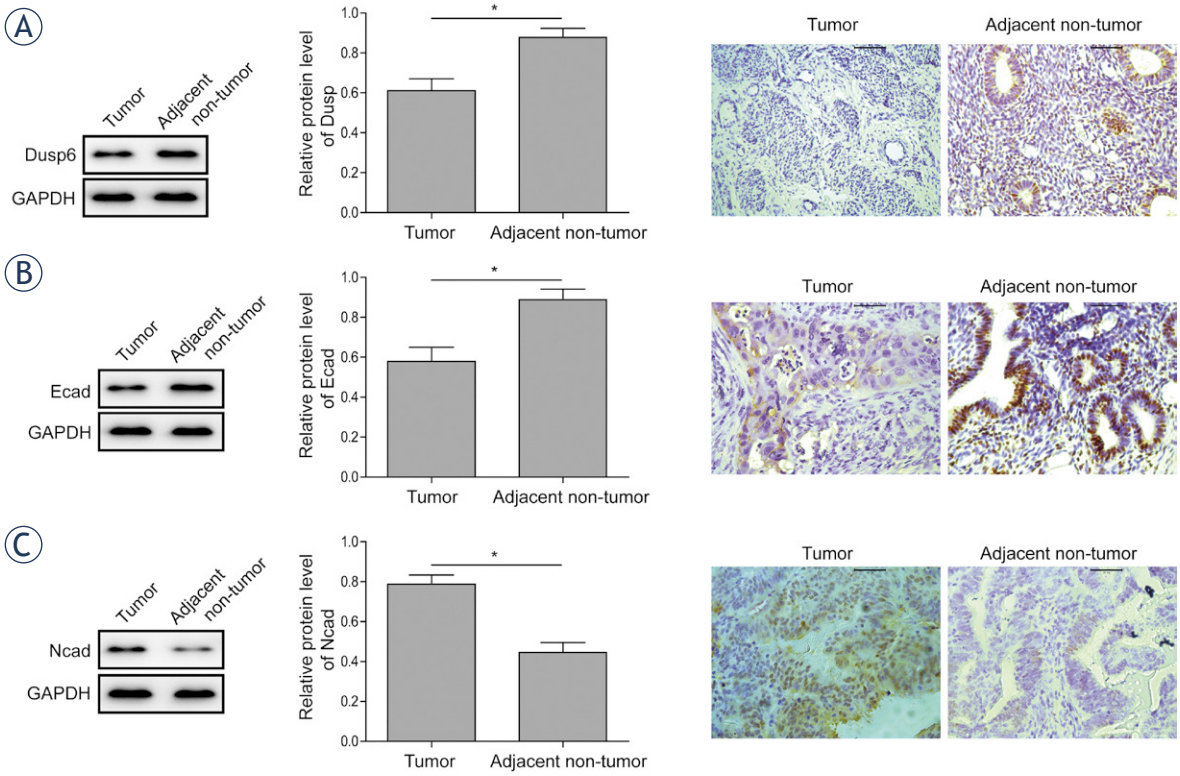


**FIGURE 1.** Expression analysis of Dusp6, E-cadherin and N-cadherin in EAC tissues and the adjacent non-tumor tissues. **(A)** The protein expression level of DUSP6 was analysed by western-blot and immunohistochemistry analysis in EAC tumor and adjacent non-tumor tissues. **(B)** The protein expression level of E-cadherin was analysed by western-blot and immunohistochemistry analysis in EAC tumor and adjacent non-tumor tissues. **(C)** The protein expression level of N-cadherin was analysed by western-blot and immunohistochemistry analysis in EAC tumor and adjacent non-tumor tissues.Data were presented as Mean ±SD. P < 0.05 was considered as significant.* P < 0.05. Each experiment was performed in triplicate.

to corresponding normal counterparts. The results of immunohistochemical staining were in line with the results of western-blot in terms of the expressions of DUSP6 and E-cadherin. Contrarily, significant up-regulation of N-cadherin was observed in cancerous tissues than that in their non-cancerous counterparts (Figure 1C).

## Overexpression of DUSP6 attenuates tumor cell growth, invasion, migration and EMT process in endometrial adenocarcinoma cell line

To investigate the role of DUSP6 in the progress of endometrial adenocarcinoma, proliferation, invasion and migration of Ishikawa 3H12 cell line was assessed after overexpressing DUSP6 by transfecting pcDNA3.1-Dusp6 vector or empty vector control. The efficiency of transfection was validated by western-blot (Figure 2A). The MTT assay demonstrated that the cell viability was significantly decreased in DUSP6 overexpressed Ishikawa 3H12 cells than that of vector (Figure 2B). Further *in vitro* Transwell Matrigel assay (Figure 2C) and wound healing assay (Figure 2D) showed the attenuated invasion and migration ability in DUSP6 overexpressed tumor cell lines. DUSP6 overexpressed clones in Matrigel culture exhibited a non-cohesive and disorganized morphology compared to polarized and well-organized spheroids shown in vector-alone clones and controls. The width of wound formed in cultures of cells overexpressing DUSP6 significantly superior to that in cultures of vector-alone clones and controls together referred to differed EMT-associated properties.[23] Increased protein level of E-cadherin and decreased Vimentin were observed in DUSP6 overexpressed cells (Figure 2E).

## Knockdown of DUSP6 enhances tumor cell growth, invasion, migration and EMT process in endometrial adenocarcinoma cell line

Cell growth, migration and invasion were evaluated after silencing DUSP6 expression by shRNA

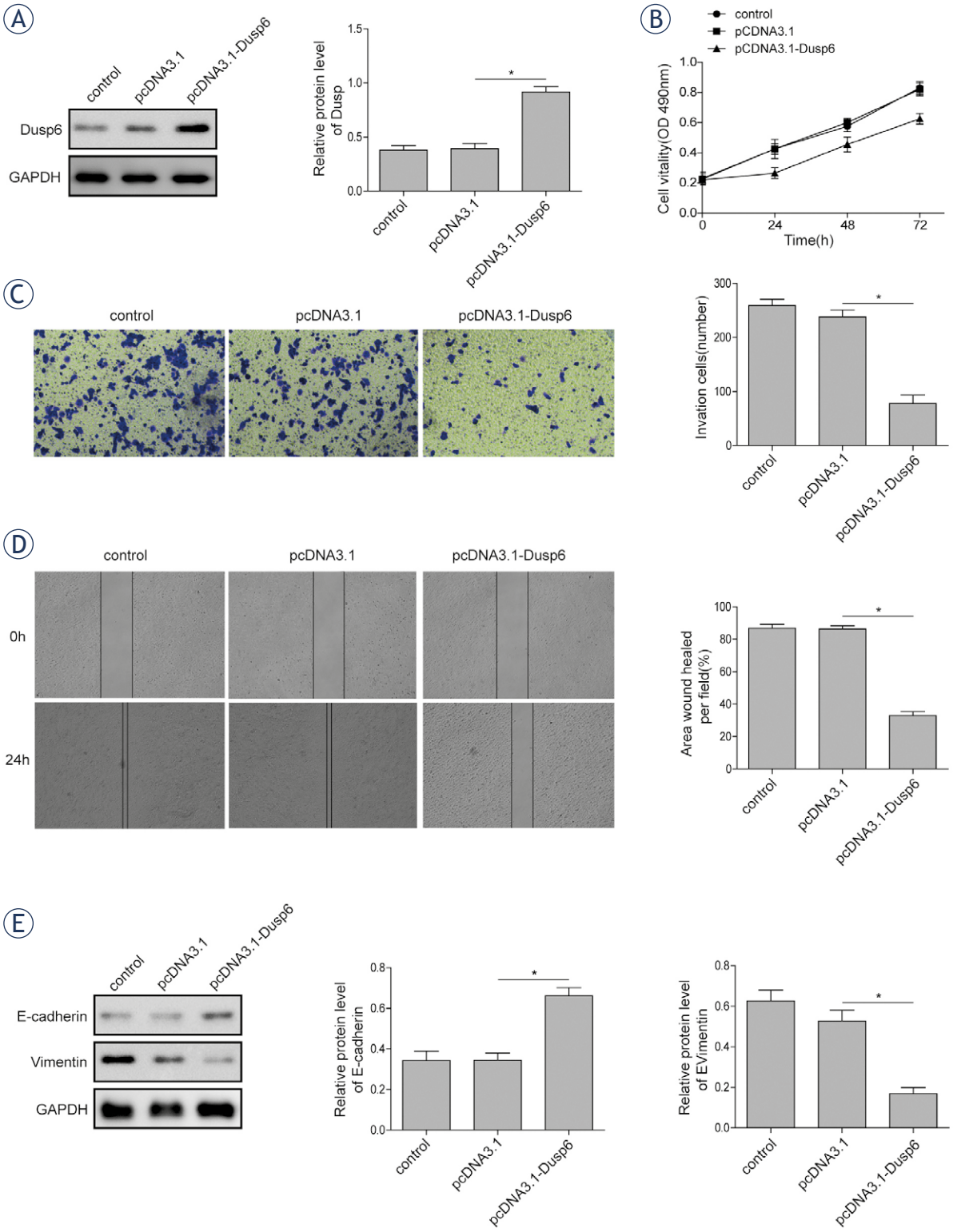


**FIGURE 2.** Effects of DUSP6 overexpression on cellular proliferation and EMT. **(A)** The protein expression level of DUSP6 was analysed using western-blot. **(B)** The cell viability of Ishikawa 3H12 cell lines measured using MTT assay. **(C)** Relative invasion ability of Ishikawa 3H12 cell lines measured using Transwell Matrigel assay. **(D)** Relative migration ability of Ishikawa 3H12 cell lines measured using wound healing assay. **(E)** The protein levels of E-cadherin and Vimentin in Ishikawa 3H12 cell lines measured using western-blot analysis. Groups: Negative control, pcDNA3.1 Vector-alone clones, pcDNA3.1-Dusp6 clones. Data were presented as Mean ±SD. $P < 0.05$ was considered as significant. *$P < 0.05$. Each experiment was performed in triplicate.

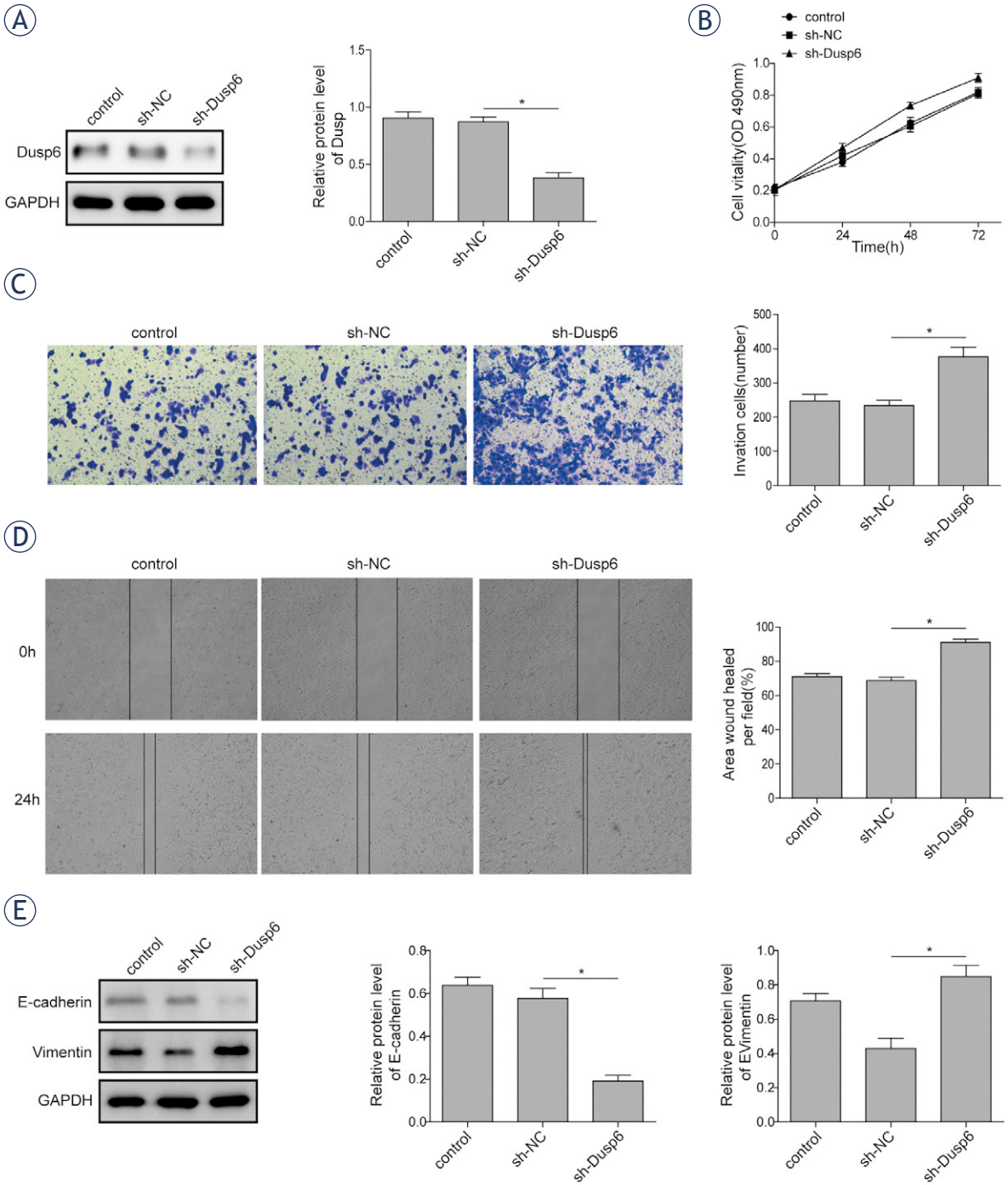


**FIGURE 3.** Effects of knocking down DUSP6 on cellular proliferation and EMT. The efficiency of transfection was validated by measuring protein expression level of DUSP6 using western blot. **(B)** The cell viability of Ishikawa 3H12 cell lines measured using MTT assay. **(C)** Relative invasion ability of Ishikawa 3H12 cell lines measured using Transwell Matrigel assay. **(D)** Relative migration ability of Ishikawa 3H12 cell lines measured using wound healing assay. **(E)** The protein levels of E-cadherin and Vimentin in Ishikawa 3H12 cell lines measured using western-blot analysis. Groups: Negative control, sh-NC clones, sh-Dusp6 clones. Data were presented as Mean ±SD. $P < 0.05$ was considered as significant. *$P < 0.05$. Each experiment was performed in triplicate.

in Ishikawa cell line. Cells were transfected with shRNA targeting DUSP6 or NC. The efficiency of knockdown was assessed by western blot (Figure 3A). Cell viability of DUSP6 knock down cell was increased significantly compared to that of NC (Figure 3B). The enhanced cell invasion and

migration ability were also observed in tumor cells with the absence of DUSP6 expression based on transwell matrigel (Figure 3C) and wound healing assay (Figure 3D) respectively. EMT process was promoted in the DUSP6 knock down tumor cells indicated by decreased protein level of E-cadherin and increased level of Vimentin (Figure 3E).

### DUSP6 inhibits activation of ERK pathway in endometrial adenocarcinoma

The activation of ERK pathway has been validated in tumorigenesis in varied human carcinoma and played an important role in endometrial adenocarcinoma. The present study confirmed that the dysregulation of DUSP6 induced aberrant activation of ERK signaling in Ishikawa 3H12 cell lines (Figure 4, Figure 5). According to western-blot results, the level of p-ERK/ERK was significantly down-regulated in overexpressed DUSP6 cells, indicating activation of ERK signaling was inhibited by DUSP6 (Figure 4). In contrary, the level of p-ERK/ERK was significantly up-regulated in DUSP6 knockdown cells compared to that in sh-NC (Figure 5).

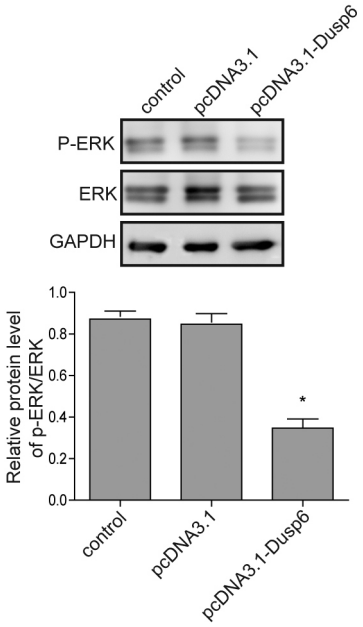


**FIGURE 4.** Assessment of effects of overexpressing DUSP6 on ERK signalling pathway. The protein level of phosphorylated ERK and ERK were measured using western blot analysis in Ishikawa 3H12 cell lines when DUSP6 was overexpressed. B-actin served as an internal control. Groups: Negative control, pcDNA3.1 Vector-alone clones, pcDNA3.1-Dusp6 clones. Data were presented as Mean ±SD. Each experiment was performed in triplicate.

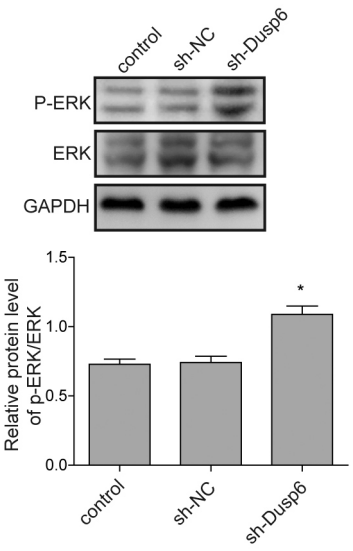


**FIGURE 5.** Assessment of effects of knocking down DUSP6 on ERK signalling pathway. The protein level of phosphorylated ERK and ERK were measured using western blot analysis in Ishikawa 3H12 cell lines when DUSP6 was knocked down. B-actin served as an internal control. Groups: Negative control, sh-NC clones, sh-Dusp6 clones. Each experiment was performed in triplicate. Data were presented as Mean ±SD.

## Discussion

Endometrial cancer usually initiates in the cells of the inner lining of the endometrium (uterus) and has developed into the most frequently occurring gynecological cancer in developed countries.[24] Endometrial adenocarcinoma (EAC) is the most frequently occurring subtype of the endometrial cancer and accounts for approximately 75% of EC cases clinically. Dual-specificity phosphatase 6 (DUSP6) was reported to dephosphorylate phosphor-tyrosine and phosphor-threonine residues on ERK1/2 to inactivate the ERK kinase. Considering the facts that DUSP6 was reported to be involved in tumorigenesis of various cancers and the lower Dusp6 expression was observed in endometrial of post-menopausal age compared with that of reproductive age[3], the potential role of Dusp6 during the development of EAC is hence worth investigating.

Accumulated evidence showed DUSP6 was down-regulated in various types of cancers, including ovarian cancer, lung cancer, esophageal squamous cell carcinoma and nasopharyngeal carcinoma.[21] However, there is discrepancy on the expression of DUSP6 exists in other cancers including endometrial cancer. Previous studies indicated that the overexpression of DUSP6 is frequent in EAC, which is associated with enhanced prone-cancer potential of estrogen in EAC.[3] Also, the overexpression of DUSP6 was observed in EAC by the same research group and was found to promote 17β-estradiol stimulation in Ishikawa cell lines.[25] It is interesting to find that DUSP6 was significantly down-regulated in Ishikawa 3H12 EAC cell lines based on the results of western blot, which was supported by results of immunochemistry analysis in EAC tumour biopsies as well in the present study. Previous evidence revealed the down-regulation of DUSP6 in cancers may be attributed to epigenetic silencing and specifically, the allelic loss of 12q21 where DUSP6 maps.[21,26,27] It is possible that the down-regulation of DUSP6 in EAC is resulted from the similar mechanism whilst further investigation is needed with a larger sample size. Despite of this discrepancy, the potentially inhibitory functions of DUSP6 on growth and proliferation of Ishikawa 3H12 cell lines were verified in the

present study, which suggested the therapeutic potential of DUSP6 in EAC.

EMT-associated properties were regulated by the overexpression and the knockdown of DUSP6 in EAC cancer cells. Since EMT causes the loss of apical-basal polarity and cell to cell adhesion[28], the formation of polarized cell spheroid in DUSP6-overexpressed clones that indicates the reversion of EMT process, which may suggest the suppressive role of DUSP6 in EMT-associated properties in EAC. Moreover, the attenuation of cell invasion and migration ability in DUSP6 overexpressed clones suggested the inhibitory function of DUSP6 on cancer cell mobility. As important indicators of cell survival and prognosis, it was previously demonstrated that the loss of E-cadherin alongside upregulated levels of N-cadherin and Vimentin are involved in EMT process and morphological changes in pancreatic carcinoma[9], also are associated with the better prognosis of tumor and lower death rate.[29] Hence, higher level of N-cadherin detected in EAC cancerous specimens compared with the adjacent normal tissues alongside the loss of E-cadherin and DUSP6 indicates the inhibitory role of DUSP6 in EMT process and metastasis of EAC. Further study should be applied to confirm the *in vivo* suppressive effect of DUSP6.

DUSP6 is demonstrated as a negative regulator of ERK signalling pathway based on the present study, which is in accordance to the previous findings. It was reported that DUSP6 functionally and physically inhibited the activity of ERK2.[30] Demonstrated promotive functions of ERK2 on cell invasiveness and EMT process in cancers[17] further indicates the suppressive role of DUSP6 during the development of tumours. Here in this study the significant down-regulated phosphorylation of ERK in DUSP6 overexpressed clones alongside the up-regulated phosphorylated ERK in DUSP6 knocking down clones suggested DUSP6 impaired the activation of ERK pathway. This is also for the first time that DUSP6 was reported to regulate EMT process through affecting ERK signalling in endometrial adenocarcinoma.

In summary, the present study explored the role of Dusp6 in regulation of EMT process via ERK signaling pathway in endometrial adenocarcinoma. The decreased level of DUSP6 was verified in the EAC tumor samples compared to the endometrial normal tissues. Overexpression of Dusp6 was demonstrated to inhibit EAC cancer EMT, cell growth, invasion and migration abilities, while the knockdown of Dusp6 inversely enhanced the malignant phenotypes mentioned above. Additionally, the present study determined that DUSP6 impaired ERK signaling. The current findings provided theoretical basis of seeking for a novel therapeutic target for treating the endometrial adenocarcinoma.

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*research article*

# Clinical relevance of the borderline results of the Hybrid Capture 2 High-Risk HPV DNA assay with cervical samples collected in Specimen Transport Medium

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**Background.** The Hybrid Capture 2 (HC2) High-Risk HPV DNA assay serves as a triage test in the Slovenian national cervical cancer screening programme ZORA. To improve the limited analytical accuracy of HC2 test results near the cut-off value (1.0 relative light units/cut-off (RLU/CO)), we follow an internal protocol of repeating the test on all samples with borderline results within the 0.7-2.0 RLU/CO interval. The aim of the study was (i) to determine the clinical relevance of HC2 test results within three different "grey zones" for samples stored in Specimen Transport Medium (STM) and (ii) to determine whether the current algorithm of retesting "grey zone" STM specimens with the HC2 assay is clinically relevant.

**Patients and methods.** The study included 594 women between 20 and 65 years of age. All participating women were referred for colposcopy, and in cases of abnormal results, biopsy was performed. We assessed the distribution of HC2 test results and the corresponding proportion of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) lesions in three different "grey zones" (1.0–2.5, 0.4–4.0 and 0.7–2.0 RLU/CO), retested specimens with results within a 0.4–4.0 RLU/CO interval and calculated the sensitivity and specificity for HC2 at different RLU/CO values.

**Results.** The proportion of specimens within 1.0–2.5, 0.4–4.0 and 0.7–2.0 RLU/CO intervals was 3.9%, 10.8% and 4.5%, respectively. The proportion of CIN2+ lesions within these "grey zones" was 2.5%, 5.6% and 1.2%, respectively. Retesting the samples did not detect any additional CIN2+ cases. Within the 1.0–2.5 RLU/CO interval, the sensitivity decreased from 93.8% to 91.4%, while the specificity increased from 63.3% to 67.5%; for the 0.4–4.0 RLU/CO interval, the sensitivity decreased from 95.1% to 89.5%, while the specificity increased from 56.8% to 69.4%; and for the 0.7–2.0 RLU/CO interval, the sensitivity remained nearly constant (94.4 vs. 93.2%), while the specificity increased from 60.6% to 66.4%.

**Conclusions.** Our results show that retesting STM samples within the "grey zones" is not necessary. Retesting samples in the negative "grey zone" does not increase sensitivity, and retesting in the positive "grey zone" is not followed by a less intensive management of women, since these women are recalled regardless of the results of the retest. Furthermore, the majority of samples retain the original HC2 results after retest, and the number of CIN2+ lesions among women with "grey zone" HC2 results is low.

Key words: Hybrid Capture 2; HPV test; borderline results; grey zone; Specimen Transport Medium

## Introduction

The Slovenian national cervical cancer screening programme ZORA uses the Hybrid Capture II (HC2) assay (Qiagen, Hilden, Germany) as a triage test that stratifies women with low-grade cervical changes into those with high and low risks for developing cervical cancer.[1] The results of the HC2 test are presented as relative light units/cut-off (RLU/CO) values, and the cut-off value for a positive result is 1.0 RLU/CO[2]. Many studies have confirmed the high reproducibility of the HC2 test results both within and between laboratories.[3-5] Nevertheless, some studies have noted that analytical accuracy is significantly lower in the vicinity of the cut-off value.[3,4,6-8] Therefore, the manufacturer has published instructions for the further management of samples with borderline results that differ regarding which medium is used for sample collection. For PreservCyt specimens (Hologic Inc., Marlborough, United States), the manufacturer proposed the implementation of a borderline RLU/CO area called the "grey zone" in the range of 1.0–2.5 RLU/CO and recommended retesting the samples when results fall within this range.[2] However, when storing the samples in the Specimen Transport Medium (STM) (Qiagen, Hilden, Germany), the manufacturer's instructions are different, recommending retesting only the samples with suspected HPV infection and those with HC2 results near but below the 1.0 RLU/CO value. Retesting can be performed with the HC2 test or using another method.[2]

Several authors[9-16] have investigated the reproducibility and clinical significance of retesting PreservCyt samples with borderline HC2 results. However, we have not found a single study where the same problem was addressed for specimens collected in STM. A previous Slovenian study by Seme *et al*.[6] evaluated the analytical accuracy of the results within 0.4–4.0 RLU/CO. Because these authors found poor reproducibility of HC2 within this range, they recommended that tests should be repeated by an alternative PCR-based method. At the Institute of Oncology Ljubljana, we adapted these instructions for our laboratory settings to repeat the test with the HC2 assay on specimens for which HC2 results fall within the 0.7–2.0 RLU/CO interval.

The aims of our study were (I) to determine the clinical relevance of the HC2 test results within three different "grey zones" for STM samples and (II) to determine whether the current algorithm for retesting "grey zone" STM specimens with the HC2 assay is clinically relevant.

## Patients and methods

### Study population

The study population included 596 women who participated in the Slovenian HPV self-sampling project L3-5512 from April 2014 to July 2016. The L3-5512 study protocol has been previously described.[17] All women were referred to colposcopy where smears for high-risk HPV testing were obtained. The indications for colposcopy followed the Slovenian national guidelines, including high-grade cytology, HPV positive triage test after repeated low-grade cytology, positive HPV test for the surveillance of the women treated for high-grade intraepithelial lesion (HSIL) and a positive HPV test on self-sampling. Women with abnormal colposcopy underwent colposcopy-guided biopsy, followed by histological evaluation according to WHO recommendations.[18] The reported high-grade histological outcomes within one year after colposcopy included cervical intraepithelial neoplasia grade 2 (CIN2), cervical intraepithelial neoplasia grade 3 (CIN3), squamous cell carcinoma, carcinoma with origin outside the cervix, cervical glandular intraepithelial neoplasia grade 2/adenocarcinoma *in situ* (CGIN2/AIS), vaginal intraepithelial neoplasia grade 3 (VAIN3) and vulvar intraepithelial neoplasia grade 3 (VIN3). All data were obtained from the Registry of the national screening programme ZORA.

The study was approved by the National Medical Ethics Committee at the Slovenian Ministry of Health (consents Nos. 155/03/13 and 136/04/14). All participating women provided written informed consent.

### HPV testing

For the detection of high-risk HPV, we used the HC2 assay (Qiagen). The HC2 assay was performed according to the manufacturer's instructions, and the results were reported as positive or negative using 1.0 RLU/CO as the cut-off value.[2] Briefly, the test is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 13 high-risk types of HPV DNA in cervical and vaginal specimens.[2] The results were also interpreted according to the "grey zone" ranges proposed by the manufacturer for PreservCyt (1–2.5 RLU/CO[2]), Seme *et al*. for STM (0.4–4.0 RLU/CO[6]) and the Department of Cytopathology Institute of Oncology Ljubljana for STM (0.7–2.0 RLU/CO). Residual samples were stored in the freezer (-30°C) after the denaturation

step. If the results for a specimen were within the 0.4–4.0 RLU/CO range, then the HC2 assay was repeated. When the results of the retest differed from the original results, we reported the final result as inconclusive.

### Statistics

The results are presented as ranges of RLU/CO values for the HPV test result; a proportion of HPV test results in a specific range for all HPV test results; a proportion of women with CIN2/3+ in a specific range for all women with CIN2/3+ in one year since colposcopy; and a risk for CIN2/3+ as a proportion of women with CIN2/3+ for all women in a specific range. Cohen's kappa with a 95% confidence interval (CI) was calculated as a measure of agreement between the original and retested HPV test results as a binary variable at a 1.0 RLU/CO cut-off value. Sensitivities and specificities were calculated with a 95% CI at different RLU/CO cut-off values, and the ROC curve was plotted. All analyses were conducted with R v3.5.3.[19]

## Results

### Study population

Our final study group included 594 women after we excluded one woman who had undergone hysterectomy prior to colposcopy and another with missing data from colposcopy. Biopsy was performed in 352 women (59.3%). Out of 594 women, 291 (49.0%) were negative either on colposcopy (242) or on histology (49). A histologically confirmed low-grade intraepithelial lesion (LSIL) was diagnosed in 141 (23.7%) women and CIN2+ was diagnosed in 162 (27.3%) women. There were 48 CIN2, 102 CIN3, 1 VAIN3, 1 VIN3, 4 AIS and 6 squamous carcinomas.

**TABLE 1.** Number of HPV test results according to "grey zones" proposed by the manufacturer (PreservCyt)[I], Seme *et al.* (STM)[II], and the Department of Cytopathology at Institute of Oncology Ljubljana (STM)[III]

| RLU/CO value [I] | N and % women ($N_{tot}$ = 594) |
|---|---|
| < 1.0 | 283 (47.6) |
| 1.0–2.5 | 23 (3.9) |
| > 2.5 | 288 (48.5) |
| **RLU/CO value [II]** | **N and % women (Tot. N = 594)** |
| < 0.4 | 253 (42.6) |
| 0.4–0.99 | 30 (5.1) |
| 1.0–3.99 | 34 (5.7) |
| ≥ 4.0 | 277 (46.6) |
| **RLU/CO value [III]** | **N and % women (Tot. N = 594)** |
| < 0.7 | 271 (45.6) |
| 0.7–0.99 | 12 (2.0) |
| 1.0–1.99 | 15 (2.5) |
| ≥ 2.0 | 296 (49.8) |

N = number; $N_{tot}$ = total number

### HPV test results based on different definitions for the "grey zone" range

The number of HPV test results within and outside the three "grey zone" ranges are presented in Table 1. The proportion of samples located in 1.0–2.5 RLU/CO, 0.4–4.0 RLU/CO, and 0.7–2.0 RLU/CO was 3.9% (23/594), 10.8% (64/594), and 4.5% (27/594), respectively.

### HPV test results for retested samples within the 0.4-4.0 RLU/CO range

All 64 samples with results between 0.4–4.0 RLU/CO values were retested. In 8/64 (12.5%) retested samples, the results differed from the original results (Figure 1). Six samples for which the results changed from positive to negative originated from patients without CIN2+ lesions, while the seventh case was obtained from a patient with CIN3+ diagnosis. The sample for which the result changed from negative to positive was from a patient without a CIN2+ diagnosis. Kappa agreement between the results before and after retesting was 0.75 (95% CI: 0.59–0.91). Retesting samples with results within the 0.4–4.0 RLU/CO range did not detect any additional CIN2+ cases.

### Detection of CIN2+ and CIN3+ at different ranges of RLU/CO values

The distribution of women with a CIN2+/3+ diagnosis and the risk for CIN2+/3+ based on the RLU/CO values of their HPV test results are presented in Table 2. The majority of women with CIN2+ (85%) had RLU/CO values above 10, and 1.2%, 2.5% % and 5.6% of CIN2+ cases were found within RLU/CO intervals of 0.7–2.0, 1.0–2.5 and 0.4–4.0, respectively. The risk for CIN2+ in women within the 0.4–0.69 and 0.7–0.99 RLU/CO ranges was 5.6% and 8.3%, respectively; however, these results represent one woman per range.

**TABLE 2.** The distribution of women with a CIN2+/3+ diagnosis and the risk for CIN2+/3+ based on the RLU/CO values of their HPV test results

| RLU/CO value | N and % women ($N_{tot}$ = 594) | N and % CIN2+ ($N_{tot}$ = 162) | Risk for CIN2+ (%) | N and % CIN3+ ($N_{tot}$ = 114) | Risk for CIN3+ (%) |
|---|---|---|---|---|---|
| RLU/CO < 0.4 | 253 (42.6) | 8 (4.9) | 3.2 | 2 (1.8) | 0.8 |
| 0.4 ≤ RLU/CO < 0.7 | 18 (3.0) | 1 (0.6) | 5.6 | 1 (0.9) | 5.6 |
| 0.7 ≤ RLU/CO < 1.0 | 12 (2.0) | 1 (0.6) | 8.3 | 1 (0.9) | 8.3 |
| 1.0 ≤ RLU/CO ≤ 2.0 | 15 (2.5) | 1 (0.6) | 6.7 | 1 (0.9) | 6.7 |
| 2.0 < RLU/CO ≤ 2.5 | 8 (1.3) | 3 (1.9) | 37.5 | 2 (1.8) | 25.0 |
| 2.5 < RLU/CO ≤ 4.0 | 11 (1.9) | 3 (1.9) | 27.3 | 2 (1.8) | 18.2 |
| 4.0 < RLU/CO ≤ 10.0 | 26 (4.4) | 7 (4.3) | 26.9 | 6 (5.3) | 23.1 |
| 10.0 < RLU/CO ≤ 100 | 112 (18.9) | 48 (29.6) | 42.9 | 33 (28.9) | 29.5 |
| 100 < RLU/CO ≤ 1000 | 105 (17.7) | 68 (42.0) | 64.8 | 53 (46.5) | 50.5 |
| 1000 < RLU/CO | 34 (5.7) | 22 (13.6) | 64.7 | 13 (11.4) | 38.2 |

CIN2+ = cervical intraepithelial neoplasia grade 2 or greater; CIN3+ = cervical intraepithelial neoplasia grade 3 or greater; N = number; $N_{tot}$ = total number

### Sensitivity and specificity of the HC2 test for CIN2+

Calculations of the sensitivity and specificity of HC2 for CIN2+ at various RLU/CO values are presented in Figure 2. At the threshold recommended by the manufacturer (RLU/CO = 1.0), the sensitivity was 93.8% (95% CI: 90.1–97.5%), and the specificity was 63.2% (95% CI: 58.6–67.6%). Increasing or decreasing the threshold in the vicinity of 1.0 RLU/CO did not significantly improve one value without lowering the other. Increasing the cut-off value from 1.0 to 2.5 RLU/CO decreased the sensitivity from 93.8% to 91.4% (95% CI: 87.0–95.7%), while the specificity increased from 63.2% to 67.6% (95% CI: 63.0–72.0%). Within the interval of 0.7–2.0 RLU/CO, the sensitivity remained nearly constant, with 94.4% (95% CI: 90.7–97.5%) at a cut-off value of 0.7 *vs.* 93.2% (95% CI: 89.5–96.9%) at a cut-off value of 2.0, while the specificity increased from 60.6% (95% CI: 56.0–65.3%) to 66.4% (95% CI: 61.8–70.8%). In the third "grey zone", the sensitivity gradually decreased from 95.1% (95% CI: 91.4–98.1%) at 0.4 RLU/CO to 89.5% (95% CI: 84.6–93.8%) at 4.0 RLU/CO, while the specificity gradually increased from 56.7% (95% CI: 51.9–61.3%) to 69.4% (95% CI: 65.0–73.8%).

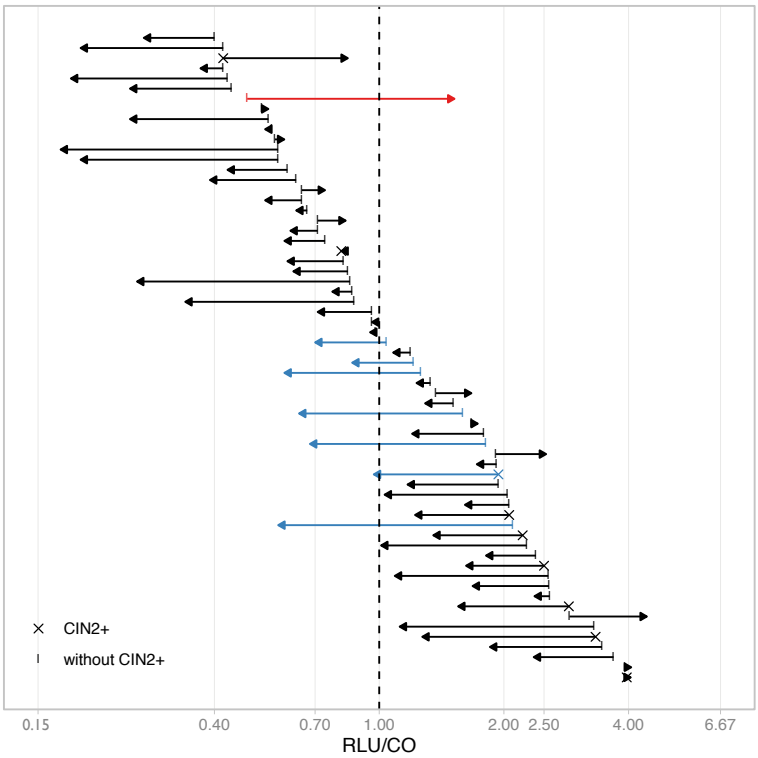


**FIGURE 1.** Changes in the RLU/CO values after retesting samples within the 0.4–4.0 RLU/CO range. The red arrow represents the samples with changes in the results from negative to positive, the blue arrows represent samples with changes in the results from positive to negative, and the black arrows represent samples that retained the original result.

## Discussion

Our results showed that relatively few HC2 test results fell within the "grey zone" ranges currently used by the Institute of Oncology Ljubljana and those proposed by the manufacturer for PreservCyt specimens (3.7% and 4.5%, respectively). The "grey zone" proposed by Seme *et al.*[6] was broader and contained 10.8% of the HC2 results. The percentages of CIN2+ diagnoses detected within the above-mentioned "grey zones" were 1.2%, 2.5% and 5.6%, respectively. Retesting the samples within the broadest "grey zone" investigated did not detect

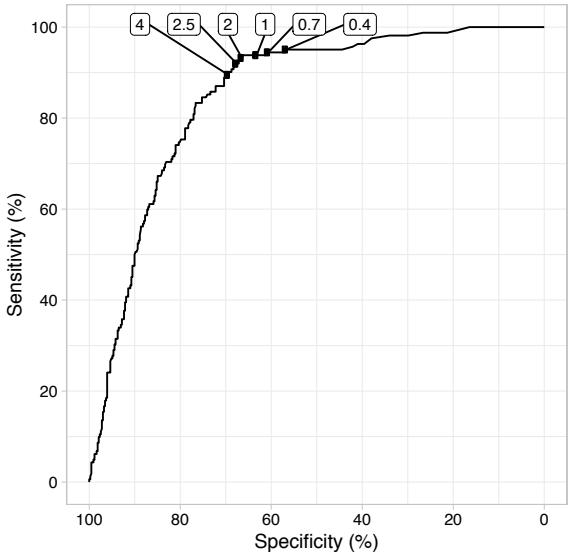


**FIGURE 2.** ROC curve demonstrating the sensitivity and specificity of the HC2 test for CIN2+ with marked RLU/CO cut-off values that represent the lower and upper borders of the "grey zone" ranges.

additional CIN2+ cases. Calculations of sensitivity and specificity at different RLU/CO values showed that increasing or decreasing the cut-off value within the three "grey zones" did not significantly improve either of the variables.

We have not found any studies that examined the proportion of STM specimens with HC2 results located in the vicinity of a cut-off value. However, a few studies have reported on the number of HC2 results found in the "grey zone" interval proposed by the manufacturer (1.0–2.5 RLU/CO) for PreservCyt specimens.[9-11] Muldrew *et al.*[9], Rao *et al.*[10] and Knoepp *et al.*[11] found 3–5.2% of their specimens within this "grey zone", which is similar to our result of 4.5%. This finding implies that the frequency of cases with HC2 results within this "grey zone" is similar, regardless of the medium used for specimen collection (STM or PreservCyt).

From a clinical point of view, the number of CIN2+ cases found within a "grey zone" is much more relevant than the number of HC2 equivocal results. Among all CIN2+ cases found in our whole study group, only 1.2% was within 0.7–2.0 RLU/CO, 2.5% was within 1.0–2.5 RLU/CO and 5.6% was within 0.4–4.0 RLU/CO. Although several studies have evaluated the proportion of women with CIN2+ diagnoses among those with HC2 results near the cut-off point, it is difficult to compare our results to theirs. The results vary to a moderate extent, and some differences could be attributed to differences in the studied populations and to the definition of the equivocal results. Knoepp *et al.*[12], for example, reported 8% of CIN2+ cases among the whole study population with equivocal HC2 results (1.0–2.5 RLU/CO) and 16.5% of CIN2+ cases of equivocal HC2 and ASC-US cytology. Origoni *et al.*[7] found only 4.6% of CIN2+ cases in women with ASC-US cytology and HC2 between 1.0 and 10.0 RLU/CO, while LaMere *et al.*[13] reported 6.8% CIN2+ among cases with low-grade cytology and 1.0–3.0 RLU/CO. Interestingly, Elkins *et al.*[14] demonstrated that the clinical relevance of the HC2 test is age dependent. In two age groups, which together ranged from 15–49 years, these authors found an approximately equal percentage of CIN2+ diagnoses (6%) within the "grey zone" of 1.0–2.5 RLU/CO, while there were no CIN2+ cases in the group aged 50 years or more. Despite variations in their results, all authors concluded that women with equivocal HC2 results should be managed as unequivocal positive results.

Since the manufacturer recommends retesting PreservCyt specimens with results within the "grey zone", several authors have already evaluated whether the retesting algorithm is effective.[11, 13-16] Some authors have performed one retest[15, 16], while others have reported two retests[13], and some retested only those samples where the results were found within the "grey zone" after the first retest[11] or below 1.0 RLU/CO.[14] The range of the "grey zone" varied from 0.8 to 3.0 RLU/CO. Some authors have found that the majority of their specimens retained the original positive (87%–97%) HC2 result.[11,13,14] These results are comparable to our results for the STM specimens, which showed that 87.5% of specimens retained the original HC2 test result after retest, even though our "grey zone" was wider. Ramirez *et al.*[15] and de Vries *et al.*[16] obtained lower values for specimens that retained original HC2 diagnoses after retest (64% and 74%, respectively). Ramirez *et al.*[15] was the only study besides ours where the "grey zone" extended below 1.0 RLU/CO, and these authors also found that two results changed from negative to positive. The low percentage of cases that retained positive HC2 results in the study of de Vries[16] is due to the age of their study population of 50 years or older. All the above-mentioned authors concluded that retest is not necessary because few results change after retest or because retests might be less reliable. For example, Ramirez *et al.*[15] mentioned that the amount of sample can influence the results, and viral loads may vary in successive tests.

The expected added value of retesting equivocal HC2 results is a potential increase in the ac-

curacy of the triage test and a better identification of women who have low-risk for developing CIN2+ lesions since these individuals could be returned to screening or to a less intensive follow-up. However, an additional reason that speaks against retesting samples within the "grey zones" is the finding that sensitivity and specificity do not change much within these "grey zone" ranges. Calculating the sensitivity of the HC2 test at different RLU/CO values demonstrated that decreasing the cut-off for positivity to the lower border of the "grey zone" range would achieve a small increase in the sensitivity and therefore would not add a higher accuracy of the test. The sensitivity was 95.1% at a cut-off value of 0.4, 94.4% at the cut-off value of 0.7 and 93.8% at the cut-off value of 1.0 RLU/CO. Several authors have performed similar studies[20-26,] and a systematic review by Rebolj *et al.*[27] concluded that the threshold could be increased to values between 2.0–10.0 RLU/CO without endangering the sensitivity level necessary for screening. This increase would avoid the problem of "grey zones". Compared to our study, the majority of the reported studies have compared the relative values of sensitivity, since HPV-negative women did not undergo colposcopy examinations. Therefore, their calculations did not include the CIN2+ lesions with HC2 results in the negative part of the RLU/CO range since such cases were missed. Thus, their values of sensitivity were higher.

The strength of our study is in reporting results within "grey zones" for STM specimens and investigating two "grey zones" that extended below 1.0 RLU/CO. The results within "grey zones" for STM samples have not been presented before, and most reports included "grey zones" above the proposed cut-off value. The limitation of our study is a small study population, comprising women invited to colposcopy, which showed a higher incidence of CIN2+ lesions. The risk for developing CIN2+ was higher within the investigated "grey zones" (7.4%, 17.4% and 14.1%, respectively) than among the general population since the prevalence of the disease was higher in women referred to colposcopy than in the population of women in our study. Therefore, our findings need to be tested on a larger population with the risk for CIN2+ comparable to that of the population where triage is recommended. An additional limitation of our study is the use of residual samples that were stored in the freezer (-30°C) after the denaturation step for HC2 retesting. This storage procedure could have caused sample degradation and might have influenced our results. These findings will be important for cervical cancer screening programmes that use the HC2 assay as a primary or triage test and collect cervical specimens in STM. The results will help specify the best protocol for handling STM specimens with results within the "grey zone".

In conclusion, our results show that retesting STM samples within the "grey zones" is not necessary for several reasons. The majority of samples within the "grey zone" retain the original HC2 results after retest. The number of CIN2+ lesions among women with "grey zone" HC2 results is low. There is limited additional value of the retesting algorithm since sensitivity and specificity of HC2 for CIN2+ do not change much within the "grey zone". Retesting samples with HC2 results in the negative range of the "grey zone" does not increase sensitivity, while retesting in the positive "grey zone" does not add to a less intensive management of women. Women with HC2 results above 1.0 RLU/CO but within the "grey zone" will be followed in the same manner, regardless of the outcome of the retest. Only women with two negative HC2 results will return to regular screening. Furthermore, according to Slovenian clinical guidelines, the management of women with discordant results between the original test and the repeated HC2 test does not allow women to be returned to screening or to a less intensive follow-up, since at least one additional test is needed before the decision could be made about further management.

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*research article*

# Transcription factors gene expression in chronic rhinosinusitis with and without nasal polyps

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**Background.** Chronic rhinosinusitis (CRS) current therapeutic approaches still fail in some patients with severe persistent symptoms and recurrences after surgery. We aimed to evaluate the master transcription factors gene expression levels of T cell subtypes in chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP) that could represent new, up-stream targets for topical DNAzyme treatment.

**Patients and methods.** Twenty-two newly diagnosed CRS patients (14 CRSwNP and 8 CRSsNP) were prospectively biopsied and examined histopathologically. Gene expression levels of T-box transcription factor *(*T-bet, *TBX21),* GATA binding protein 3 *(GATA3),* Retinoic acid-related orphan receptor C *(RORC)* and Forkhead box P3 (*FOXP3)* were analyzed by real-time quantitative polymerase chain reaction (RT-qPCR).

**Results.** Eosinophilic CRSwNP was characterized by higher level of *GATA3* gene expression compared to noneosinophilic CRSwNP, whereas there was no difference in T-bet, *RORC* and *FOXP3* between eosinophilic and noneosinophilic CRSwNP. In CRSsNP, we found simultaneous upregulation of T-bet, *GATA3* and *RORC* gene expression levels in comparison to CRSwNP; meanwhile, there was no difference in *FOXP3* gene expression between CRSwNP and CRSsNP.

**Conclusions.** In eosinophilic CRSwNP, we confirmed the type 2 inflammation by elevated *GATA3* gene expression level. In CRSsNP, we unexpectedly found simultaneous upregulation of T-bet and *GATA3* that is currently unexplained; however, it might originate from activated CD8+ cells, abundant in nasal mucosa of CRSsNP patients. The elevated *RORC* in CRSsNP could be part of homeostatic nasal immune response that might be better preserved in CRSsNP patients compared to CRSwNP patients. Further data on transcription factors expression rates in CRS phenotypes are needed.

Key words: chronic rhinosinusitis; nasal polyps; Th1 cells; Th2 cells; Th17 cells; Transcription factors

## Introduction

The estimated prevalence of chronic rhinosinusitis (CRS) is more than 10% in the European and US adult population.[1] CRS can be subdivided into chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). Histopathologically, CRSwNP can be further classified into eosinophilic CRSwNP (with pronounced eosinophilic mucosal infiltration and type-2 inflammation) and noneosinophilic CRSwNP.[2]

Studies on cytokine profiles and transcription factor expression rates characterized eosinophilic CRSwNP by mucosal eosinophilia, Th2 and type 2 innate cell inflammation.[3,4] Type 2 inflammation cytokines in CRSwNP are IL-4, IL-5 and IL-13, eosinophilic cationic protein (ECP) or Charcot Leyden Crystal protein.[4-6] IL-5 is a key mediator in type 2 inflammation, providing survival, maturation and

activation of eosinophils at the bone marrow and the site of inflammation.[7] Higher levels of mucosal and/or peripheral blood eosinophils are correlated with poor life quality, asthma comorbidity, recurrences after endoscopic surgery and CRSwNP disease severity, associated with inadequate defence against bacteria and viruses.[8,9] Additionally, eosinophilic CRSwNP is associated with disrupted epithelial barrier and Staphylococcus aureus presence, which intramucosaly releases enterotoxins (SE). SE are immunogenic superantigens that bind to T cell receptors with unrestricted antigen specificity to activate T and B cells, finally leading to specific SE IgE production.[10] SE IgE amplify the type 2 inflammation in eosinophilic CRSwNP and are an independent risk factor for asthma comorbidity.[9,10] Staphylococcus aureus presence in nasal mucosal biofilm was associated with worse postoperative outcome after endoscopic surgery.[11]

Noneosinophilic CRSwNP was characterized by mixed Th1/ Th2/ Th17 pattern.[4] A flow cytometric study showed a predominant Th1 endotype in CRSwNP in addition to Th2; however, in basal conditions, those Th1 cells were not activated, did not release cytokine IFNγ and thus the authors hypothesized that an increased number of Th1 in CRSwNP was not related to inflammation pathogenesis.[12] Similarly, in our recent flow cytometric study, we found more abundant Th1 cells in CRSwNP compared to CRSsNP; however, we didn't find any impact of Th1 cell count on disease control and speculated that Th1 cells were not important for the pathogenesis in CRSwNP.[13]

CRSsNP was found to have more heterogeneous endotypes, with either Th1, Th2 or Th17 cytokines; furthermore, the remodelling process was not in correlation with CRSsNP endotype.[4,9,14] Th17 cells can secrete IL-17, IL-22 or IFN-γ upon stimulation.[15] Moreover, in our recent flow cytometric study, we found that Th17 cells in CRSwNP were associated with well-controlled CRSwNP.[13] Similarly, Th17 were reported to have a potentially protective immune homeostatic role in CRSwNP and to be a part of the normal homeostatic immune response in healthy nasal mucosa by IL-17 production.[15]

Regulatory T cells (Treg) control the CRS inflammation; the regulatory cells deficiency or dysfunction can lead to exaggerated inflammation; on the other hand, an elevated Treg number might be a sign of an unsuccessful inflammation control by immune system.[16]

Current therapeutic approaches still fail in a portion of patients with severe persistent CRS symptoms and recurrences after surgery.[17] Future treatment concepts focus on up-stream targets like transcription factors; *GATA3* DNAzyme topical therapy attenuated Th2-regulated inflammatory responses in asthma.[18]

Therefore, we decided to evaluate the gene expression of intrinsic cellular, lineage-defining, master transcription factors for effector T helper lymphocytes (Th) in relation to CRS phenotypes (CRSwNP compared to CRSsNP, eosinophilic CRSwNP compared to noneosinophilic CRSwNP): T-box transcription factor (T-bet, *TBX21*) for Th1 cells, GATA binding protein 3 *(GATA3)* for Th2 cells and Retinoic acid-related orphan receptor C *(RORC)* for Th17 cells. Additionally, we aimed to characterize Forkhead box P3 (*FOXP3)*, the master transcription factor for the regulatory T cell (Treg) gene expression level to evaluate Treg cell bias in CRS phenotypes.

## Patients and methods

### Study subjects

Twenty-two newly diagnosed, adult CRS patients (14 CRSwNP, 8 CRSsNP) were prospectively included at their first visit at the Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Centre, Ljubljana, Slovenia. CRS diagnosis was established by symptoms, nasal endoscopy and a 2 or 3 mm computed tomography (CT) scan of paranasal sinuses. Included patients have never before used intranasal or systemic steroids. We took a biopsy of the middle meatal nasal polyp in CRSwNP patients or uncinate process mucosa in CRSsNP patients. Detailed patient inclusion data are shown in the flowchart in Figure 1 and detailed demographic data are summarized in Table 1. This study was conducted in accordance with the amended Declaration of Helsinki. The study was approved by the Slovenian National Medical Ethics Committee (approval number 34/10/12) and patients gave their written informed consent.

### Histopathology

After sampling, the biopsy specimen was immediately fixed in 10% neutral-buffered formalin. Each paraffin-embedded specimen was sectioned at 5 micrometers thickness and stained by hematoxylin-eosin. The histopathologic evaluation of nasal biopsy specimens was performed using a structured CRS inflammation report as described by.[2] On hematoxylin-eosin-stained sections, the specimens were analyzed for eosinophil count per high

**TABLE 1.** Demographic features, investigations and histopathological findings

| Demographic features, investigations and histopathology | CRSwNP study population (n = 14) | CRSwNP (n = 14) | | | CRSsNP study population (n = 8) | *P** |
|---|---|---|---|---|---|---|
| | | eosinophilic CRSwNP (n = 10) | noneosinophilic CRSwNP (n = 4) | *P* | | |
| Age (years) | 52.1 (39.3–62.4) | 49.4 (39.3–58.4) | 63.3 (37.6–68.3) | 0.18 | 45.2 (33.3–51.3) | 0.23 |
| Female sex, no. (%) | 5 (35.7) | 4 (40) | 1 (25) | 0.60 | 4 (50) | 0.51 |
| Allergy, no. (%) | 3 (21.4) | 1 (10) | 2 (50) | 0.10 | 1 (12.5) | 0.60 |
| Asthma, no. (%) | 3 (21.4) | 1 (10) | 2 (50) | 0.31 | 1 (12.5) | 0.84 |
| COPD, no. (%) | 1 (7.1) | 1 (10) | 0 (0) | 0.89 | 0 (0) | 0.71 |
| Smoking, no. (%) | 3 (21.4) | 3 (30) | 0 (0) | 0.22 | 1 (12.5) | 0.60 |
| CRS duration (y) | 4 (3–10) | 5.5 (2.5–10) | 4 (3–10) | 0.91 | 3 (2.3–9.3) | 0.37 |
| VAS (0–10) at inclusion | 8 (7.8–9.3) | 8.5 (7.8–10) | 8 (5.8–8.8) | 0.42 | 9.5 (7.3–10) | 0.38 |
| CT Lund MacKay score at inclusion | 15 (12.8–18) | 14.5 (12.8–18) | 17.5 (12.8–23) | 0.36 | 12 (8.3–12.8) | N.A. |
| Endoscopic Lund Kennedy score at inclusion | 8 (7–9.3) | 8 (7–8.3) | 9 (8–10) | 0.15 | 4 (3–4) | N.A. |
| Tissue eosinophilia > 10 / HPF, no. (%) | 10 (71.4) | 10 (100) | 0 (0) | N.A. | 1 (13) | **0.008** |
| Neutrophil infiltration, no. (%) | 6 (42.9) | 4 (40) | 2 (50) | 0.73 | 2 (25) | 0.40 |
| Basement membrane thickening ≥ 7.5µm, no. (%) | 11 (78.6) | 10 (100) | 1 (25) | **0.002** | 8 (100) | 0.16 |
| Moderate / severe subepithelial oedema, no. (%) | 12 (85.7) | 9 (90) | 3 (75) | 0.47 | 1 (12.5) | **0.002** |
| Hyperplastic / papillary change, no. (%) | 5 (35.7) | 4 (40) | 1 (25) | 0.51 | 2 (25) | 0.53 |
| Squamous metaplasia, no. (%) | 4 (28.6) | 3 (30) | 1 (25) | 0.85 | 4 (50) | 0.31 |
| Fibrosis, no. (%) | 10 (71.4) | 7 (70) | 3 (75) | 0.85 | 7 (87.5) | 0.39 |

* *P* refers to the comparison between CRSwNP and CRSsNP; values are expressed as numbers (percentages) or medians (Q1–Q3); $P < 0.05$ are boldface; CRS = chronic rhinosinusitis; HPF = high power field; N.A. = not applicable, values are not indicated because these characteristics are the basis for the patient's classification; VAS = visual analogue scale

power field (HPF) (< 5, 5–10, > 10), neutrophilic infiltration, basement membrane thickening (≥ 7.5µm), subepithelial edema (absent to mild, moderate to severe) and presence of hyperplastic / papillary change, squamous metaplasia and fibrosis.

## Tbet *(TBX21)*, *GATA3*, *RORC* and *FOXP3* gene expression levels

After sampling, tissue biopsy was stored in RNA*later* solution (Qiagen, Hilden, Germany) at –40ºC. Total RNA was extracted using miRNeasy Mini Kit (Qiagen), according to the manufacturer's instructions, and reverse transcribed to cDNA using the High-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-qPCR was performed on ABI PRISM 7500 Fast Real-Time PCR System at standard conditions utilizing TaqMan Fast Advanced Master Mix (Applied Biosystems). The Taqman assays T-bet (*TBX21*) (Hs00203436_m1), *GATA3* (Hs00231122_m1), *RORC* (Hs01076122_m1) and *FOXP3* (Hs00203958_m1) were utilized to determine the mRNA expression levels of transcription factors and glyceraldehyde-

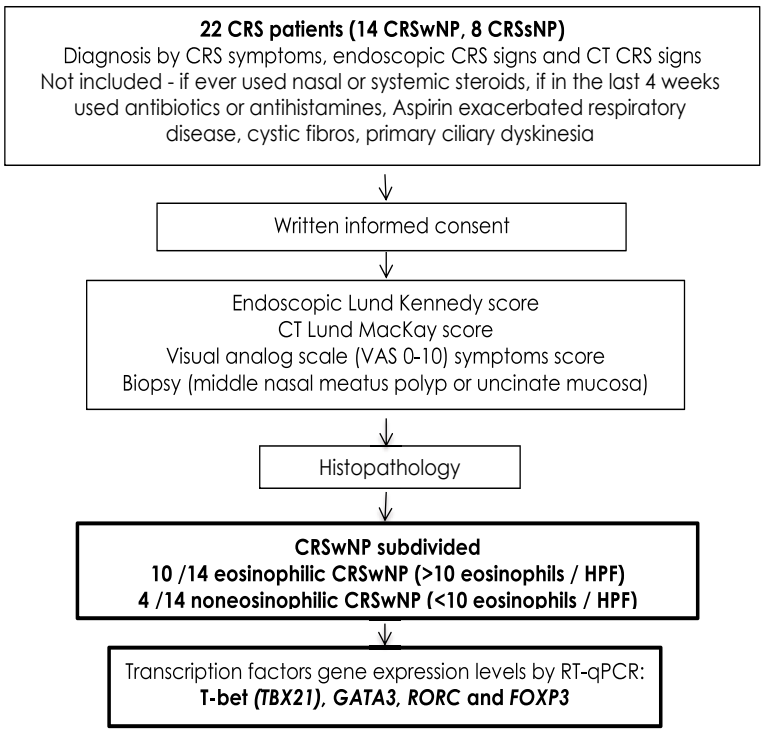


**FIGURE 1.** Flowchart of the inclusion of the study subjects.

**TABLE 2.** T-bet *(TBX21), GATA3, RORC* and *FOXP3* gene expression (relative mRNA levels) in CRS nasal mucosa

| | CRSwNP study population (n = 14) | CRSwNP (n = 14) | | | CRSsNP study population (n = 8) | *P** |
|---|---|---|---|---|---|---|
| | | eosinophilic CRSwNP (n = 10) | noneosinophilic CRSwNP (n = 4) | *P* | | |
| ***T-bet*** | 1.6 (0.5–2.4) | 2.0 (0.5–3.9) | 1.1 (0.5–2.0) | 0.43 | 7.9 (4.4–11.8) | **0.0003** |
| ***GATA3*** | 0.6 (0.3–0.8) | 0.7 (0.6–1.3) | 0.3 (0.1–0.4) | **0.02** | 4.1 (2.3–9.6) | **0.0003** |
| ***RORC*** | 1.7 (0.5–4.0) | 1.7 (0.5–5.0) | 1.3 (0.3–3.3) | 0.71 | 6.9 (3.8–13.3) | **0.006** |
| ***FOXP3*** | 1.6 (1.2–3.7) | 1.6 (1.1–3.7) | 1.6 (1.2–4.5) | 0.76 | 3.8 (1.0–10.8) | 0.73 |

* *P* refers to the comparison between CRSwNP and CRSsNP; values are expressed as medians (Q1–Q3); $P < 0.05$ are boldface

3-phosphate dehydrogenase (GAPDH; 4333764F) as an endogenous control (all Applied Biosystems). All measurements were performed in triplicate for each sample and relative expression was analyzed using the ΔΔCt method.[19] Briefly, the relative expression of each mRNA was calculated by subtracting the Ct value of GAPDH from the Ct value of the gene analyzed to calculate the ΔCt.[19] Then the relative expression of the sample was calculated using the formula RQ sample = $2^{-(\Delta Ct\ sample - \Delta Ct\ calibrator)}$. Nasal mucosa from the uncinate process of a control subject, 55 years old healthy female without CRS was used as a calibrator.

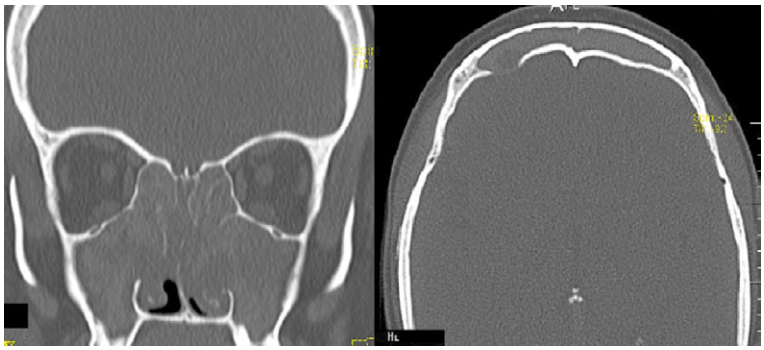

**FIGURE 2.** A representative CT scan of one CRSwNP patient in frontal and axial plane, CT Lund MacKay score 24.

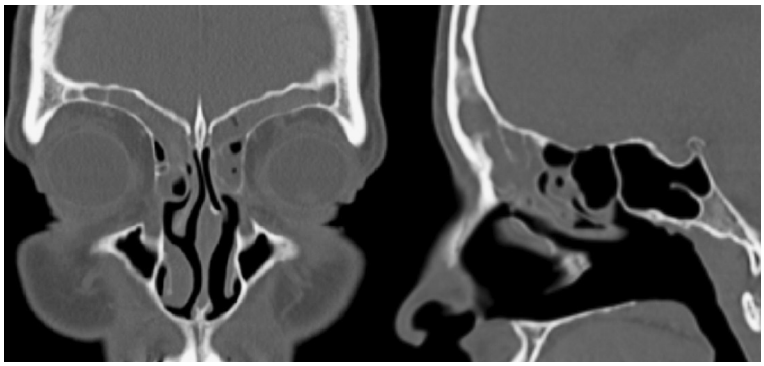

**FIGURE 3.** A representative CT scan of one CRSsNP patient in frontal view (left) and sagittal view (right), CT Lund MacKay score 8.

### Statistical analysis

The data generated in the study were analyzed using GraphPad Prism 6.0 (San Diego, CA, USA). Statistical analysis was performed using the Mann-Whitney and Chi-square tests as appropriate with 2-tailed p. The data are expressed as numbers (percentages) or medians (first quartile (Q1) – third quartile (Q3)). The significance level was set at a p value of 0.05.

## Results

### Clinical analysis of CRSwNP compared to CRSsNP

Twenty-two newly diagnosed CRS patients (14 CRSwNP and 8 CRSsNP) were prospectively included in the study. They have not been treated yet and have never used intranasal or systemic steroids. Both groups were comparable in age, sex, smoking, allergy, asthma, chronic obstructive pulmonary disease (COPD) and severity of CRS symptoms on visual analog scale 0–10. As expected, patients with CRSwNP had higher endoscopic Kennedy Lund scores than patients with CRSsNP. Exact clinical data are summarized in Table 1.

### Paranasal sinus CT scan

In concordance with CRS phenotypes, patients with CRSwNP had higher CT Lund MacKay scores at inclusion than patients with CRSsNP. Exact CT

scores are summarized in Table 1. Representative CT scans of one CRSwNP and one CRSsNP patient are shown (Figure 2,3).

### Histopathological analysis of CRSwNP compared to CRSsNP and eosinophilic CRSwNP compared to noneosinophilic CRSwNP

Tissue eosinophilia > 10 per HPF was significantly more frequent (71%) in CRSwNP patients compared to CRSsNP patients (13%); meanwhile, there was no difference in tissue neutrophil infiltration between groups. As expected, moderate to severe subepithelial oedema was more pronounced in CRSwNP patients compared to CRSsNP patients. 10 CRSwNP patients with > 10 tissue eosinophils per HPF represented the eosinophilic CRSwNP group, the other 4 CRSwNP patients with < 10 tissue eosinophils per HPF represented the noneosinophilic CRSwNP group. More eosinophilic CRSwNP patients had basement membrane thickening ≥ 7.5μm compared to noneosinophilic CRSwNP patients. There was no difference in hyperplastic or papillary change, squamous metaplasia and fibrosis between groups. Exact histopathological data are summarized in Table 1.

### Transcription factors gene expression levels analysis in eosinophilic CRSwNP compared to noneosinophilic CRSwNP patients and in CRSwNP compared to CRSsNP

*GATA3* gene expression level was significantly upregulated in eosinophilic CRSwNP compared to noneosinophilic CRSwNP patients (Table 2 and Figure 4B). There was no difference in T-bet, *RORC* and *FOXP3* expression levels between eosinophilic and noneosinophilic CRSwNP patients (Figures 4A, C, D). Effector Th cell master transcription factors T-bet, *GATA3* and *RORC* gene expression levels in nasal mucosal were significantly elevated in CRSsNP compared to CRSwNP patients (Table 2 and Figures 5A, B, C). On the other hand, Treg master transcription factor *FOXP3* gene expression level was equalized in CRSwNP and CRSsNP patients (Figure 5D).

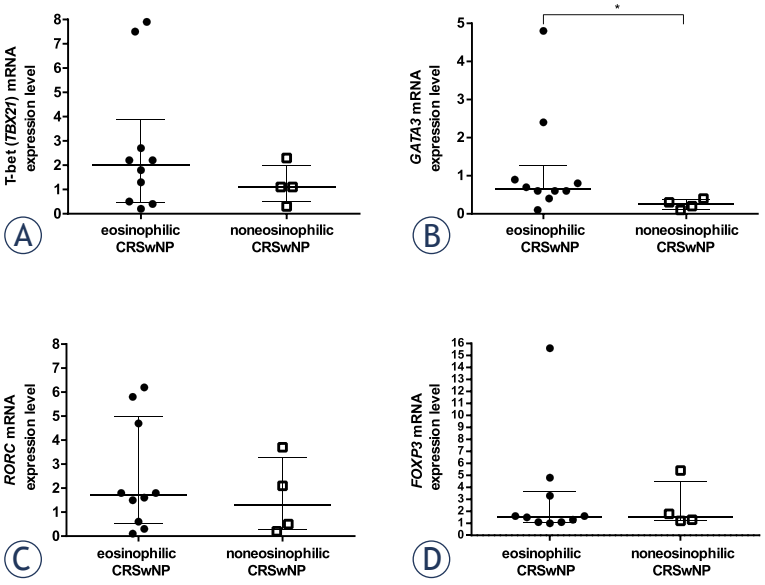


**FIGURE 4.** Comparison of mRNA expression levels between eosinophilic CRSwNP and noneosinophilic CRSwNP in **(A)** T-bet *(TBX21)* mRNA, **(B)** *GATA3* mRNA, **(C)** *RORC* mRNA and **(D)** *FOXP3* mRNA expression. *P< 0.05 by the Mann–Whitney test.

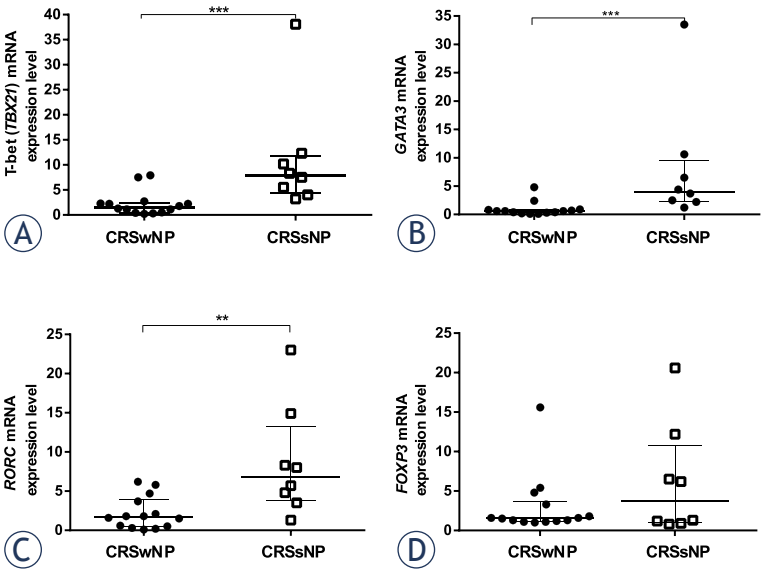


**FIGURE 5.** Comparison of mRNA expression levels between CRSwNP and CRSsNP in **(A)** T-bet *(TBX21)* mRNA, **(B)** *GATA3* mRNA, **(C)** *RORC* mRNA and **(D)** *FOXP3* mRNA expression. *P < 0.05, **P < 0.001, and ***P < 0.0001 by the Mann–Whitney test.

## Discussion

In this prospective study, we analyzed histopathological characteristics and master transcription factors gene expression levels of nasal mucosa T cells in newly diagnosed CRSwNP and CRSsNP patients, who have not been treated yet and have never used intranasal or systemic steroids.

## Mucosal eosinophilia and histopathological differences

We confirmed previous findings that CRSwNP is characterized by mucosal eosinophilia and pronounced subepithelial oedema.[2] Additionally, basement membrane thickening ≥ 7.5μm as a sign of remodeling and more severe inflammation was more pronounced in eosinophilic CRSwNP patients with > 10 tissue eosinophils per HPF compared to noneosinophilic CRSwNP patients with < 10 tissue eosinophils per HPF, in concordance with previous reports.[17] Tissue eosinophilia in CRSwNP mucosa is known to be associated with type 2 cytokines and inflammation.[9,17]

## T-bet transcription factor gene expression

Surprisingly, we found significantly upregulated T-bet gene expression level in CRSsNP patients compared to CRSwNP. In contrast, one earlier study has reported downregulated T-bet in healthy patients inferior turbinates mucosa and CRSsNP mucosa compared to CRSwNP mucosa[6], meanwhile, other studies haven't found any difference in T-bet expression between CRSwNP and CRSsNP groups.[20,21] T-bet is the master transcription factor of Th1 cells and type 1 innate lymphoid cells (ILC1), it promotes their differentiation, activation and interferon-γ (IFN-γ) secretion.[22,23] In response to IFN-γ signaling, T-bet can either activate type 1 inflammation or modulate it in case of exaggerated type 1 response.[24] Additionally, in effector cytotoxic type CD8+ T cells, T-bet is highly expressed.[25] Furthermore, in many acute and chronic viral infections, T-bet expression in effector CD8+ cells correlates with infection clearance or improved infection control.[26-28] In our recent study, we found significantly depleted Th1 and abundant, effector cytotoxic CD8+ cells in CRSsNP nasal mucosa compared to CRSwNP.[13] We might speculate here that highly upregulated T-bet in CRSsNP patients could originate from expanded effector cytotoxic CD8+ cells in CRSsNP nasal mucosa.

## *GATA3* transcription factor gene expression

In this analysis, significantly elevated *GATA3* gene expression level in patients with eosinophilic CRSwNP compared to patients with noneosinophilic CRSwNP was found, in concordance with previous reports.[21,29] *GATA3* is the master transcription factor of Th2 cells[30,31] and type 2 innate lymphoid cells (ILC2)[32,33], enriched in CRS mucosa.[3] Obviously, upregulated *GATA3* expression in the eosinophilic CRSwNP group is related to type 2 (Th2 and ILC-2) inflammation. However, activated effector CD4-CD8- double negative T (DN T) cells can also secrete type 2 cytokines.[34,35] Importantly, in our recent study, elevated inflammatory DN T cells in the nasal mucosa of patients with uncontrolled CRSwNP were found. The master transcription factor of DN T cells is currently unknown. The impact and contribution of DN T cells to the type 2 inflammation in CRSwNP mucosa needs further investigation.

Unexpectedly, *GATA3* gene expression level was significantly higher in patients with CRSsNP compared to patients with CRSwNP, in line with one earlier rtPCR study[3], meanwhile, in contrast to two immunohistochemic studies.[6,20] In healthy sinus mucosa, *GATA3* expression level was upregulated compared to CRSwNP mucosa, whereas there was no difference in *GATA3* between CRSsNP mucosa and healthy sinus mucosa.[3] Other studies found downregulated *GATA3* expression level in healthy patient's inferior turbinates mucosa compared to CRSwNP mucosa[6,36]; however, inferior turbinate mucosa is not the same as sinus mucosa. Interestingly, CD8+ T cells were previously shown to express *GATA3* constitutively, upregulate *GATA3* upon T cell receptor (TCR) activation and further increase it by IL-4 and IL-2 stimulation.[37] Moreover, *GATA3* expressing and type 2 cytokines producing effector memory CD8+ T cells were confirmed to be elevated in asthma[38] and tuberculosis infection.[39] Similarly to high T-bet expression, we might speculate here, that upregulated *GATA3* in CRSsNP group could originate from activated effector CD8+ T cells, that were present abundantly in CRSsNP nasal mucosa in our previous study.[13]

## *RORC* transcription factor gene expression

We found significantly higher *RORC* expression in patients with CRSsNP compared to patients with CRSwNP, in line with previous reports.[6,20] Master transcription factor RORC is expressed by Th17[40] and type 3 innate lymphoid cells (ILC3).[41] In our previous flow cytometric study, we reported the association of Th17 cells with well-controlled CRSwNP.[13] The Th17 response was earlier found to be present in the healthy nasal mucosa and therefore, the authors proposed the Th17 cells were a part of normal homeostatic nasal mucosa immune

response.[15] Similarly, we could speculate here that upregulated *RORC* expression level in patients with CRSsNP is part of homeostatic nasal mucosa immune response, that might be better preserved and more functional in CRSsNP mucosa compared to CRSwNP.

### *FOXP3* transcription factor gene expression level

*FOXP3* expression level was equalized in eosinophilic CRSwNP compared to noneosinophilic CRSwNP as well as in CRSwNP compared to CRSsNP; these observations suggest that regulatory T cells are not significantly involved in inflammatory bias between CRSwNP and CRSsNP patients. Previously, contradictory results were reported with either no difference in *FOXP3* expression levels between CRSwNP and CRSsNP[20] or downregulated *FOXP3* in CRSwNP compared to CRSsNP.[6,42] *FOXP3* gene expression was found downregulated in CRSwNP patients compared to healthy patients inferior turbinate mucosa.[6,36] *FOXP3* is the master transcription factor for Treg[43]; in contrast to the other master transcription factors, it has not been identified in ILCs.[23] Interestingly, some *FOXP3*+ Treg cells can even co-express another master transcription factor (T-bet, *GATA3* or *RORC*), however, at much lower levels than corresponding effector Th cells. This way, Tregs may co-localize with corresponding Th effector cells to control their activation[23]; T-bet and *FOXP3* co-expression specifically inhibits Th1 and CD8+ T cell activation[44], *GATA3* and *FOXP3* co-expression controls type 2 inflammation and also co-modulates Treg function.[45] Additionally, co-expression of master transcription factors in effector Th cells was noticed in various chronic inflammatory diseases and can play a beneficial or a detrimental role.[46-48] Moreover, upon in vitro stimulation of CRSwNP mucosa with Staphylococcus aureus enterotoxin (SE), upregulation of Treg was reported; the authors concluded, that Treg cells might be unsuccessful in inflammation control by Th2 activation and Th1 supression.[12] In the present study, we haven't found any difference in FOXP3 expression levels between eosinophilic and nonesosinophilic CRSwNP patients and therefore, we cannot make any speculations about SE presence and stimulation in the eosinophilic CRSwNP patients mucosa.

Importantly, transcription factor-targeted future treatment strategies might have a positive impact on CRS mucosa by blocking the inflammatory pathway and, on the other hand, a negative impact by targeting co-expression in Tregs and their corresponding inflammation suppression.

### Limitations of the study

This study has some limitations. No control group was included. The groups of patients were relatively small. However, all participants were newly diagnosed and have not been treated with intranasal or systemic steroids yet. Finally, we have only analyzed master transcription factors gene expression levels and not protein expression levels. We plan to expand gene expression investigation in CRS mucosa further to RNA sequence analysis.

To conclude, the type-2 inflammation was confirmed by elevated *GATA3* gene expression level in patients with eosinophilic CRSwNP in comparison to patients with noneosinophilic CRSwNP. The unexpectedly found, simultaneous upregulation of T-bet and *GATA3* transcription factors gene expression levels in patients with CRSsNP compared to patients with CRSwNP is currently unexplained; however, it might originate from activated CD8+ cells in CRSsNP nasal mucosa, in concordance with our recent findings of expanded effector cytotoxic CD8+ cells in the mucosa of patients with CRSsNP.[13] The *RORC* upregulation in CRSsNP could be part of normal homeostatic nasal mucosa immune response that might be better preserved and more functional in CRSsNP mucosa compared to CRSwNP. In the perspective of future transcription factor-targeted topical treatments development, further data on transcription factors expression rates in CRS phenotypes are needed.

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*research article*

# Factors affecting the morbidity and mortality of diverting stoma closure: retrospective cohort analysis of twelve-year period

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**Background.** Diverting stoma is often performed in rectal cancer surgery for reducing the consequences of possible anastomotic failure. Closing of stoma follows in most cases after a few months. The aim of our study was to evaluate morbidity and mortality after diverting stoma closure and to identify risk factors for complications of this procedure.

**Patients and methods.** At our department, we have performed a retrospective cohort analysis of data for 260 patients with diverting stoma closure from 2003 to 2015. Age, stoma type, patient's preoperative ASA score, surgical technique and time to stoma closure were investigated as factors which could influence the complication rate.

**Results.** 218 patients were eligible for investigation. Postoperative complications developed in 54 patients (24.8%). Most common complications were postoperative ileus (10%) and wound infection (5%). Four patients died (1.8%). There was no effect on complication rate regarding type of stoma, closing technique, patient's ASA status and patient age. The only factor influencing the complication rate was the time to stoma closure. We found that patients which had the stoma closed prior to 8 months after primary surgery had lower overall complication rate ($p<0.05$).

**Conclusions.** To reduce overall complication rate, our data suggest a shorter period than 8 months after primary surgery before closure of diverting stoma. As diverting stoma closure is not a simple operation, all strategies should be taken to reduce significant morbidity and mortality rate.

Key words: low anterior resection; surgical stoma closure; risk factors; morbidity; mortality

## Background

There is no doubt that anastomotic leakage is one of the most important surgical complications of rectal surgery.[1] Due to high morbidity and mortality, it is a major issue and is certainly affecting long-term survival. Clinically manifested anastomotic leaks are seen after 3 – 30% of low anterior resection for carcinoma and may even be associated with a higher local recurrence rate.[2] The mortality rate associated with symptomatic anastomotic leaks varies between 6 and 22%.[3] Risk factors for anastomotic leakage are numerous and not well-explored: neoadjuvant treatment, patient age, comorbidity, operation length, male sex, anastomotic height, peripheral arterial disease with different stages of reduced vascular supply after radiotherapy and systemic chemotherapy, and many others.

To avoid the severe consequences of anastomotic problems, it is crucial to take all available actions to prevent a symptomatic anastomotic leakage. One of the well-established procedures to prevent such a complication is diverting enterostomy. Nowadays, this is a common surgical choice to secure an anastomosis after low anterior resection for cancer, especially after neoadjuvant treatment. This procedure was also proposed by Working Group for Colon/Rectum Carcinoma (WGCRC) in 2002, who suggested the use of a diverting stoma for lower rectal carcinomas, especially in patients in poor general condition.[3,4]

The critics raised many questions about such recommendations. One of them is the problem with diverting stoma closure. One must understand that stoma closure is not just a simple operation reserved for young surgeons and residents but a serious procedure with quite moderate morbidity and mortality.[5-7] Overall postoperative morbidity in systematic reviews is reported as high as 17%, with a rate of 7.2-7.6% for postoperative bowel obstruction, 1.4-2.0% for anastomotic leak and 1.2% for bowel perforation.[5-8]

Type of stoma closure depends on surgical preferences and skills. There are at least three different approaches: the anterior wall (AWT) or fold-over technique, resection with end-to-end anastomosis (RWA) or latero-lateral anastomosis, hand sewn or stapled.

The aim of our study was to evaluate morbidity and mortality after diverting stoma closure and to identify risk factors for complications of stoma closure procedure regarding various factors.

## Patients and methods

We performed a retrospective cohort analysis of the patients operated in our department between 2003 and 2015. Patients with diverting ileostomy and colostomy were included. At the time of primary procedure, it was the surgeon's personal decision which type of stoma had to be made. Institutional Review Board approved the study.

Only patients after rectal cancer surgery with diverting stoma made were included in the study. Gastrografin enema or colonoscopy were performed in all patients prior to stoma closure. Patients had routine mechanical preparation of the proximal and distal bowel with the cessation of oral feeding the day before surgery. All patients underwent single-shot parenteral antibiotic treatment (cefuroxime and metronidazole) one hour prior to operation. Elementary data included age, sex, American Society of Anaesthesiologists (ASA) score at primary and stoma closure operation, and time from the primary operation to closure. Operative and postoperative data included type of operation, time until release and postoperative complications.

Two main closure techniques were used. The anterior wall technique (AWT) was performed, leaving the mesenteric side of the bowel intact and closing the bowel enterostomy in a transverse fashion by using a double-layer technique with absorbable suture material. The other technique, resection with anastomosis (RWA), represents the resection of bowel with hand-sewn entero-entero anastomosis in the same fashion.

Complications were classified according to Clavien-Dindo classification (Table 1), but there were also more specific complications accessed separately: postoperative ileus requiring reoperation, paralytic ileus that did not require reoperation, wound surgical site infection (SSI) and anastomotic leakage.[9]

Intestinal obstruction was defined by a combination of the following findings: abdominal distention, abdominal pain, vomiting and the presence of air-fluid levels with imaging techniques during the postoperative period. Wound infection was defined by the presence of purulent discharge, erythema, and induration of the wound. Anastomotic leakage was defined by the presence of clinical or laboratory signs of acute abdomen and was confirmed by an ultrasound, contrast enema, or computed tomography scan.

**TABLE 1.** Clavien-Dindo classification

| Grade | Explanation |
|---|---|
| 1 | Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Acceptable therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside. |
| 2 | Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions, antibiotics and total parenteral nutrition are also included. |
| 3 | Requiring surgical, endoscopic or radiological intervention. |
| a | Intervention under regional/local anesthesia. |
| b | Intervention under general anesthesia. |
| 4 | Life-threatening complication requiring intensive care/intensive care unit management. |
| a | Single organ dysfunction. |
| b | Multi-organ dysfunction. |
| 5 | Patient demise. |

According to the time of stoma closure, we divided patients into two groups. In the first group, there were patients where we managed to close the stoma prior to eight months after rectal surgery, and in the second group, there were patients where the stoma was closed later than eight months after the initial surgery. Patients were accessed for complications during the hospitalisation, at the dismissal and at the control at the outpatient clinic 5 weeks after dismissal. None of the patients was lost.

All of the statistical analyses were performed using the SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA). Continuous data are expressed as means ± SD, while categorical variables are given as percentages. The Shapiro-Wilk test was used to determine whether the continuous data were normally distributed. Comparisons of continuous variables were carried out with Student's t-test for parametric data and Mann–Whitney U test for nonparametric data. The Chi-square test was used for comparison of discrete variables. A p-value of <0.05 was considered as statistically significant.

## Results

The medical records of 260 patients with stoma closure at the University Clinical Centre Maribor between January 2003 and December 2014 were identified and reviewed. We included only patients with diverting stoma after rectal cancer surgery. Basic clinical characteristics are presented in Table 2.

There was 116 colostomy and 102 ileostomy closures, 76 by AWT technique and 142 by RWA technique (Table 3). Median time to closure was 248 days (30-911 days).

**TABLE 2.** Clinical characteristics

| | |
|---|---|
| N = 218 | |
| Age | 64.6 years (26 – 90) |
| Sex | |
| Male | 136 (62%) |
| Female | 82 (38%) |
| ASA score | |
| 1 | 60 (27%) |
| 2 | 102 (47%) |
| 3 | 26 (12%) |
| 4 | 1 (0.5%) |
| Time to closure | 248 days (30 – 911) |

**TABLE 3.** Type of stoma and closure technique

| | Anterior Wall Sutures | Resection With Anastomosis | Together |
|---|---|---|---|
| Ileostomy | 7 (7%) | 95 (93%) | 102 |
| Colostomy | 69 (59%) | 47 (41%) | 116 |
| Together | 76 (35%) | 142 (65%) | 218 |

**TABLE 4.** Complications according to Clavien-Dindo

| Clavien- Dindo | n (%) |
|---|---|
| 0 | 164 (75.2) |
| 1 | 20 (9.2) |
| 2 | 18 (8.3) |
| 3b | 9 (4.1) |
| 4a | 3 (1.4) |
| 5 | 4 (1.8) |

Morbidity was 24.8%. Postoperative ileus (12%) and wound infection (5%) were the most common surgical complications. According to Clavien-Dindo classification, there were mostly Clavien-Dindo 1 and 2 (17.5%), but there were also 16 grave complications (7.3), Clavien-Dindo 3b and higher. Indications for operative treatment were postoperative ileus non-responding to conservative measurements, anastomotic leakage, and enterovaginal and enterocutaneal fistula. Mortality was 1.8%. Four patients died, three after colostomy closure and one after ileostomy closure.

Table 5 demonstrates the effect of different variables on the total complication rate and separately on most severe complications Clavien-Dindo 3b and higher. There was no effect on the complication rate regarding type of stoma (ileostomy vs. colostomy), closing technique (AWS vs. RWA), ASA status of the patient (ASA 1-2 vs. ASA 3-4), and patient age (less than 65 years vs. more than 65 year). The only factor influencing the complication rate was the time to stoma closure. We found that patients which had the stoma closed prior to 8 months after the formation had lower overall complication rate ($p<0.05$).

## Discussion

There is no definitive consensus about the routine use of diverting stoma in rectal cancer surgery. Until today, there has been no evidence-based data that diverting stoma influences survival after rectal

**TABLE 5.** The effect of different variables on complication rate

| Type of closure | AWS | RWA | p |
|---|---|---|---|
| All complications | 16 (7.3%) | 38 (17.4%) | > 0.05 |
| Severe complications | 5 (2.2%) | 11 (5%) | > 0.05 |
| **Age** | < 65 years | > 65 years | p |
| All complications | 23 (10.5%) | 31 (14.2%) | > 0.05 |
| Severe complications | 4 (1.8%) | 12 (5.5%) | > 0.05 |
| **ASA status** | ASA 1 & 2 | ASA 3 & 4 | p |
| All complications | 38 (17.4%) | 6 (3%) | > 0.05 |
| Severe complications | 13 (6%) | 3 (1.3%) | > 0.05 |
| **Type of stoma** | Ileostomy | Colostomy | p |
| All complications | 27 (12.4%) | 27 (12.4%) | > 0.05 |
| Severe complications | 9 (4.1%) | 7 (3.2%) | > 0.05 |
| **Time to closure** | <240 days | >240 days | p |
| All complications | 14 (6.4%) | 40 (18%) | **= 0.044** |
| Severe complications | 4 (1.8%) | 12 (5.5%) | > 0.05 |

cancer surgery, but many surgeons perform this procedure because it reduces septic consequences of a possible leak.[10,11]

If we presume that anastomotic leakage rate after low anterior resection for cancer is 5-10%, we are creating around 90% of unnecessary stomas which need to be closed.[12] One possible cause of anastomotic leakage is insufficient vascular supply. Poor vascular perfusion seems to play a key role in determining anastomotic viability. Until recently, the most common technique to evaluate tissue perfusion was the surgeon's intraoperative visual judgment based on clinical findings such as colour, pulsation and bleeding of resected margins. Unfortunately, many studies have suggested that the surgeon's clinical judgment is not enough to successfully predict the possibility of anastomotic leakage.[13] Recently, fluorescent angiography with indocyanine green has emerged as an innovative modality for intraoperative perfusion assessment. Fluorescent angiography with indocyanine green can be performed before or after intestinal resection or, alternatively, after creation of the anastomosis.[14] The technique shows very promising results.

Although the closure of diverting stoma might seem as a rather simple operation, different studies have shown a varying frequency and pattern of complications which could even lead to death.[5-7] The problem with most of those studies is a great heterogeneity of patients, considering the indication for stoma construction, patient age, presence of comorbidities, type of stoma (ileo vs. colo) and other factors.[15-22] A systematic review of 48 studies from 2009, including 6,107 patients, showed a 17.3% overall morbidity following the closure of loop ileostomy with a mortality rate of 0.4%. There was considerable range in morbidity (from 3 to 38.5%) and mortality (from 0 to 6.9%).[23] It is very interesting that such a straightforward and technically simple operation shows such different results in a relatively short time span. In our study, complication rates were rather high; morbidity was 24.8% and mortality 1.8%.

We tried to identify the factors which may have contributed to relatively high complication rate and had closely investigated the patient's age, stoma type, ASA status, surgical technique of stoma closure, and time from stoma construction to closure. The data showed that neither stoma type (ileostomy or colostomy) nor surgical technique of stoma closure had impact on complication rate. The only variable we found to have an impact on postoperative morbidity is time to stoma closure.

There are still controversies about ideal time for stoma closure. There are some studies which advocate early stoma closure, but the majority of operations are still done relatively late. One must consider that the majority of rectal cancer patients are scheduled for postoperative chemotherapy which may postpone any surgical treatment. Optimal time to start chemotherapy after surgery is not well established, but it is usually accepted that it should begin within 6-8 weeks after surgery and usually for a total of about six months. It is also unknown whether adjuvant therapy in patients with rectal cancer has an impact on the morbidity of loop ileostomy closure[24], but the strategy in our institution is to postpone the closure until the end of adjuvant therapy, which is approximately 8 months after initial surgery. According to our data, patients which had stoma closed prior to 8 months after primary operation had significantly less complications than patients where stoma was closed later.

Late stoma closure is connected with stoma related complications such as parastomal hernia, prolapse, retraction, peristomal dermatitis and peristomal fistula.[25] A prospective study from 2005 has shown an increase in the number of parastomal hernias, prolapses and skin irritations from the 10-day follow-up to the 3-month follow-up and again

to the 2-year follow-up. The same applied to general ostomy problems such as leakages and the need for frequent emptying.[26] A prospective randomised study by Alvez *et al.* demonstrated a much higher number of stoma-related complications in patients whose stoma was closed after two months compared with those whose stoma was closed after only eight days. Late closure led to complications in 12% of the patients in comparison to only 1% of early closure patients.[27]

In a multicentre pilot study in Germany, stoma closure was performed 5.1 months after the creation on 171 patients from 17 surgical centres.[28] They stated that there is no recommendation for optimal timing for stoma closure available and they started a very interesting study in 2013 where they "compare completeness of adjuvant chemotherapy after early versus late protecting stoma closure in low anterior resection for rectal cancer – CoCStom trial".[28] They believe that early stoma closure has a beneficial effect for a patient. While we are awaiting the final results of this study, there are already some researches and articles in literature about early vs. late stoma closure.

Several prospective studies and a single randomized controlled trial have shown that closure in less than two weeks after stoma creation was associated with lower or equal morbidity compared with later closure. Thus, some authors support the early closure of temporary ileostomy performed to cover rectal anastomosis in routine clinical practice.[25] On the other hand, Perez *et al.*, performed a study on 93 patients undergoing ileostomy closure and concluded that the interval between primary operation and ileostomy closure should be no shorter than 8.5 weeks if morbidity of this procedure is to be reduced.[20]

Regarding other investigated parameters, one could expect that younger patients and patients in better physical condition according to ASA status would have lower complication rate and that there could also be some differences regarding stoma type (colo- or ileostomy) and closing technique. In literature, we found many studies that did identify some connections between various other factors and complication rate after stoma closure, but the results were not uniform.

Man *et al.*, presented their result in 2016. They recruited 213 patients with diverting ileostomy after low anterior resection. Overall complication rate was 16.4% and mortality was zero. The majority of stomas were closed after 12 weeks, mostly by stapler. They discussed and investigated possible risk factors for post closure complications: patient's age (less or more than 80 years old), influence of postoperative chemotherapy, body mass index, patient's general condition and diseases (diabetes, pre-existing respiratory or cardiac disease, operating time and anaemia. According to their data, elderly patients (p=0.002) and patients with a pre-existing respiratory disease (p=0.04) were more likely to develop postoperative complications, but elderly were defined as older than 80 years.[29]

In a retrospective study, Poskus *et al.*, performed a retrospective analysis of 132 patients who underwent ileostomy closure. There were mostly patients after rectal cancer surgery but also with some other benign conditions. The complication rate was 18.2% and mortality 1.5%. They found that the experience of a surgeon and preliminary condition which required diverting stoma were independent factors for complications.[30]

Schneider *et al.*, published a very interesting article about surgical interventions after the ileostomy closure. In three months period, 106 patients after ileostomy closure were analysed. 12 patients required operative management due to Clavien-Dindo 3b complications. Higher body mass index and anaemia were associated with immediate re-operations. There was no mortality.[31]

According to our data, ileostomy closure technique has no impact on the complication rate. Attaallah *et al.*, investigated the postoperative course of patients treated with fold-over technique and end-to-end anastomosis as two closing techniques. They did not find any differences between the two groups of patients.[32] Similar findings have been shown by Cheong *et al.* They concluded that fold-over technique and the conventional resection with anastomosis have similar short-term clinical outcomes for diverting ileostomy reversal.[33]

One of the most common and important complications of stoma closure is also surgical site infection. The reported incidence may be as high as 40%.[34,35] Therefore, some authors suggest leaving the wound open, whereas others found lower infection rates after primary closure. A modification of wound closure is the so-called purse string approximation in which a circumferential approximation of the wound is performed. Providing a hole in the centre of the wound, this approximation follows the intention to drain wound liquids and therefore reduce SSI rates.[36]

The limitations of our study lie in its retrospective. Although the patients in a group where stoma was closed earlier had less complications, the reason for this could be that those patients had shorter or no adjuvant therapy because of less advanced

primary disease. We also did not explore other factors that, according to some studies, might contribute to stoma closure complications like higher body mass index, anaemia and diabetes.

## Conclusions

Diverting stoma closure is not a simple operation and should not be taken lightly because it is associated with significant morbidity and mortality. According to our data, the only factor that contributed to lower complication rate in our group of patients was time to stoma closure.

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*research article*

# Health-related quality of life in Croatian general population and multiple myeloma patients assessed by the EORTC QLQ-C30 and EORTC QLQ-MY20 questionnaires

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**Background.** The impact of disease and treatment on the patient's overall well-being and functioning is a topic of growing interest in clinical research and practice. The aim of this study is to obtain reference data on quality of life of Croatian general population. Further, we aim to assess the impact of the disease and its primary systemic treatment on their health related quality of life (HrQoL) in multiple myeloma (MM) patients.

**Patients and methods.** Participants for the first part of the study were randomly selected from adult Croatian population. In the clinical part of the study MM patients were included as prospectively diagnosed within two years in two major Croatian haematological centres. The EORTC QLQ-C30 in both trials and QLQ-MY20 in MM patients only were applied for HrQoL assessment.

**Results.** Gender, age and place of residence have great impact on quality of life scores in Croatian population. The MM patients at the time of diagnosis have lower QLQ-C30 scores for global quality of life, functional and symptom scale scores, as well as single items. The type of disease followed by the choice of therapy options are important HrQoL determinants.

**Conclusions.** The norm values available now for Croatian population will help to interpret HrQoL for clinicians and aid in planning cancer care interventions. This study identified treatment effect consistent with those from other observational studies and provided new data on HrQoL across two different treatment choices for MM patients.

Key words: health-related quality of life; multiple myeloma; EORTC QLQ-C30; EORTC MY-20; reference data; Croatian population

## Introduction

Cancer incidence is increasing both in developed and developing countries. According to the Croatian Institute for Public Health, in Croatia cancer incidence has been steadily rising from 1990 on. In 2016, there were 23,650 newly diagnosed cancer cases, less than 1 percent are younger than 20 years at diagnoses. Among adult patients 3 to 4 percent are diagnosed before the age of 40.[1] Statistic data of the International Agency for Research on Cancer show that the incidence of multiple myeloma (MM) in Croatia for men is 4.0 with mortality of 2.1 in 100,000 citizens, and for women the incidence is 3.4 with mortality of 2.1 in 100,000 citizens. This means that every year we have 225–260 new MM cases in Croatia. MM is more frequent in males; the incidence increases with age.[1] In 2016, there were

no case of MM before the age of 40 in Croatia, and most patients are aged 70 years or more at the time of diagnosis.[1] Given the number of patients, there is a great need for assessing the health-related quality of life (HrQoL) to improve the care of oncology patients. Therefore, quality of life assessment is becoming more common in oncology.[2]

Health is one of the most important variables affecting well-being.[3] World Health Organisation defines quality of life as the individuals' perception of their position in life in the context of the culture and value system in which they live and in relation to their goals, expectations, standards and concerns.[4] The impact of disease and treatment on the patient's overall well-being and functioning is a topic of growing interest in clinical research and practice.[5] Indicators of quality of life can provide evidence that is based on comparable and standardized measures and that can be used to improve the health care system. Conducting studies using various health-related quality of life measures is a basis for improvement of health care delivery.[6] In the last decades, the measurement of health-related quality of life has gained acceptance as a primary or secondary endpoint in cancer research worldwide.[7]

Evaluation of quality of life is conducted by using standardized questionnaires.[8] European Organization for Research and Treatment of Cancer (EORTC) has purposed a development of an integrated, modular approach for evaluating the quality of life of cancer patient. They have launched a core EORTC quality of life questionnaire for cancer patients that consists of 30 questions (EORTC QLQ-C30). The EORTC QLQ-C30 is one of the most widely used instrument for assessing health-related quality of life in cancer patients[9] and has been used so far in more than 3000 studies worldwide. The core questionnaire is supplemented by disease-specific modules.[10] So far disease specific modules were developed for 13 different malignant conditions. The EORTC QLQ-MY20 is specific questionnaire adjusted for patients with multiple myeloma. The EORTC QLQ questionnaires have been translated and validated into 81 languages. The EORTC QLQ-C30 and EORTC QLQ-MY20 exist also in Croatian language.[11,12]

The normative (reference) values of QLQ-C30 questionnaire for general healthy population are already available for some countries, for example Germany[5], the Netherlands[13], Denmark[7], Sweden[14] and Slovenia.[15] Typically, they demonstrate the decrease of HrQoL on all levels with age in both sexes. On the contrary, some symptoms such as pain and fatigue increase with age. The population norms are applied in clinical practice as an aid to the clinical assessment of an individual patient and in research to assist in the overall interpretation of results from clinical studies of HrQoL.

The knowledge on HrQoL in MM patients is scarce. The findings of the recent European cohort study provide a better understanding on how to improve the treatment of patients with MM in order to improve their QoL. The data indicated that there is a need for better management of the treatment of fatigue and bone related symptoms since those are the strongest HrQoL predictors. The study also suggested that specific QoL aspects can be notably improved by treatment which targets specific symptoms.[16]

The aim of this study is to obtain reference data on quality of life in a representative sample of Croatian general population older than 40 years, and obtaining data on quality of life in MM patients with the aid of EORTC QLQ-C30 and QLQ-MY20 questionnaires. The norm values will help to interpret health-related quality of life data for clinicians, and aid in planning interventions for symptoms in the early stages of the disease. In MM patients, we aim to assess the impact of the disease and its primary systemic treatment on their HrQoL.

## Patients and methods

### Population sample

The study was conducted in the second half of 2016 and during 2017. For the initial part of our study, eligible respondents were adults, aged over 40 at the entry, residents of Croatia with no history of a malignant disease. A random sample of adult inhabitants in all six regions of Croatia: Dalmatia, Slavonia, Istria, Podravina, Zagreb region and Medimurje has been obtained. The EORTC QLQ-C30 questionnaire supplemented by a demographic inquiry was personally delivered to randomly selected individuals. The data collection was carried out by specially trained medical nurses in health centres, homes for the elderly, in the street and in other highly frequented places. For any randomly chosen adult we firstly determined their age and health status, and, in the case of not having a malignant disease and being older than 40, we continued with collecting the quality of life data. From the 362 initially contacted, 310 respondents were included in our population sample. Of 51 not included respondents, 35 had cancer, while 16 of them chose not to participate for different

personal reasons. The Survey Monkey Sample Size Calculator[17] was used to estimate that such a sample size is sufficient for our study to reach adequate power.

## Multiple myeloma patient sample

In the second part of the study MM patients older than 40 were included. A prospective study was conducted in the second half of 2016, 2017 and the first half of 2018. EORTC QLQ-C30 and EORTC QLQ-MY20 questionnaires were filled in by respondents before and three months after treatment with chemotherapy and stem cell transplantation in two major haematological centres in Croatia: Clinical Hospital Dubrava and Clinical Hospital Merkur. Questionnaires were distributed to the respondents during their stay in hospital, at the moment when transplantation or chemotherapy is determined as a therapeutic procedure. The second evaluation was conducted three months after therapeutic procedures. The sample consisted of 25 respondents before and after stem cell transplantation and 26 respondents before and after chemotherapy, which corresponds to a similar Czech research which evaluated the quality of life among malignant lymphoma and MM patients undergoing autologous stem cell transplantation.[16] Additional five people were invited to the study but they chose not to participate.

## Questionnaires

In both samples we used the EORTC QLQ-C30 questionnaire which contains 30 questions. The official Croatian translation of the questionnaire was used in assessing individual HrQoL during previous week. Respondents were offered four response alternatives: 1 "not at all", 2 "a little", 3 "quite a bit" and 4 "very much". In the last two questions the range was 1 to 7 in which 1 is "very poor" and 7 is "excellent". The EORTC QLQ-C30 questionnaire consists of a general health/quality of life scale, and five functional scales: physical functioning, role functioning, cognitive functioning, emotional functioning and social functioning, and 13 symptom items. Functional scale includes cognitive, emotional, physical, role and social functioning dimension. Symptom scale includes fatigue, nausea or vomiting and pain, and single items include loss of appetite, constipation, diarrhoea, dyspnoea, financial impact and sleep disturbance. For general quality of life and functional scale, higher scores mean better quality of life while higher scores for symptom scale and single items mean lower quality of life.[18] In addition to EORTC QLQ-C30 all the participants also responded to a questionnaire on their demographic data including age, gender and place of residence.

The EORTC QLQ-MY20 is specific questionnaire adjusted for patients with multiple myeloma. The official Croatian translation of the questionnaire was used in assessing individual HrQoL during the previous week. It consists of functional scales which include future perspective and body image, and symptom scale which includes disease symptoms and side effects of treatment. Respondents were offered four response alternatives: 1 "not at all", 2 "a little", 3 "quite a bit" and 4 "very much". The higher the number of an item means poorer functioning.[18]

## Internal consistency

Some dimensions are composed of more than one answer, and the others include only one categorical answer. Internal consistency of multiple answers within single dimension was proved by using the Cronbach alpha coefficient of reliability. The high value of the Cronbach alpha coefficients suggests a very high reliability. The coefficients were as follows: general health status/quality of life (0,87), physical functioning (0,84), role functioning (0,86), emotional functioning (0,87), cognitive functioning (0,70), social functioning (0,88), fatigue (0,83), nausea/vomiting (0,69) and pain (0,81). In case of a lack of response to a question, the average of the other answers was used, but only if at least half of the questions were answered.[19-21]

To investigate if there is a correlation between EORTC QLQ-C30 and QLQ-MY20 scores, Pearson correlation coefficient calculations were performed.

## Statistical analysis

Demographic characteristics were analysed as categorical variables and are represented in numbers and relative frequencies. Four age categories (40-49, 50-59, 60-69 and 70+) and six geographical regions were applied. The chi-square test was applied for determining the statistical significance of difference in demographical characteristics among groups of individuals in the sample. The answers, which were recorded in the EORTC QLQ-C30 and EORTC QLQ-MY20 were converted into dimensions, which evaluate the quality of life associated with health. Dimensions are ranged from 0–100 according to the EORTC scoring instructions.[18]

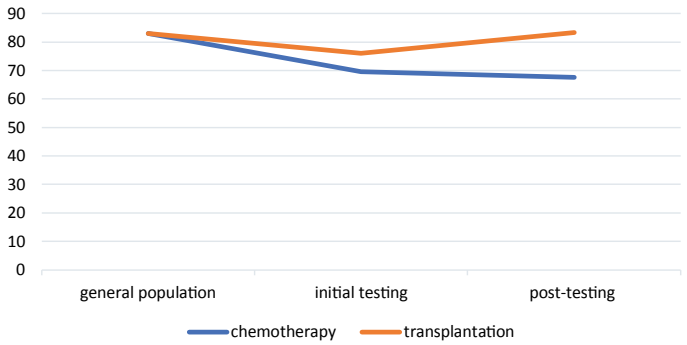


**FIGURE 1.** Summary score for EORTC QLQ-C30 in general population, initial testing and post-testing.

Dimensions are described by the arithmetic mean and standard deviation. As suggested by Nielsen *et al.*[22] Mann-Whitney U test was used to compare the dimensions within two groups (gender, treatment). Where there were more than two groups for comparison (age, region) we used the Kruskal-Wallis test. For testing the statistical significance of the change in dimensions before and after treatment Wilcoxon signed rank test was used.

Furthermore, for the first time to the best of our knowledge, the QLQ-C30 summary score of the EORTC QLQ-C30 was calculated for the population reference scores. QLQ-C30 summary score is a single higher-order model based on 27 of the 30 items of the QLQ-C30, excluding global quality of life and financial difficulties proposed by Giesinger *et al.*[23] In our study we investigated the ability of the QLQ-C30 summary score to distinguish between groups formed according to treatment choice (chemotherapy, transplantation) and general population.

The values of $p < 0.05$ were evaluated as statistically significant. Statistical analysis was performed using the 24.0 SPSS Inc., Chicago, IL, SAD software.

## Compliance with ethical standards

The data collection, preparation, implementation and presentation were in accordance with legal requirements for protecting the confidentiality of personal information: in Croatia non-drug trials are reviewed by ethics committees at an institutional level. Approval for the study was obtained from the Medical Ethics Committee of the Clinical Hospital Dubrava and Clinical Hospital Merkur (03/1-6234). All participants were fully informed about the aims of the study and provided written informed consent for participation in the study.

**TABLE 1.** Mean score (MS) and standard deviations (SD) for all EORTC QLQ-C30 dimensions and scales by age for general population

| | 40–49 | | 50–59 | | 60–69 | | 70 and older | | p* |
|---|---|---|---|---|---|---|---|---|---|
| | MS | SD | MS | SD | MS | SD | MS | SD | |
| Global health status/quality of life | 69.1 | 18.6 | 71.1 | 20.7 | 67.4 | 19.9 | 56.9 | 23.9 | .035 |
| Physical functioning | 86.2 | 12.9 | 83.9 | 16.9 | 81.8 | 21.7 | 61.2 | 30.2 | .000 |
| Role functioning | 86.1 | 20.6 | 85.9 | 20.2 | 80.8 | 28.6 | 69.1 | 37.2 | .128 |
| Emotional functioning | 71.9 | 22.4 | 78.2 | 19.7 | 78.6 | 19.7 | 66.7 | 26.4 | .034 |
| Cognitive functioning | 80.6 | 21.7 | 85.7 | 17.8 | 85.6 | 17.4 | 80.8 | 26.3 | .402 |
| Social functioning | 86.8 | 20.1 | 90.6 | 15.5 | 88.5 | 20.8 | 76.5 | 31.0 | .049 |
| Fatigue | 33.7 | 20.8 | 28.0 | 22.7 | 29.5 | 22.8 | 43.8 | 29.2 | .014 |
| Nausea/vomiting | 6.9 | 14.2 | 4.9 | 11.3 | 4.6 | 10.4 | 10.3 | 20.1 | .276 |
| Pain | 25.8 | 24.3 | 23.3 | 24.5 | 24.1 | 27.8 | 40.2 | 32.3 | .028 |
| Dyspnoea | 18.8 | 24.6 | 17.3 | 23.6 | 22.1 | 25.9 | 26.5 | 30.6 | .362 |
| Insomnia | 24.4 | 28.7 | 26.3 | 30.2 | 30.7 | 30.5 | 41.2 | 37.7 | .094 |
| Appetite loss | 6.9 | 15.2 | 5.3 | 13.7 | 8.2 | 15.6 | 24.5 | 34.1 | .001 |
| Constipation | 8.2 | 18.0 | 8.3 | 19.2 | 13.8 | 21.9 | 14.7 | 28.7 | .164 |
| Diarrhoea | 7.9 | 15.8 | 6.5 | 13.9 | 6.2 | 13.0 | 13.7 | 26.1 | .595 |
| Financial difficulties | 7.6 | 17.7 | 7.4 | 17.7 | 14.4 | 26.9 | 22.5 | 33.6 | .009 |
| Summary score | 83.1 | 12.5 | 85.8 | 12.2 | 82.9 | 15.0 | 72.6 | 23.7 | .030 |

*p = Kruskal-Wallis test

## Results

From the general population there were 310 questionnaires eligible for analysis; 68.4% women (212 persons) and 31.6% men (98 persons). The largest number of the respondents were in the age range of 50–59 years, at 36.8% (114 persons). Most of the respondents were from the Zagreb region, at 33.9% (105 persons). Furthermore, 51 MM patients were included in the second part of the research. The largest number of the MM respondents were in the age range of 60–69 years, 20 patients. 25 of the respondents were treated with chemotherapy while 26 of the respondents underwent transplantation. Supplement table 1 shows how the groups differ in their demographic characteristics.

### Scale and item scores

Results illustrate the transformed values of general quality of life, functional and symptom scales in the range 1–100. Table 1 shows the results for the EORTC QLQ-C30 for all scales, single items, as well as the summary score in general population according to age. In many dimensions there is a statistically significant difference in age in our sample. The scores for general health status are highest for the respondents aged 50–59 and lowest for the respondents who are 70 and older. Furthermore, there is also a statistically significant difference according to age for physical functioning, emotional functioning and social functioning. The scores are significantly lower for respondents aged 70 and older. EORTC QLQ-C30 summary scores also indicate that there is a statistically significant difference according to age, where scores are lower with increasing age.

Supplement Figure 1 shows mean scores of EORTC QLQ-C30 scores for all scales and items in general population according to age and gender. Men reported better general quality of life, as well as better physical and emotional functioning. There is a statistically significant difference if we compare the results for symptom scale and single items for fatigue, pain, dyspnoea and insomnia by gender, where the ranges are higher for women. Men reported better functioning for all items on the symptom scale. If we compare the results for single items, women reported less constipation, diarrhoea and financial problems. Supplement Figure 2 shows EORTC QLQ-C30 summary score in Croatian general population according to age and gender. Supplement Table 2 shows results for the EORTC QLQ-C30 for all scales and single items according to a place of residence. There is a statistically significant difference in general health status according to a place of residence; the scores are higher for respondents from Istria and Medimurje, and lowest for respondents from Dalmatia. Furthermore, there is a statistically significant difference in physical functioning, role functioning, emotional functioning, cognitive functioning and social functioning according to a place of residence; the scores are again the highest for respondents from Istria and Medimurje.

**TABLE 2.** Mean score (MS) and standard deviation (SD) for all EORTC QLQ-C30 dimensions and scales in general population and multiple myeloma (MM) patients at diagnosis at the time of setting the diagnosis

| | MM patients at diagnosis | | General population | | |
|---|---|---|---|---|---|
| | MS | SD | MS | SD | P* |
| Global health status/ quality of life | 58.9 | 19.4 | 68.2 | 20.6 | .003 |
| Physical functioning | 63.0 | 26.2 | 81.7 | 20.1 | .000 |
| Role functioning | 52.6 | 38.6 | 83.1 | 25.1 | .000 |
| Emotional functioning | 75.8 | 21.7 | 75.1 | 21.6 | .820 |
| Cognitive functioning | 82.0 | 22.0 | 83.5 | 20.1 | .725 |
| Social functioning | 60.1 | 34.0 | 87.4 | 20.6 | .000 |
| Fatigue | 41.0 | 24.3 | 31.9 | 23.4 | .014 |
| Nausea/vomiting | 7.5 | 13.9 | 6.2 | 13.3 | .346 |
| Pain | 38.2 | 33.4 | 26.1 | 26.5 | .020 |
| Dyspnoea | 27.5 | 29.6 | 19.7 | 25.2 | .078 |
| Insomnia | 31.4 | 32.9 | 28.3 | 30.9 | .556 |
| Appetite loss | 21.6 | 34.5 | 8.5 | 18.7 | .010 |
| Constipation | 13.7 | 25.9 | 10.1 | 20.8 | .392 |
| Diarrhoea | 9.8 | 20.3 | 7.7 | 16.2 | .712 |
| Financial difficulties | 39.2 | 36.9 | 10.6 | 22.6 | .000 |
| Summary score | 72.9 | 17.8 | 83.0 | 14.8 | .000 |

*p = Mann-Whitney test

Table 2 presents the comparison between general population and multiple myeloma patients for all EORTC QLQ-C30 dimensions. There is a statistically significant difference for global health status, physical functioning, physical functioning, role functioning, social functioning, fatigue, pain, appetite loss and financial difficulties. The values for global health status, physical functioning, role functioning and social functioning are higher in general population while values for fatigue, pain, appetite loss and financial difficulties are higher in MM patients.

There is a statistically significant difference in physical functioning, cognitive functioning, social

**TABLE 3.** Mean score (MS) and standard deviation (SD) for all EORTC QLQ-C30 dimensions and scales in multiple myeloma (MM) patients before and after chemotherapy and transplantation

| | MM–before chemotherapy | | MM–after chemotherapy | | p* | MM–before transplantation | | MM–after transplantation | | p* |
|---|---|---|---|---|---|---|---|---|---|---|
| | MS | SD | MS | SD | | MS | SD | MS | SD | |
| Global health status/ quality of life | 53.3 | 21.4 | 49.7 | 22.5 | .283 | 64.4 | 15.7 | 74.7 | 18.0 | .008 |
| Physical functioning | 53.9 | 26.3 | 38.9 | 21.3 | .011 | 71.8 | 23.4 | 71.8 | 19.1 | .955 |
| Role functioning | 50.7 | 39.8 | 46.7 | 31.9 | .458 | 54.5 | 38.2 | 77.6 | 24.5 | .009 |
| Emotional functioning | 73.3 | 27.6 | 78.0 | 21.9 | .776 | 78.2 | 14.5 | 84.3 | 23.3 | .107 |
| Cognitive functioning | 78.0 | 27.1 | 57.3 | 28.1 | .007 | 86.0 | 14.9 | 91.0 | 14.3 | .083 |
| Social functioning | 57.3 | 37.6 | 787 | 26.6 | .007 | 62.8 | 30.7 | 78.0 | 26.1 | .026 |
| Fatigue | 43.1 | 27.3 | 64.0 | 22.3 | .001 | 39.1 | 21.5 | 40.2 | 24.6 | .782 |
| Nausea/vomiting | 7.3 | 15.3 | 5.3 | 13.4 | .603 | 7.7 | 12.7 | 0.0 | 0.0 | .010 |
| Pain | 39.3 | 36.9 | 54.7 | 25.2 | .039 | 37.2 | 30.3 | 31.4 | 28.0 | .323 |
| Dyspnoea | 28.0 | 32.9 | 33.3 | 30.4 | .590 | 26.9 | 26.7 | 17.9 | 23.5 | .100 |
| Insomnia | 40.0 | 36.0 | 14.7 | 23.7 | .010 | 23.1 | 27.9 | 20.5 | 31.4 | .637 |
| Appetite loss | 28.0 | 38.1 | 21.3 | 35.8 | .463 | 15.4 | 30.2 | 7.7 | 17.1 | .286 |
| Constipation | 16.0 | 32.1 | 20.0 | 28.9 | .564 | 11.5 | 18.7 | 1.3 | 6.5 | .011 |
| Diarrhoea | 9.3 | 22.6 | 6.7 | 19.2 | .414 | 10.3 | 18.3 | 1.3 | 6.5 | .035 |
| Financial diffuclities | 41.3 | 41.1 | 14.7 | 28.9 | .005 | 37.2 | 33.1 | 20.5 | 29.9 | .018 |
| Summary score | 69.6 | 19.5 | 67.7 | 14.5 | .181 | 76.0 | 15.7 | 83.3 | 9.7 | .097 |

*p = Wilcoxon signed rank test

functioning, fatigue, pain, insomnia and financial difficulties in the measurements before and after chemotherapy where the results are worse in measurements after chemotherapy. Furthermore, there is statistically significant difference for global health status, role functioning, social functioning, nausea and vomiting, diarrhoea and financial difficulties in measurements after transplantation. For global health status in post-testing positive ranks in 15 cases were observed which means that they indicate better results. For role functioning positive ranks in 15 cases were observed while in social functioning positive ranks in 14 cases were observed which means that they indicated worse results. While for nausea and vomiting in 8 cases, constipation in 7 cases, diarrhoea in 7 cases and financial difficulties in 12 cases negative ranks were observed which means that they indicate better results (Table 3).

When analysing QLQ-MY20 questionnaire, there is a statistically significant difference for body image and disease symptoms in measurements before and after chemotherapy. In post-testing negative ranks in 7 cases for body image were observed which means that they indicate better results. For disease symptoms in post-testing positive ranks in 16 cases were observed which means that the values were higher in post-testing in accordance to initial

**TABLE 4.** Mean score (MS) and standard deviation (SD) for all QLQ-MY20 scales in multiple myeloma (MM) patients before and after chemotherapy and transplantation

| | MM–before chemotherapy | | MM–after chemotherapy | | p* | MM–before transplantation | | MM–after transplantation | | p* |
|---|---|---|---|---|---|---|---|---|---|---|
| | MS | SD | MS | SD | | MS | SD | MS | SD | |
| Future perspective | 2.2 | 0.7 | 2.0 | 0.7 | .285 | 2.2 | 0.7 | 1.8 | 0.7 | .036 |
| Body image | 1.9 | 1.3 | 1.4 | 0.9 | .023 | 1.4 | 0.6 | 1.4 | 0.6 | 1.00 |
| Disease symptoms | 1.9 | 0.6 | 2.2 | 0.6 | .039 | 1.7 | 0.6 | 1.8 | 0.7 | .571 |
| Side effects of treatment | 2.1 | 0.7 | 1.8 | 0.4 | .361 | 1.9 | 0.6 | 1.5 | 0.3 | .144 |

*p = Wilcoxon signed rank test

testing. Furthermore, there is a statistically significant difference for future perspective in measurements before and after transplantation (Table 4).

We investigated the ability of the QLQ-C30 summary score to distinguish between groups formed according to treatment choice (chemotherapy, transplantation) and general population status. Using the treatment choice as a variable, the QLQ-C30 summary score showed the changes from the initial testing to post-testing. For MM patients on chemotherapy, results showed that in comparison to general population and transplanted patients, their functional scale was worse in the initial testing and it further deteriorated in post-testing. For transplanted patients the results showed worse results in comparison to general population but better when compared to patients on chemotherapy. In post-testing the results were improved. Three months after treatment the summary score was comparable to general population and significantly better when compared to patients on chemotherapy.

### Correlation between EORTC QLQ-C30 and QLQ-MY20 in MM patients before treatment

The correlations between the EORTC QLQ-C30 dimensions and scales and 4 items of the QLQ-MY20 before treatment are shown in Table 5 for patients on chemotherapy, and Table 6 for transplanted patients. The strongest positive correlation was found for pain (0.779) and disease symptoms, and strongest negative correlation for fatigue (-0.808) and physical functioning for patients on chemotherapy. Furthermore, for transplanted patients the strongest positive correlation was found for pain (0.707) and disease symptoms, and strongest negative correlation for physical functioning (-0.710) and side effects of treatment.

## Discussion

Quality of life is now widely recognized as a central outcome of many clinical trials.[3] It is a multi-dimensional concept based on a holistic view of human well-being. It considers a number of domains of people's lives and the interplay between these dimensions. The principal domains include: subjective well-being, health, income and standard of living, relationship with family and friends, work and quality of jobs, sense of inclusion in one's local community, and personal safety.[24]

In this study we presented the reference data of the EORTC QLQ-C30 quality of life dimensions for the general Croatian population. This study begun with the aim of obtaining reference data of the EORTC QLQ-C30 quality of life dimension in a sample of Croatian adults older than 40 years who were not suffering from a malignant disease. In Croatia, there is around 30% of population younger than 40 years, however there are only around 4% of cancer patients that are diagnosed below this age.[1] The collected data are transformed into expected mean HRQL scores for distinctive demographic population groups.

Our results illustrate that mean scores vary with age, gender and place of residence. Men reported better general health status as well as physical and emotional functioning. On the symptom scale they reported less symptoms when compared to women. Women reported significantly higher scores for fatigue, pain, dyspnoea and insomnia. These findings are similar to those of the Norwegian study, which was the first to be conducted in a general population. Their study also showed that men reported fewer symptoms and better quality of life on all scales.[25] Moreover, a study conducted in German general population showed that men reported better quality of life on all scales and fewer symptoms in comparison to women.[5] The first study conducted on a South-eastern European population was in neighbouring Slovenia, also showed that men report better quality of life on the majority of specific scales and that they report fewer symptoms, but the results were mostly not statistically significant.[15]

Our study also highlights that all scores deteriorated with age and older respondents reported more symptoms present. Scores were statistically higher for respondents 70 and more years old for physical, emotional and social functioning. The study conducted in the Norwegian population also showed that increasing age influences the scores; both QoL scales and all functional scales, except for emotional, showed a gradual decline in mean scores with increasing age for both sexes.[25] The study conducted in Slovenia also showed that older participants report more symptoms.[15] For general health status the ranges are interestingly highest for the age group 50–59 in the Croatian population.

The results indicate that place of residence has great impact on quality of life. Two of the areas in Croatia report better global functioning and less symptoms present. The ranges for physical, emotional, cognitive and social functioning are the highest for the respondents from Istria and

**TABLE 5.** Correlation between dimensions and scales in the EORTC QLQ-C30 and QLQ-MY20 (chemotherapy)

| | FP | BI | DS | SET | GHS | PF | RF | EF | CF | SF | F | NV | P | D | I | AL | C | DIA | FD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FP** | 1 | .369 | .330* | .395* | -.213 | -.254 | -.443 | -.478 | -.057 | -.410 | .086 | .149 | .322* | .119 | -.104 | .309* | .004 | .017 | .264 |
| **BI** | .369 | 1 | .208 | .586 | -.135 | -.155 | -.333* | -.428 | -.101 | -.396 | 131 | .299* | .164 | -.044 | .107 | .186 | -.074 | .229 | .270 |
| **DS** | .330* | .208 | 1 | .669 | -.459 | -.678 | -.429 | -.369 | -.545 | -.400 | .642 | .289* | .779 | .255 | -.127 | .512 | .150 | .274 | .243 |
| **SET** | .395* | .586 | .669 | 1 | -.277 | -.513 | -.462 | -.579 | -.577 | -.568 | .560 | .599 | .482 | .116 | .305 | .644 | -.121 | .451 | .469 |
| **GHS** | -.213 | -.135 | -.459 | -.277 | 1 | .427 | .341* | .503 | .336* | .468 | -.399 | -.205 | -.332* | -.283* | .013 | -.528 | -.033 | -.489 | -.335* |
| **PF** | -.254 | -.155 | -.678 | -.513 | .427 | 1 | .588 | .367 | .524 | .430 | -.807 | -.277 | -.725 | -.337* | .165 | -.389 | -.156 | -.136 | -.147 |
| **RF** | -.443 | -.333* | -.429 | -.462 | .341* | .588 | 1 | .384 | .359* | .491 | -.564 | -.373 | -.554 | -.376 | -.094 | -.449 | -.113 | -.259 | -.316* |
| **EF** | -.478 | -.428 | -.369 | -.579 | .503 | .367 | .384 | 1 | .329* | .692 | -.266 | -.616 | -.408 | .115 | .048 | -.596 | -.117 | -.424 | -.414 |
| **CF** | -.057 | -.101 | -.545 | -.577 | .336* | .524 | .359* | .329* | 1 | .176 | -.625 | -.383 | -.502 | -.170 | -.088 | -.424 | .043 | -.349* | -.262 |
| **SF** | -.410 | -.396 | -.400 | -.568 | .468 | .430 | .491 | .692 | .176 | 1 | -.225 | -.498 | -.353* | -.134 | .007 | -.589 | .064 | -.304* | -.534 |
| **F** | .086 | .131 | .642 | .560 | -.399 | -.807 | -.564 | -.266 | -.625 | -.225 | 1 | .272 | .717 | .456 | .066 | .374 | .127 | .369 | .119 |
| **NV** | .149 | .299* | .289* | .599 | -.205 | -.277 | -.373 | -.616 | -.383 | -.498 | .272 | 1 | .227 | -.139 | .083 | .605 | .125 | .361* | .317* |
| **P** | .322* | .164 | .779 | .482 | -.332* | -.725 | -.554 | -.408 | -.502 | -.353* | .717 | .227 | 1 | .350* | -.178 | .389 | .161 | .222 | .182 |
| **D** | .119 | -.044 | .255 | .116 | -.283* | -.337* | -.376 | .115 | -.170 | -.134 | .456 | -.139 | .350* | 1 | .204 | .078 | -.187 | .206 | .160 |
| **I** | -.104 | .107 | -.127 | .305 | .013 | .165 | -.094 | .048 | -.088 | .007 | .066 | .083 | -.178 | .204 | 1 | .238 | -.186 | .271 | .194 |
| **AL** | .309* | .186 | .512 | .644 | -.528 | -.389 | -.449 | -.596 | -.424 | -.589 | .374 | .605 | .389 | .078 | .238 | 1 | .000 | .329* | .375 |
| **C** | .004 | -.074 | .150 | -.121 | -.033 | -.156 | -.113 | -.117 | .043 | .064 | .127 | .125 | .161 | -.187 | -.186 | .000 | 1 | -.089 | -.133 |
| **DIA** | .017 | .229 | .274 | .451 | -.489 | -.136 | -.259 | -.424 | -.349* | -.304* | .369 | .361* | .222 | .206 | .271 | .329* | -.089 | 1 | .517 |
| **FD** | .264 | .270 | .243 | .469 | -.335* | -.147 | -.316* | -.414 | -.262 | -.534 | .119 | .317* | .182 | .160 | .194 | .375 | -.133 | .517 | 1 |

AL = appetite loss; BI = body image; C = constipation; CF = cognitive functioning; D = dyspnoea; DIA = diarrhoea; DS = disease symptoms; EF = emotional functioning; F = fatigue; FD = financial difficulties; FP = future perspective; GHS = global health status; I = insomnia; NV = nausea/vomiting; P = pain; PF = physical functioning; RF = role functioning; SET = side effects of treatment; SF = social functioning; * Correlation is significant at the 0.05 level (2-tailed)

**TABLE 6.** Correlation between dimensions and scales in the EORTC QLQ-C30 and QLQ-MY20 (transplantation)

| | FP | BI | DS | SET | GHS | PF | RF | EF | CF | SF | F | NV | P | D | I | AL | C | DIA | FD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FP** | 1 | .451** | .029 | .289 | -.383 | -.246 | -.203 | -.499 | -,106 | -.333* | .287* | .238 | -.035 | .145 | .296* | .371 | .309* | .049 | .053 |
| **BI** | .451 | 1 | -.142 | .026 | -.109 | -.047 | -.009 | -.083 | ,105 | .016 | .038 | -.006 | -.151 | -.048 | .333* | .109 | .053 | -.029 | -.195 |
| **DS** | .029 | -.142 | 1 | .447 | -.312* | -.589 | -.449 | -.339* | ,228 | -.397 | .527 | -.051 | .707 | .406 | .145 | .035 | -.060 | -.072 | .188 |
| **SET** | .289 | .026 | .447 | 1 | -.615 | -.710 | -.627 | -.397 | -,299 | -.455 | .570 | .597 | .519 | .642 | .377* | .523 | .470 | .485 | .337* |
| **GHS** | -.383 | -.109 | -.312* | -.615 | 1 | .629 | .518 | .349* | ,159 | .498 | -.436 | -.467 | -.423 | -.443 | -.071 | -.192 | -.429 | -.371 | -.370 |
| **PF** | -.246 | -.047 | -.589 | -.710 | .629 | 1 | .562 | .403 | ,147 | .506 | -.645 | -.375 | -.643 | -.447 | -.379 | -.325* | -.232 | -.171 | -.443 |
| **RF** | -.203 | -.009 | -.449 | -.627 | .518 | .562 | 1 | .346* | -,015 | .580 | -.563 | -.341* | -.626 | -.403 | -.194 | -.200 | -.209 | -.127 | -.421 |
| **EF** | -.499 | -.083 | -.339* | -.397 | .349* | .403 | .346* | 1 | ,188 | .378 | -.347* | -.217 | -.146 | -.191 | -.395 | -.120 | -.123 | -.054 | -.258 |
| **CF** | -.106 | .105 | .228 | -.299 | .159 | .147 | -.015 | .188 | 1 | .024 | -.002 | -.348* | .023 | -.041 | -.105 | -.033 | -.304* | -.143 | -.250 |
| **SF** | -.333* | .016 | -.397 | -.455 | .498 | .506 | .580 | .378 | ,024 | 1 | -.539 | -.245 | -.376 | -.178 | -.188 | -.078 | -.158 | -.080 | -.292* |
| **F** | .287* | .038 | .527 | .570 | -.436 | -.645 | -.563 | -.347* | -,002 | -.539 | 1 | .299* | .563 | .294* | .423 | .441 | .154 | .266 | .336* |
| **NV** | 238 | -.006 | -.051 | .597 | -.467 | -.375 | -.341* | -.217 | -,348* | -.245 | .299* | 1 | .258 | .263 | .197 | .587 | .660 | .464 | .299* |
| **P** | -.035 | -.151 | .707 | .519 | -.423 | -.643 | -.626 | -.146 | ,023 | -.376 | .563 | .258 | 1 | .563 | .077 | .182 | .188 | .143 | .376 |
| **D** | .145 | -.048 | .406 | .642 | -.443 | -.447 | -.403 | -.191 | -,041 | -.178 | .294* | .263 | .563 | 1 | .149 | .346* | .190 | .356 | .178 |
| **I** | .296* | .333* | .145 | .377* | -.071 | -.379 | -.194 | -.395 | -,105 | -.188 | .423 | .197 | .077 | .149 | 1 | .398 | .123 | .264 | .288* |
| **AL** | .371** | .109 | .035 | .523 | -.192 | -.325* | -.200 | -.120 | -,033 | -.078 | .441 | .587 | .182 | .346* | .398 | 1 | .271 | .363 | .148 |
| **C** | .309* | .053 | -.060 | .470 | -.429 | -.232 | -.209 | -.123 | -,304* | -.158 | .154 | .660 | .188 | .190 | .123 | .271 | 1 | .540 | .152 |
| **DIA** | .049 | -.029 | -.072 | .485 | -.371 | -.171 | -.127 | -.054 | -,143 | -.080 | .266 | .464 | .143 | .356 | .264 | .363 | .540 | 1 | .104 |
| **FD** | .053 | -.195 | .188 | .337* | -.370 | -.443 | -.421 | -.258 | -,250 | -.292* | .336* | .299* | .376 | .178 | .288* | .148 | .152 | .104 | 1 |

AL = appetite loss; BI = body image; C = constipation; CF = cognitive functioning; D = dyspnoea; DIA = diarrhoea; DS = disease symptoms; EF = emotional functioning; F = fatigue; FD = financial difficulties; FP = future perspective; GHS = global health status; I = insomnia; NV = nausea/vomiting; P = pain; PF = physical functioning; RF = role functioning; SET = side effects of treatment; SF = social functioning; * Correlation is significant at the 0.05 level (2-tailed)

Medimurje. It is hard to define what is the predictor for this result because one area is continental and the other is a coastal area. Both areas are developed and it could be that socio-economic circumstances in these areas have influenced the results. Development index is a composite indicator calculated as a customized average of standardized values of socio-economic indicators for measuring the degree of development in a given period. In Croatia, the following indicators are used to calculate the development index: average income per capita, average source income per capita, the average unemployment rate, movements of general population, degree of education of the population (tertiary education) and the aging index. Local self-government units are classified into categories considering development index. Third and fourth level represent above-average ranking units. Istria currently belongs to the forth level and Medimurje to the third level, which means that they are above-average developed.[26,27] For global health status the ranges are the lowest for respondents from Dalmatia.

In addition, in this study we also aimed to assess the impact of the disease and its primary systemic treatment on their HrQoL in a prospective cohort of multiple myeloma patients older than 40 years at the time of diagnosis. If we compare the results for general population and MM patients which were obtained using the EORTC QLQ-C30 questionnaire, it can be seen that during the initial testing patients suffering from multiple myeloma have lower scores for global health status, physical functioning, role functioning and social functioning, while they have higher scores for fatigue, pain, appetite loss and financial difficulties. It can be concluded that they have more symptoms present.

According to Delforge *et al.*, about 70% of the patients suffer from pain at the time of diagnosis.[28] Our results indicate that pain is a great predictor of global health status in general population, as well as in MM patients. In general population, pain and fatigue were the most expressed symptoms, while in MM patients pain, fatigue, insomnia, dyspnoea and appetite loss were the most expressed symptoms. Severity, type of disease, symptoms and treatment are important determinants of HrQoL in patients with multiple myeloma. Advanced disease and treatment related symptoms are associated with lower HrQoL.[16]

Our descriptive and exploratory analysis suggests a beneficial effect of transplanted patients three months after therapy on HrQoL. HrQoL scores were higher among transplanted patients, compared to those who underwent chemotherapy. Respondents who underwent chemotherapy were mostly older than 70 years with a different comorbidity. They also indicate that approximately 9 months passed from the occurrence of the first symptoms and diagnosis, due to the attribution of symptoms to other diseases, most commonly to spinal diseases. Also, patients starting treatment for the first time are affected by the psychological burden of their recent diagnosis and experience treatment related toxicities which they have not previously been exposed to.[29] Furthermore, the period of three months was sufficient for transplanted patients to recover from high doses of chemotherapy that preceded transplantation. On the other hand, patients who underwent chemotherapy, regardless of the small dosage of therapy, noticed that they have deterioration in symptoms.

The Netherlands study, also conducted on 51 transplanted patients during the treatment and 12 months after, shown that after 12 months the results were better for physical, role, emotional, cognitive and social functioning. Additionally, for symptoms, the results were better for fatigue, pain and appetite loss while nausea/vomiting and diarrhoea deteriorated in results.[30] Our results illustrate that scores for global health status, physical functioning, role functioning and cognitive functioning are higher three months after transplantation, while results for fatigue, pain, dyspnoea and constipation are higher after chemotherapy. Patients who underwent chemotherapy had more symptoms present three months after therapy in comparison to patients who underwent transplantation.

An additional valuable output from our study is the correlation analysis of the EORTC QLQ-C30 scores and 4 items of the EORTC QLQ-MY20. This method can provide reliable, accurate descriptions of the HrQoL of patients with multiple myeloma.[29] Correlation analysis for patients on chemotherapy from our study shows that side effects of treatment strongly correlate with body image and disease symptoms; furthermore, physical functioning strongly correlates with disease symptoms, side effects of treatment and role functioning, while emotional functioning correlates with side effects of treatment and social functioning. Cognitive functioning strongly correlates with disease symptoms, side effects of treatment and physical functioning. When symptoms are taken into consideration fatigue correlates with disease symptoms, side effects of treatment, physical functioning and cognitive functioning, while pain strongly correlates with disease symptoms, physical functioning,

role functioning, cognitive functioning and fatigue. Appetite loss strongly correlates with disease symptoms, side effects of treatment, global health status, emotional functioning, social functioning, and nausea/vomiting.

Correlation analysis for transplanted patients from our study shows that global health status strongly correlates with side effects of treatment; furthermore, physical functioning strongly correlates with disease symptoms, side effects of treatment and global health status but also with role functioning; while role functioning and physical functioning correlate with social functioning. When symptoms are taken into consideration the pain strongly correlates with the largest number of items. Pain strongly correlates with disease symptoms, side effects of treatment, physical functioning, role functioning and fatigue. Other symptoms correlate only with one item as follows: dyspnoea with side effects of treatment, appetite loss with nausea/vomiting, constipation with nausea/vomiting and diarrhoea with constipation.

The study conducted in France shows the strongest correlation between EORTC QLQ-C30 global health status and QLQ-MY20 disease symptoms. They also highlight that information such as this may be useful in studies of HrQoL in multiple myeloma by helping to establish the factors that have greatest influence on the global health status score.[29] These data do not represent the course of an individual patient's disease. Nonetheless, they offer a basis for hypotheses regarding the factors that could influence HrQoL throughout the disease course.[29]

Improvements in overall survival achieved in recent years with new therapies for MM patients are a great achievement. Data from clinical trials suggest that the benefits of multiple myeloma treatment may outweigh the negative effects of toxicities and disease progression.[29] Quality of life is influenced by the ability to adapt to unfortunate conditions or it can be said that that changes in HQL scores are subordinate to the individual subjective assessment of life situations which they consider to be important.[31,32] However, the updating and improving QoL measure is essential to remain relevant in new treatments.[33,34] Quality of life could be monitored objectively, excluding the impact of important socio-demographic factors.[15]

In conclusion, this study is the first to present Croatian general population reference values for the EORTC QLQ-C30 questionnaire. Age, gender and place of residence are important predictors of quality of life in Croatian population. For MM patients, our study identified treatment effects consistent with those from other observational studies and provide new data on HrQoL across two different treatment choices for patients with multiple myeloma treated in Croatian clinical centres. According to available information there has been no research on MM patients before and after transplantation using the combination of EORTC QLQ-C30 and EORTC MY-20 questionnaires. It was recognized once again, that there is a need to compare the patient's quality of life to the quality of life in the general population.

Similarly as in the same research in our neighbouring Slovenia,[15] our results are applicable to more than 95% of the entire pool of Croatian cancer patients. Still, the HrQoL in Croatian general population could be further explored on larger sample sizes, and also with a wider range of age groups, where younger respondents could also be included. This might improve the applicability in patients that are diagnosed as children, adolescents or young adults, especially given the socio-demographic and political changes that have taken place in Croatia over the past 30 years.

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*research article*

# Inquiry and computer program Onko-Online: 25 years of clinical registry for breast cancer at the University Medical Centre Maribor

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**Background.** High-quality routine care data collected in the clinical registry play a significant role in improving the management of cancer patients. Clinical cancer registries record important data in the course of cancer diagnosis, treatment, follow-up and survival. Analyses of such comprehensive data pool make it possible to improve the quality of patients care and compare with other health care providers.

**Methods.** The first inquiry at the Department of Gynaecologic and Breast Oncology of the then General Hospital Maribor to follow breast cancer patients has been introduced in 1994. Based on our experience and new approaches in breast cancer treatment, the context of inquiry has been changed and extended to the present form, which served as a model for developing a relevant computer programme named *Onko-Online* in 2014.

**Results.** During the 25-year period, we collected data from about 3,600 breast cancer patients. The computer program *Onko-Online* allowed for quick and reliable collection, processing and analysis of 167 different data of breast cancer patients including general information, medical history, diagnostics, treatment, and follow-up.

**Conclusions.** The clinical registry for breast cancer *Onko-Online* provides data that help us to improve diagnostics and treatment of breast cancer patients, organize the daily practice and to compare the results of our treatment to the national and international standards. A limitation of the registry is the potentially incomplete or incorrect data input by different healthcare providers, involved in the treatment of breast cancer patients.

Key words: clinical registry; computer program; breast cancer

## Introduction

In Slovenia, we have one of the oldest population-based cancer registries in Europe named the Cancer Registry of Republic of Slovenia. It was founded at the Institute of Oncology in Ljubljana in 1950. This registry monitors the population burden for all malignant and non-malignant oncological diseases.[1] Clinical registers in Slovenia are needed for collecting additional information on certain cancers.[2] The Clinical Register of Skin Melanoma was founded in 2017 as the first special clinical registry for Slovenia.[3]

At our Department of Gynaecologic and Breast Oncology we introduced seven different inquiries for gynaecological (vulvar, vaginal, cervical, endometrial, ovarian, fallopian tube cancer) and breast cancer in 1994. For all of them, a computer program running in Microsoft Access has been designed and we published two articles on the use of this software for follow-up of patients with ovarian malignancies in 1996 and 1999.[4,5]

## Methods

In the last decades, treatment of the most common female carcinoma, breast cancer, changed dramatically in terms of surgery and systemic

treatment. Regarding previous experience with collecting data of cancer patients and including relevant data, the context of the inquiry for breast cancer has been changed and extended to achieve the form, which we use nowadays. The updated inquiry served as a model for developing an adequate computer program named *Onko-Online* in 2014, which records data during diagnostics, treatment and follow-up.

The paper inquiry was completed during diagnostic and treatment procedures. Included in the program were all breast cancer patients at first presentation who started treatment at our institution irrespective of the disease stage. If a patient underwent diagnostic procedures at a different institution, it was possible to collect data based on medical records. Therefore, these patients were also included to the program in case their first treatment was initiated at our institution. General data were partly collected when the diagnosis of breast malignancy was established.

After completing primary treatment, data were recorded using the computer program *Onko-Online*, which allowed for processing and analysing of the obtained data. Hard copies were completed by the doctor in charge. The data from hard copies were put into the computer program by a clerk with adequate training.

The documentation was also kept in the form of printed copies as part of health records.

## Results

The inquiry for breast cancer covered 167 different information, divided into 11 sections: general data (G), medical history (MH), clinical examination (CE), mammography (M), ultrasound (US), preoperative investigations (PI), surgery (S), radiotherapy (RT), histopathology (H), systemic treatment (ST), and follow-up (FU).

General data consisted of the identification data and data regarding treatment collected at the end of primary treatment (Figure 1).

The data were recorded using the computer program when patients completed their primary treat-

**BREAST CANCER**

**G1 year/no.:**

**G2 NAME AND G3. FAMILY NAME:** | **G4 GENDER:**

**G5 PERSONAL IDENTIFICATION NUMBER:** | **G6 AGE:**

**G7 DATE OF BIRTH:**

**G8 CARD NO. OF CBD (BREAST DISEASE CENTER):** | **G9 CARD NO. GIN:** | **PC NO.:**

**G10 DATE OF LAST EXAMINATION (or EX):** _____
(last check-up, field S1.)

**G11 STATUS AT LAST FOLLOW-UP (or EX):**
(last check-up, field S8.)

0 alive, no symptoms
1 alive, partial remission (PR)
2 alive, stable disease (SD)
3 alive, relapse
4 alive, progressive disease (PD)
5 alive, condition unknown
6 ex due to breast malignancy
7 ex during treatment
8 ex due to other disease, no breast cancer symptoms
9 ex due to other disease, breast cancer symptoms present
10 ex, cause unknown
11 condition unknown

**G12 DG:** _____ | **G13 DATE OF DG:** _____

| | | | | | |
|---|---|---|---|---|---|
| 1 DCIS | ☐R | ☐ L | 6 mucinous carcinoma | ☐R | ☐ L |
| 2 ductal carcinoma | ☐R | ☐ L | 7 tubular carcinoma | ☐R | ☐ L |
| 3 LCIS | ☐R | ☐ L | | ☐R | ☐ L |
| 4 lobular carcinoma | ☐R | ☐ L | | | |
| 5 medullary carcinoma | ☐R | ☐ L | 8 other (please, specify) _____ | | |

**G14 STAGE:**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TX | T0 | TIS | T1 | *T1mi* | *T1a* | *T1b* | *T1c* | T2 | T3 | T4a | T4b | T4c | T4d |
| NX | N0 | N1 | N2 | *N2a* | *N2b* | N3 | *N3a* | *N3b* | *N3c* | | | | |
| MX | M0 | M1 | | | | | | | | | | | |

**G15 DIFFERENTIATION:** 1 G1 2 G2 3 G3

**G16 INTRINSIC TUMOR SUBTYPE:** 1 luminal A 2 luminal B, HER2 negative 3 luminal B, HER2 positive 4 HER2 positive non-luminal 5 triple negative

**G17 TREATMENT:**

| | | | |
|---|---|---|---|
| 0 no | ☐R ☐ L | 5 complete/full chemotherapy | 9 hormone therapy |
| 1 tumorectomy | ☐ R ☐ L | 6 non-complete chemotherapy | 10 other (please, specify) |
| 2 mastectomy | ☐R ☐ L | 7 non-adjuvant chemotherapy | |
| 3 SNB | ☐R ☐ L | 8 beam radiation | |
| 4 axillary clearance | ☐R ☐ L | | |

| G18 DATE OF 1st RELAPSE | G21 DATE OF 2nd RELAPSE | G24 DATE of 3rd RELAPSE | G27 DATE of 4th RELAPSE |
|---|---|---|---|
| _____ | _____ | _____ | _____ |
| **G19 SITE OF 1st RELAPSE** | **G22 SITE OF 2nd RELAPSE** | **G25 SITE of 3rd RELAPSE** | **G28 SITE of 4th RELAPSE** |
| 1 bones 7 same breast | 1 bones 7 same breast | 1 bones 7 same breast | 1 bones 7 same breast |
| 2 axilla 8 other breast | 2 axilla 8 other breast | 2 axilla 8 other breast | 2 axilla 8 other breast |
| 3 lungs 9 soft tissues | 3 lungs 9 soft tissues | 3 lungs 9 soft tissues | 3 lungs 9 soft tissues |
| 4 liver 10 chest wall | 4 liver 10 chest wall | 4 liver 10 chest wall | 4 liver 10 chest wall |
| 5 brain 11 other | 5 brain 11 other | 5 brain 11 other | 5 brain 11 other |
| 6 local relapse | 6 local relapse | 6 local relapse | 6 local relapse |
| **G20 1st LINE TREATMENT** | **G23 2nd LINE TREATMENT** | **G26 3rd LINE TREATMENT** | **G29 4th LINE TREATMENT** |
| 0 no | 0 no | 0 no | 0 no |
| 1 surgical | 1 surgical | 1 surgical | 1 surgical |
| 2 systemic chemotherapy | 2 systemic chemotherapy | 2 systemic chemotherapy | 2 systemic chemotherapy |
| 3 systemic-targeted | 3 systemic-targeted | 3 systemic-targeted | 3 systemic-targeted |
| 4 systemic hormone therapy | 4 systemic hormone therapy | 4 systemic hormone therapy | 4 systemic hormone therapy |
| 5 beam radiation | 5 beam radiation | 5 beam radiation | 5 beam radiation |
| 6 other (please, specify) | 6 other (please, specify) | 6 other (please, specify) | 6 other (please, specify) |

**FIGURE 1.** General data.

**MEDICAL HISTORY**

**MH1 FAMILY HISTORY**
0 none
1 tuberculosis
2 diabetes
3 allergies
4 mental disorders
5 STDs
6 other (_____)

**MH2 FAMILY HISTORY OF CANCER**
0 none (go to A5)
1 breast
2 ovary
3 uterus
4 GIT
5 other (_____)

**MH3 FAMILY RELATIONSHIP**
1 mother
2 sister
3 other (_____)

**MH4 AGE AT DISEASE ONSET (in years) (see A3)**
1 _____
2 _____
3 _____

**MH5 FIRST PERIOD (age in years)**
_____

**MH6 NUMBER OF PREGNANCIES**
_____

**MH7 NUMBER OF MISCARRIAGES**
_____

**MH8 NUMBER OF INDUCED ABORTIONS**
_____

**MH9 NUMBER OF DELIVERIES/BIRTHS**
_____

**MH13 AGE AT FIRST BIRTH (years)**
_____

**MH11 BREASTFEEDING**
0 no (go to A13)
1 yes

**MH12 TOTAL DURATION OF BREASTFEEDING**
_____

**MH13 HORMONAL CONTRACEPTION**
0 never (go to A15)
1 before
2 now

**MH14 NUMBER OF YEARS of OCP USE**
_____

**MH15 FERTILITY TREATMENT**
0 no (go to A18)
1 yes

**MH16 DURATION OF FERTILITY TREATMENT (months)**
_____

**MH17 NUMBER OF STIMULATED CYCLES**
_____

**MH18 MENOPAUSE**
0 not yet (go to A20)
1 natural
2 artificial/triggered

**MH13 AGE AT MENOPAUSE (years)**
_____

**MH20 HORMONE THERAPY (PERI- OR POSTMENOPAUSE)**
0 never (go to A23)
1 estrogen
2 estrogen-progesterone
3 other (_____)

**MH21 NUMBER OF YEARS of HRT USE**
_____

**MH22 NUMBER OF YEARS since DISCONTINUED HRT**
_____

**MH23 SMOKING**
0 never (go to A25)
1 before
2 now

**MH24 NUMBER OF PACKAGES-YEARS (number of years x no. of packages daily)**
_____

**MH25 ALCOHOL CONSUMPTION**
0 never
1 moderate (< 20g [1 unit] per day)
2 excessive (> 20g per day)

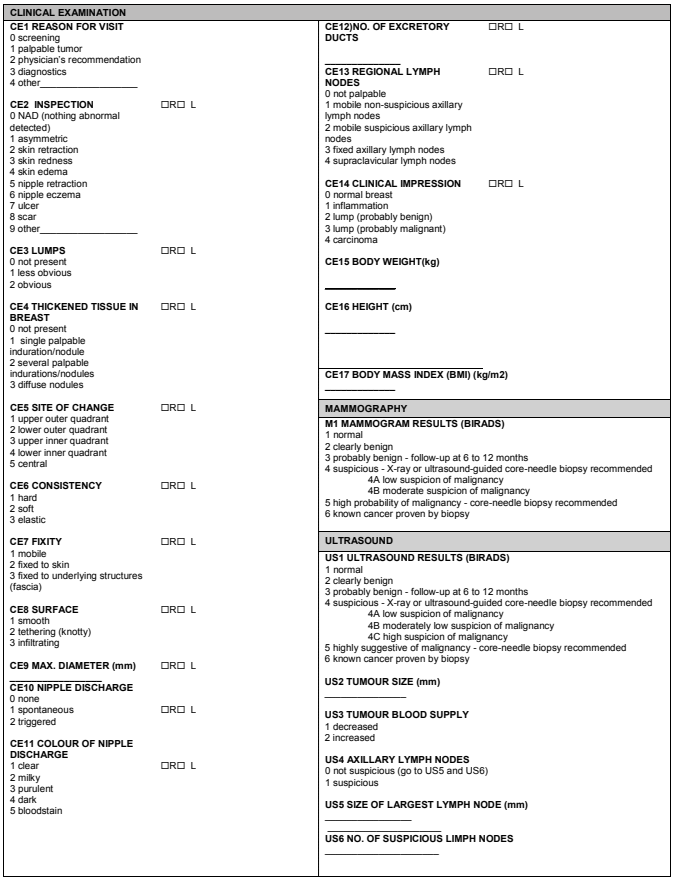
**MH26 PREVIOUS OR PRESENT CONDITIONS**
0 none
1 arterial hypertension
2 diabetes
3 obesity
4 coronary heart disease
5 other_____

**MH27 PREVIOUS OR CURRENT CANCER DISEASES**
0 none
1 other breast
2 ovary
3 GIT _____
4 other_____

**MH28 SIGNS AND SYMPTOMS**
0 none
1 palpable tumor
2 painful breast
3 skin changes
4 nipple discharge
5 palpable lymph nodes
6 pain in bones
7 abdominal pain
8 dyspnea
9 coughing
10 neurological symptoms
11 losing weight
12 other _____

**MH29 DURATION OF SIGNS AND SYMPTOMS (in months)**
_____

**FIGURE 2.** Medical history.



**CLINICAL EXAMINATION**

**CE1 REASON FOR VISIT**
0 screening
1 palpable tumor
2 physician's recommendation
3 diagnostics
4 other_____

**CE2 INSPECTION** ☐R☐ L
0 NAD (nothing abnormal detected)
1 asymmetric
2 skin retraction
3 skin redness
4 skin edema
5 nipple retraction
6 nipple eczema
7 ulcer
8 scar
9 other_____

**CE3 LUMPS** ☐R☐ L
0 not present
1 less obvious
2 obvious

**CE4 THICKENED TISSUE IN BREAST** ☐R☐ L
0 not present
1 single palpable induration/nodule
2 several palpable indurations/nodules
3 diffuse nodules

**CE5 SITE OF CHANGE** ☐R☐ L
1 upper outer quadrant
2 lower outer quadrant
3 upper inner quadrant
4 lower inner quadrant
5 central

**CE6 CONSISTENCY** ☐R☐ L
1 hard
2 soft
3 elastic

**CE7 FIXITY** ☐R☐ L
1 mobile
2 fixed to skin
3 fixed to underlying structures (fascia)

**CE8 SURFACE** ☐R☐ L
1 smooth
2 tethering (knotty)
3 infiltrating

**CE9 MAX. DIAMETER (mm)** ☐R☐ L
_____

**CE10 NIPPLE DISCHARGE**
0 none
1 spontaneous ☐R☐ L
2 triggered

**CE11 COLOUR OF NIPPLE DISCHARGE**
1 clear ☐R☐ L
2 milky
3 purulent
4 dark
5 bloodstain

**CE12)NO. OF EXCRETORY DUCTS** ☐R☐ L
_____

**CE13 REGIONAL LYMPH NODES** ☐R☐ L
0 not palpable
1 mobile non-suspicious axillary lymph nodes
2 mobile suspicious axillary lymph nodes
3 fixed axillary lymph nodes
4 supraclavicular lymph nodes

**CE14 CLINICAL IMPRESSION** ☐R☐ L
0 normal breast
1 inflammation
2 lump (probably benign)
3 lump (probably malignant)
4 carcinoma

**CE15 BODY WEIGHT(kg)**
_____

**CE16 HEIGHT (cm)**
_____

**CE17 BODY MASS INDEX (BMI) (kg/m2)**
_____

**MAMMOGRAPHY**

**M1 MAMMOGRAM RESULTS (BIRADS)**
1 normal
2 clearly benign
3 probably benign - follow-up at 6 to 12 months
4 suspicious - X-ray or ultrasound-guided core-needle biopsy recommended
4A low suspicion of malignancy
4B moderate suspicion of malignancy
5 high probability of malignancy - core-needle biopsy recommended
6 known cancer proven by biopsy

**ULTRASOUND**

**US1 ULTRASOUND RESULTS (BIRADS)**
1 normal
2 clearly benign
3 probably benign - follow-up at 6 to 12 months
4 suspicious - X-ray or ultrasound-guided core-needle biopsy recommended
4A low suspicion of malignancy
4B moderately low suspicion of malignancy
4C high suspicion of malignancy
5 highly suggestive of malignancy - core-needle biopsy recommended
6 known cancer proven by biopsy

**US2 TUMOUR SIZE (mm)**
_____

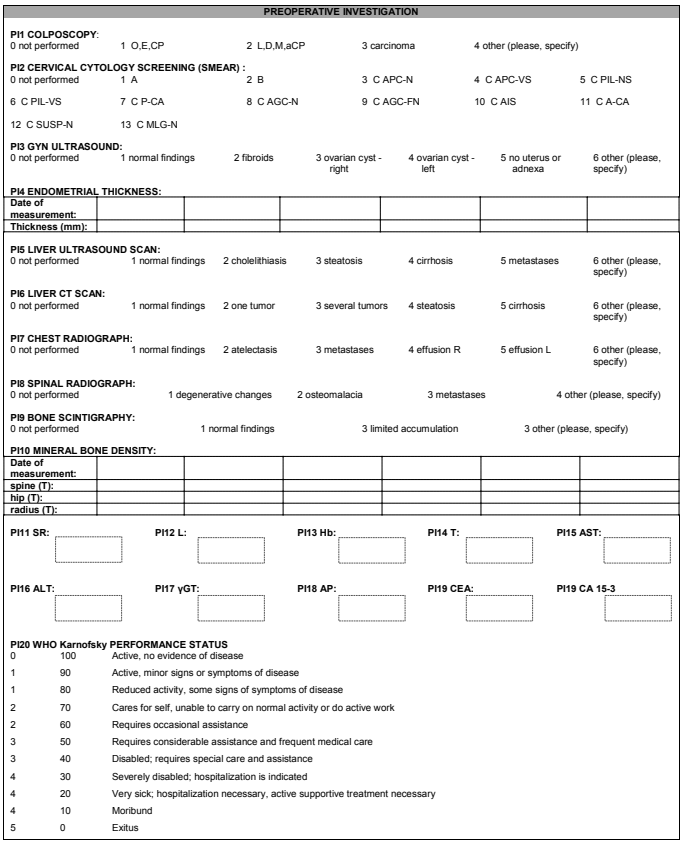
**US3 TUMOUR BLOOD SUPPLY**
1 decreased
2 increased

**US4 AXILLARY LYMPH NODES**
0 not suspicious (go to US5 and US6)
1 suspicious

**US5 SIZE OF LARGEST LYMPH NODE (mm)**
_____

**US6 NO. OF SUSPICIOUS LIMPH NODES**
_____

**FIGURE 3.** Clinical examination and breast imaging.



**PREOPERATIVE INVESTIGATION**

**PI1 COLPOSCOPY**:
0 not performed 1 O,E,CP 2 L,D,M,aCP 3 carcinoma 4 other (please, specify)

**PI2 CERVICAL CYTOLOGY SCREENING (SMEAR) :**
0 not performed 1 A 2 B 3 C APC-N 4 C APC-VS 5 C PIL-NS
6 C PIL-VS 7 C P-CA 8 C AGC-N 9 C AGC-FN 10 C AIS 11 C A-CA
12 C SUSP-N 13 C MLG-N

**PI3 GYN ULTRASOUND:**
0 not performed 1 normal findings 2 fibroids 3 ovarian cyst - right 4 ovarian cyst - left 5 no uterus or adnexa 6 other (please, specify)

**PI4 ENDOMETRIAL THICKNESS:**

| Date of measurement: | | | | | | |
|---|---|---|---|---|---|---|
| Thickness (mm): | | | | | | |

**PI5 LIVER ULTRASOUND SCAN:**
0 not performed 1 normal findings 2 cholelithiasis 3 steatosis 4 cirrhosis 5 metastases 6 other (please, specify)

**PI6 LIVER CT SCAN:**
0 not performed 1 normal findings 2 one tumor 3 several tumors 4 steatosis 5 cirrhosis 6 other (please, specify)

**PI7 CHEST RADIOGRAPH:**
0 not performed 1 normal findings 2 atelectasis 3 metastases 4 effusion R 5 effusion L 6 other (please, specify)

**PI8 SPINAL RADIOGRAPH:**
0 not performed 1 degenerative changes 2 osteomalacia 3 metastases 4 other (please, specify)

**PI9 BONE SCINTIGRAPHY:**
0 not performed 1 normal findings 3 limited accumulation 3 other (please, specify)

**PI10 MINERAL BONE DENSITY:**

| Date of measurement: | | | | | | |
|---|---|---|---|---|---|---|
| spine (T): | | | | | | |
| hip (T): | | | | | | |
| radius (T): | | | | | | |

**PI11 SR:** **PI12 L:** **PI13 Hb:** **PI14 T:** **PI15 AST:**

**PI16 ALT:** **PI17 yGT:** **PI18 AP:** **PI19 CEA:** **PI19 CA 15-3**

**PI20 WHO Karnofsky PERFORMANCE STATUS**

| | | |
|---|---|---|
| 0 | 100 | Active, no evidence of disease |
| 1 | 90 | Active, minor signs or symptoms of disease |
| 1 | 80 | Reduced activity, some signs of symptoms of disease |
| 2 | 70 | Cares for self, unable to carry on normal activity or do active work |
| 2 | 60 | Requires occasional assistance |
| 3 | 50 | Requires considerable assistance and frequent medical care |
| 3 | 40 | Disabled; requires special care and assistance |
| 4 | 30 | Severely disabled; hospitalization is indicated |
| 4 | 20 | Very sick; hospitalization necessary, active supportive treatment necessary |
| 4 | 10 | Moribund |
| 5 | 0 | Exitus |

**FIGURE 4.** Investigations before treatment.

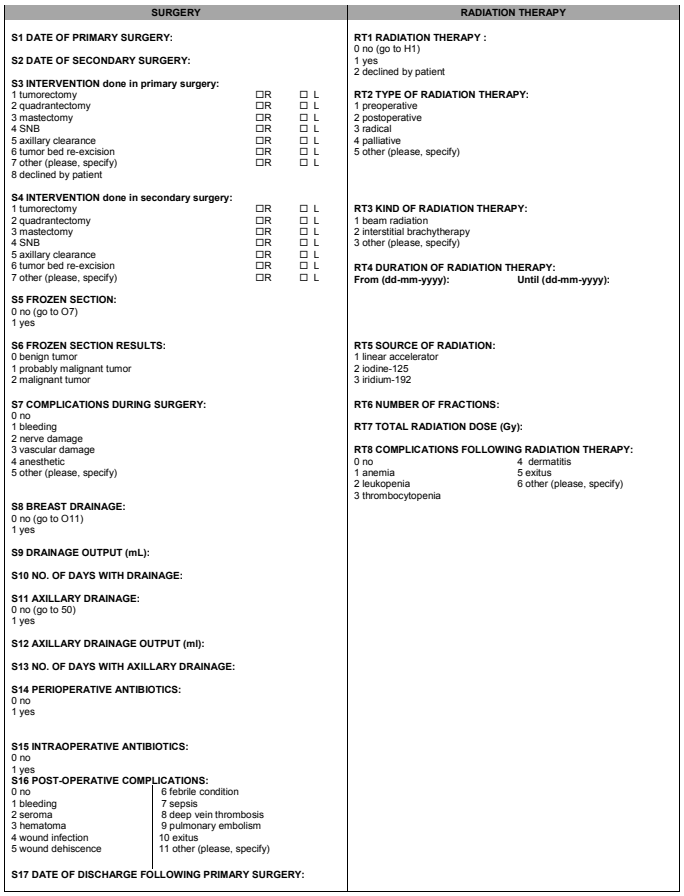
ment. Until now, data about 3,600 patients have been included in this computer program.

Twenty-nine anamnestic data focus on known risk factors for breast cancer as well as current symptoms and signs. Among the risk factors, detailed data on family history of breast cancer and other malignancies, reproductive data, use of hormonal therapy, smoking, and use of alcohol were recorded. Detailed data are listed in Figure 2. The anamnestic data ended with signs and symptoms in the breast, such as breast lump, pain, skin changes, nipple discharge, enlarged axillary lymph nodes as well as their duration and general symptoms, such as bone pain, abdominal pain, dyspnoea, cough, neurological symptoms, and loss of weight.

Next section covered a clinical examination with 17 parameters, including inspection and palpation of the breasts and regional lymph nodes, including axillary and supraclavicular lymph nodes. Body mass index data were recorded and data on breast imaging, mammography and ultrasonography of the breast and axillary lymph nodes were collected (Figure 3).

The following section contained data about different extended investigations before treatment: gynaecological examinations (colposcopy, gynaecological ultrasound), imaging examinations of liver, lung and bones and certain laboratory testing with the focus on the most common sites of metastases. At the end of this section, WHO and Karnofsky performance status was recorded (Figure 4).

The section containing data about the surgical procedure and postoperative care included 16 parameters. Date of procedure, type of surgery, use of frozen section, complications during procedure, and placement of drains were recorded immediately after the surgery. Later, the removal of drains, antibiotic therapy and possible complications were added before the patient leaves hospital (Figure 5). For an easy and fast completion of the inquiry, six types of surgical procedures were listed with separate marks for the right and left breast. The most common complications during and after surgery were also listed, including the complications in the breasts, such as bleeding or hematoma, seroma,



**SURGERY**

**S1 DATE OF PRIMARY SURGERY:**

**S2 DATE OF SECONDARY SURGERY:**

**S3 INTERVENTION done in primary surgery:**
1 tumorectomy ☐R ☐ L
2 quadrantectomy ☐R ☐ L
3 mastectomy ☐R ☐ L
4 SNB ☐R ☐ L
5 axillary clearance ☐R ☐ L
6 tumor bed re-excision ☐R ☐ L
7 other (please, specify) ☐R ☐ L
8 declined by patient

**S4 INTERVENTION done in secondary surgery:**
1 tumorectomy ☐R ☐ L
2 quadrantectomy ☐R ☐ L
3 mastectomy ☐R ☐ L
4 SNB ☐R ☐ L
5 axillary clearance ☐R ☐ L
6 tumor bed re-excision ☐R ☐ L
7 other (please, specify) ☐R ☐ L

**S5 FROZEN SECTION:**
0 no (go to O7)
1 yes

**S6 FROZEN SECTION RESULTS:**
0 benign tumor
1 probably malignant tumor
2 malignant tumor

**S7 COMPLICATIONS DURING SURGERY:**
0 no
1 bleeding
2 nerve damage
3 vascular damage
4 anesthetic
5 other (please, specify)

**S8 BREAST DRAINAGE:**
0 no (go to O11)
1 yes

**S9 DRAINAGE OUTPUT (mL):**

**S10 NO. OF DAYS WITH DRAINAGE:**

**S11 AXILLARY DRAINAGE:**
0 no (go to 50)
1 yes

**S12 AXILLARY DRAINAGE OUTPUT (ml):**

**S13 NO. OF DAYS WITH AXILLARY DRAINAGE:**

**S14 PERIOPERATIVE ANTIBIOTICS:**
0 no
1 yes

**S15 INTRAOPERATIVE ANTIBIOTICS:**
0 no
1 yes

**S16 POST-OPERATIVE COMPLICATIONS:**
0 no
1 bleeding
2 seroma
3 hematoma
4 wound infection
5 wound dehiscence
6 febrile condition
7 sepsis
8 deep vein thrombosis
9 pulmonary embolism
10 exitus
11 other (please, specify)

**S17 DATE OF DISCHARGE FOLLOWING PRIMARY SURGERY:**

**RADIATION THERAPY**

**RT1 RADIATION THERAPY :**
0 no (go to H1)
1 yes
2 declined by patient

**RT2 TYPE OF RADIATION THERAPY:**
1 preoperative
2 postoperative
3 radical
4 palliative
5 other (please, specify)

**RT3 KIND OF RADIATION THERAPY:**
1 beam radiation
2 interstitial brachytherapy
3 other (please, specify)

**RT4 DURATION OF RADIATION THERAPY:**
**From (dd-mm-yyyy):** **Until (dd-mm-yyyy):**

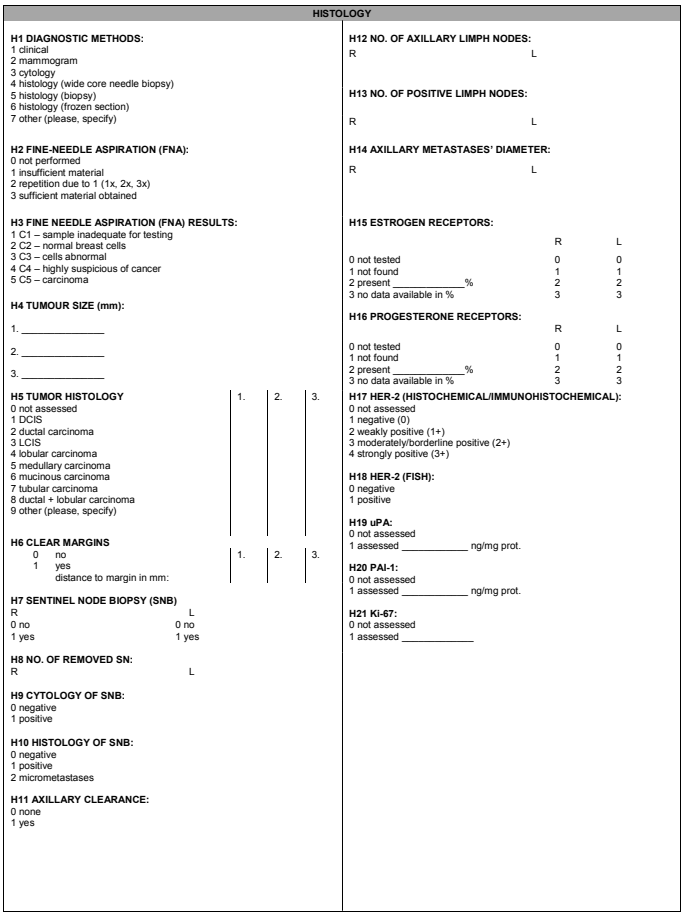
**RT5 SOURCE OF RADIATION:**
1 linear accelerator
2 iodine-125
3 iridium-192

**RT6 NUMBER OF FRACTIONS:**

**RT7 TOTAL RADIATION DOSE (Gy):**

**RT8 COMPLICATIONS FOLLOWING RADIATION THERAPY:**
0 no
1 anemia
2 leukopenia
3 thrombocytopenia
4 dermatitis
5 exitus
6 other (please, specify)

**FIGURE 5.** Surgery and radiotherapy.



**HISTOLOGY**

**H1 DIAGNOSTIC METHODS:**
1 clinical
2 mammogram
3 cytology
4 histology (wide core needle biopsy)
5 histology (biopsy)
6 histology (frozen section)
7 other (please, specify)

**H2 FINE-NEEDLE ASPIRATION (FNA):**
0 not performed
1 insufficient material
2 repetition due to 1 (1x, 2x, 3x)
3 sufficient material obtained

**H3 FINE NEEDLE ASPIRATION (FNA) RESULTS:**
1 C1 – sample inadequate for testing
2 C2 – normal breast cells
3 C3 – cells abnormal
4 C4 – highly suspicious of cancer
5 C5 – carcinoma

**H4 TUMOUR SIZE (mm):**

1. _____

2. _____

3. _____

**H5 TUMOR HISTOLOGY** 1. 2. 3.
0 not assessed
1 DCIS
2 ductal carcinoma
3 LCIS
4 lobular carcinoma
5 medullary carcinoma
6 mucinous carcinoma
7 tubular carcinoma
8 ductal + lobular carcinoma
9 other (please, specify)

**H6 CLEAR MARGINS** 1. 2. 3.
0 no
1 yes
distance to margin in mm:

**H7 SENTINEL NODE BIOPSY (SNB)**
R L
0 no 0 no
1 yes 1 yes

**H8 NO. OF REMOVED SN:**
R L

**H9 CYTOLOGY OF SNB:**
0 negative
1 positive

**H10 HISTOLOGY OF SNB:**
0 negative
1 positive
2 micrometastases

**H11 AXILLARY CLEARANCE:**
0 none
1 yes

**H12 NO. OF AXILLARY LIMPH NODES:**
R L

**H13 NO. OF POSITIVE LIMPH NODES:**

R L

**H14 AXILLARY METASTASES' DIAMETER:**

R L

**H15 ESTROGEN RECEPTORS:**

| | R | L |
|---|---|---|
| 0 not tested | 0 | 0 |
| 1 not found | 1 | 1 |
| 2 present _____% | 2 | 2 |
| 3 no data available in % | 3 | 3 |

**H16 PROGESTERONE RECEPTORS:**

| | R | L |
|---|---|---|
| 0 not tested | 0 | 0 |
| 1 not found | 1 | 1 |
| 2 present _____% | 2 | 2 |
| 3 no data available in % | 3 | 3 |

**H17 HER-2 (HISTOCHEMICAL/IMMUNOHISTOCHEMICAL):**
0 not assessed
1 negative (0)
2 weakly positive (1+)
3 moderately/borderline positive (2+)
4 strongly positive (3+)

**H18 HER-2 (FISH):**
0 negative
1 positive

**H19 uPA:**
0 not assessed
1 assessed _____ ng/mg prot.

**H20 PAI-1:**
0 not assessed
1 assessed _____ ng/mg prot.

**H21 Ki-67:**
0 not assessed
1 assessed _____

**FIGURE 6.** Histopathology.

wound infection, wound dehiscence and systemic complications, such as fever, deep vein thrombosis and pulmonary embolism.

For radiation therapy, eight boxes were designed: type, dates of starting and ending radiotherapy and possible complications (Figure 5). As in the case of surgery, the most common type and complications of radiotherapy were provided in the inquiry. Because radiotherapy was performed at the Department of Oncology, data about this part of treatment were filled after complete treatment, at the first follow-up visit at the latest.

In the next section, data on cytological and histopathological examination of tumour and lymph nodes were collected. The first part of this section included data on preoperative diagnostics, which could be collected prior to the primary treatment. The inquiry included data on the tumour histology before and after surgery, cytology and histology of sentinel node biopsy (SNB) and/or axillary node dissection and the main predictive and prognostic biomarkers, oestrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2) and proliferation marker Ki67 (Ki67) (Figure 6). Full data on histopathology were usually available after the patient leaves the hospital; hence, this part of the inquiry was completed later on.

Since the systemic therapy represented an important part of breast cancer treatment in the control and cure of breast cancer, a relatively large part of the inquiry was dedicated to this issue.

Detailed information about adjuvant or neoadjuvant chemotherapy was collected in the special section of the inquiry boxes during treatment (Figure 7). Among others, this data included the date of each chemotherapy cycle and chemotherapy regimen. The presence of the adverse events during chemotherapy was collected in the Chemotherapy section. Detailed data regarding the type and severity of adverse events were collected in the section Adverse events.

A separate sheet contained data on systemic anti-cancer treatment, including chemotherapy,

hormonal and targeted therapy, applied as neoadjuvant or adjuvant treatment. The same page contained boxes for systemic treatment in case of recurrent disease. The most frequently used agents were already listed and categorized for chemotherapy, hormonal therapy, and targeted therapy. Over the past decades, adjunctive and supportive therapy of breast cancer have evolved substantially. In the inquiry, the data on bisphosphonates, erythropoietin and granulocyte colony-stimulating factor (G-CSF) were collected during the systemic treatment (Figure 8).

The last section of the inquiry was follow-up sheet (Figure 9). All nine boxes were completed at every follow-up visit. Data collected at follow-up were limited to performance status, pain, clinical examination, mammography, laboratory tests, and the clinical state of the patient.

All data collected with the paper inquiry were recorded using the computer program *Onko-Online* for processing data and statistical analysis. The program enables to find, list and sort data in a quick and easy manner. The existing data could be modified or new data could be added, if necessary.

| **ST1 CHEMOTHERAPY CYCLE / TREATMENT LEVEL:** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **ST2 DATE:** | | | | | | |
| **ST3 BODY WEIGHT (kg):** | | | | | | |
| **ST4 HEIGHT (cm):** | | | | | | |
| **ST5 SURFACE ($m^2$):** | | | | | | |
| **ST6 PERFORMANCE STATUS:** (See P21)<br>0 3<br>1 4<br>2 5 | | | | | | |
| **ST7 EXAMINATION:**<br>0 NAD 3 lymphedema<br>1 tumor 4 metastasis<br>2 hydrothorax 5 other (specify) | | | | | | |
| **ST8 CHEST RADIOGRAPH:**<br>0 NAD 2 hydrothorax<br>1 metastases3 other (specify) | | | | | | |
| **ST9 LIVER ULTRASOUND SCAN:**<br>0 NAD 2ascites<br>1metastases3 other (specify) | | | | | | |
| **ST10 BONE SCINTIGRAPHY:**<br>0 NAD (nothing abnormal detected)<br>1 metastases (site)<br>2 diffuse accumulation (site) | | | | | | |
| **ST11 BONE RADIOGRAPHY:**<br>0 NAD (nothing abnormal detected)<br>1 metastases (site)<br>2 diffuse changes (please, specify) | | | | | | |
| **ST12 Ca 15-3** | | | | | | |
| **ST13 DOSE REDUCTION (%)** | | | | | | |
| **ST14 REASON FOR REDUCTION**<br>a ↓L c liver dysfunction<br>b ↓T d renal dysfunction | | | | | | |
| **ST15 CYTOTOXIC 1:** (mg) | | | | | | |
| **ST16 CYTOTOXIC 2:** (mg) | | | | | | |
| **ST17 CYTOTOXIC 3:** (mg) | | | | | | |
| **ST18 G-CSF** (dose) | | | | | | |
| **ST19 ANTIEMETIC** (mg) | | | | | | |
| **ST20 PATHOLOGY LAB. RESULTS**<br>biochemistry (AP, GT…)<br>marker (CEA)<br>other (please, specify) | | | | | | |
| **ST21 VOMITING:**<br>0 no 2 6x–10x<br>1 1x–5x 3 > 10x | | | | | | |
| **ST22 ADVERSE EVENT:** (See page 6)<br>0 no<br>1 yes | | | | | | |

**FIGURE 7.** Adjuvant or neoadjuvant chemotherapy.

## Discussion

The breast cancer inquiry collected extended information on altogether 167 questions about breast cancer patient medical history, clinical status, treatment, and its outcome.

Among the risk factors, we recorded data known to be associated with high risk for breast cancer. It is well known that there is a two-fold increase in the risk of developing breast cancer for women with breast cancer in their first-degree family, especially among women with a first-degree relative diagnosed before the age of 50.[6,7] Among the reproductive data, young age at menarche, late menopause, late age at first pregnancy, low number of deliveries, spontaneous or induced abortions, and lack of breastfeeding are known to increase the risk of breast cancer.[8,9] Known risk factors also include hormonal contraception and hormonal replacement therapy, although the absolute increase in risk, especially for contraception, is small.[10,11] Some studies reported a link between infertility and increased breast cancer risk, while others were not able to find a connection.[12, 13] The results of recently published data in literature strongly support the role of cigarette smoking in breast cancer etiology.[14] The risk of breast cancer is significantly increased by alcohol consumption as well.[15] Data on body mass index were included, since it is known that obesity is associated with an increased relative risk, especially for postmenopausal receptor-positive breast cancer.[16] Known risk factors for breast cancer were included to determine the frequency of these risk factors in our population. Moreover, the knowledge of these risk factors in a subset of patients could lead to a better understanding of different factors involved in the breast cancer development.

Typical local signs and symptoms for breast cancer are: a breast lump, usually painless; skin retraction, nipple retraction, nipple discharge, and swelling in the armpit.[17] All these signs were listed in the inquiry as well as palpable lymph nodes in the axilla.

We also added some typical signs of a metastatic disease (bone pain, dyspnoea, persistent cough, abdominal pain, weigh loss), although primary metastatic cancer is relatively rare. According to our registry, in Slovenia 7.1% of patients were presented with primary metastatic disease in 2015.[1] The data in the literature for developed countries

| TREATMENT SCHEME (TS1) LEVEL OF TREATMENT | (ST2 – ST7) CHEMOTHERAPY | | | | (ST8 – ST12) HORMONAL THERAPY | | | | (ST13 – ST19) TARGETED (BIOLOGICAL) TREATMENT | | | | (ST20 – ST22) THERAPY | ADJUVANT | ST23 OUTCOMES, RESPONSE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 no<br>1 yes | 1 cyclophosphamide<br>2 methotrexate<br>3 5-fluorouracil<br>4 capecitabine<br>5 doxorubicin<br>6 epirubicin<br>7 paclitaxel<br>8 docetaxel<br>9 cisplatin<br>10 carboplatin<br>11 vinorelbine<br>12 other (specify) | No. of cycles<br>Frequency of cycles | Date - since<br>Date - since | 0 no<br>1 yes | 1 tamoxifen (Nolvadex)<br>2 anastrazole (Arimidex)<br>3 exemestane (Aromasin)<br>4 letrozole (Femara)<br>5 fulvestrant (Faslodex)<br>6 GnRH (Zoladex)<br>7 other (specify) | Dose | Date - since<br>Date - until | 0 no<br>1 trastuzumab<br>2 lapatinib<br>3 bevacizumab<br>4 other (specify) | Dose<br>cumulative dose | No. of cycles<br>Frequency of cycles | Date - since<br>Date - until | 1 Bisphosphonates<br>2 Erythropoietins<br>3 GCSF<br>4 other (specify) | Date - since<br>Date - until | |
| NON-ADJUVANT | | | | | | | | | | | | | | | 0 disease-free<br>1 progress during chemotherapy and/or targeted (biological) treatment<br>2 progress following chemotherapy and/or targeted (biological) treatment<br>3 condition unknown |
| ADJUVANT | | | | | | | | | | | | | | | 0 disease-free<br>1 progress during chemotherapy and/or targeted (biological) treatment<br>2 progress following chemotherapy and/or targeted (biological) treatment<br>3 condition unknown |
| PRIMARY METASTATIC DISEASE | | | | | | | | | | | | | | | |
| 1. RELAPSE (LINE)<br>0 no<br>1 yes, clinical<br>2 yes, biochemical<br>3 yes, x-ray, ultrasound, scintigraphy<br>4 yes, confirmed by biopsy<br>DATE 1. RELAPSE | | | | | | | | | | | | | | | 0 complete remission (CR)<br>1 partial remission (PR)<br>2 stable disease (SD)<br>3 progressive disease (PD)<br>4 condition unknown |
| 2. RELAPSE (LINE)<br>0 no<br>1 yes, clinical<br>2 yes, biochemical<br>3 yes, x-ray, ultrasound, scintigraphy<br>4 yes, confirmed by biopsy<br>DATE 2. RELAPSE | | | | | | | | | | | | | | | 0 complete remission (CR)<br>1 partial remission (PR)<br>2 stable disease (SD)<br>3 progressive disease (PD)<br>4 condition unknown |
| 3. RELAPSE (LINE)<br>0 no<br>1 yes, clinical<br>2 yes, biochemical<br>3 yes, x-ray, ultrasound, scintigraphy<br>4 yes, confirmed by biopsy<br>DATE 3. RELAPSE | | | | | | | | | | | | | | | 0 complete remission (CR)<br>1 partial remission (PR)<br>2 stable disease (SD)<br>3 progressive disease (PD)<br>4 condition unknown |
| 4. RELAPSE (LINE)<br>0 no<br>1 yes, clinical<br>2 yes, biochemical<br>3 yes, x-ray, ultrasound, scintigraphy<br>4 yes, confirmed by biopsy<br>DATE 4. RELAPSE | | | | | | | | | | | | | | | 0 complete remission (CR)<br>1 partial remission (PR)<br>2 stable disease (SD)<br>3 progressive disease (PD)<br>4 condition unknown |

CR = complete response (disappearance of all target lesions); PD = progressive disease (20% increase of sum of the longest target lesions dimension); PR = partial response (30% decrease of sum of all target lesions dimension); SD = stable disease (minor lesions not qualifying for CR/PR/PD)

**FIGURE 8.** Treatment scheme.

are similar, approximately 5-10% of all breast cancer patients were presented with distant metastases at initial diagnosis.[18]

Clinical breast examination is not a reliable diagnostic tool[19], but it has to be performed in all known breast cancer patients when planning primary treatment - surgical or neoadjuvant systemic therapy. Ultrasound preoperative examination of axilla was routinely performed to avoid two-stage axillary surgery in selected patients.[20, 21] At the moment, MRI was not included in the inquiry. Since both MRI and digital breast tomosynthesis are nowadays common diagnostic procedures in breast diagnostics, we intended to add both procedures to the pre-treatment diagnostics.

According to Slovenian recommendations for stage I and II breast cancer, laboratory tests, including blood count, liver function tests, alkaline phosphatase, calcium levels, and chest X-ray were routinely performed.[22] In case of clinical symptoms and/or pathological laboratory results as well as in all stage III and IV patients, thoracic and abdominal CT scan and bone scintigraphy were performed.[22]

In the inquiry section covering a surgical procedure, breast reconstruction was not included, since this type of procedure was performed at the Department of Plastic and Reconstructive Surgery at the University Medical Centre Maribor and not within our department. Breast reconstruction is an important part of breast cancer management which has evolved significantly in the past decades because of advances in reconstructive strategy.[23] It is oncologically safe and associated with high satisfaction rates.[24] In the case of breast reconstruction, data was recorded in the inquiry during the first follow-up visit.

Over the last two years, radiation therapy for breast cancer patients has mostly been administered at our hospital at the Department of Oncology at the University Medical Centre Maribor, but some patients still receive therapy at the Institute of Oncology in Ljubljana. All data concerning radiotherapy, including complications, were collected at the first follow-up visit.

According to the data in literature, fine-needle aspiration cytology (FNAC) and core needle biopsy (CNB) have similar values of diagnostic accura-

| S1 DATE: | S2 TYPE OF EXAMINATION: | S3 WHO PERFORMANCE STATUS: | S4 PAIN: | S5 EXAMINATION | S6 MAMOGRAPHY: | S7 LABORATORY: | S8 CONDITION ASSESSMENT: | S9 NOTES: |
|---|---|---|---|---|---|---|---|---|
| | 1 outpatient clinic<br>2 hospital | 0 asymptomatic, but completely ambulatory<br>1 symptomatic,<br>2 symptomatic, up and about more than 50% of waking hours<br>3 symptomatic, confined to bed or chair more than 50% of waking hours<br>4 confined to bed | 0 no pain<br>1 mild pain<br>2 moderate pain<br>3 severe pain | 1 NAD (nothing abnormal detected)<br>2 tumor (size)<br>3 lymph nodes in axilla<br>4 lymph nodes above collar bone<br>5 hand edema<br>6 other (describe) | 0 N/A D<br>1 NAD (nothing abnormal detected) 1<br>2 suspicious findings 2<br>3 carcinoma 3<br>4 other (specify) 9 | 1 NAD (nothing abnormal detected)<br>2 high ESR levels<br>3 anemia<br>4 leukopenia<br>5 leukocytosis<br>6 high AST levels<br>7 high ALT levels<br>8 high γGT levels<br>9 high ALP levels<br>10 high CEA levels<br>11 high CA 15-3<br>12 other (specify) | 0 alive, no symptoms (CR or DF)<br>1 alive, partial remission (PR)<br>2 alive, stable disease (SD)<br>3 alive, relapse<br>4 alive, progressive disease (PD)<br>5 alive, condition unknown<br>6 ex due to breast malignancy<br>7 ex during treatment<br>8 ex due to other disease, no breast symptoms<br>9 ex due to other disease, breast symptoms present<br>10 ex, cause unknown<br>11 condition unknown | |
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**FIGURE 9.** Follow-up.

cy.[25, 26] We routinely used CNB as the first method in breast cancer diagnostics, because hormonal receptor (HR) status and expression of HER2 can be tested. Sometimes, this information was crucial for planning the treatment, e.g. neoadjuvant systemic therapy.

TNM classification of breast cancer was not included in the computer program and it served as a tool to define the correct stage in the general data (Figure 1 – G14).

The data set about the systemic treatment has been designed to provide access to quick and transparent information on systemic therapy for patients and enable easier decision-making processes for further treatment in case of disease progression. Every list of chemotherapy, hormonal and targeted therapy was given the option "others" to name drugs, which were not included. Novel therapeutic approaches included immunologic therapies, PARP inhibitors, PI3K inhibitors, and CDK4/6 inhibitors, and others to be added to the inquiry at any time.

In the inquiry, information on date of diagnosis and date of starting (different) treatment were included. The inquiry collected the date of first and second surgery, date of all neoadjuvant or adjuvant chemotherapy cycles, beginning and ending date of radiotherapy, and beginning and ending date for all types of systemic treatments. There are data in the literature suggesting that time to start of adjuvant treatment might have an influence on survival.[27] Delays to adjuvant radiotherapy are also related with decreases in survival of patients with locally advanced tumours.[28]

The purpose of a follow-up was surveillance for recurrence, management of long-term effects of cancer treatment, and management of medication side effects. At our department, follow-up was performed over a time period of 10 years. According to Slovenian recommendations[22], follow-up visits for asymptomatic patients were performed every six months for the first 3 years and then annually. At each visit, clinical examination was performed. Patients underwent mammography on

a yearly basis. Laboratory tests were indicated in case of clinical symptoms. Liver ultrasound, chest radiography, bone scan, and other investigations were performed only in case of clinical symptoms or pathological laboratory tests. At the end of the follow-up visit, treatment response rate was estimated. Treatment response rates were mostly evaluated on the basis of WHO criteria[29], although new and updated criteria had been published for more precise and objective response.[30,31]

There is no evidence that the detection of asymptomatic distant metastases leads to a longer survival.[32] Some data indicated that the detection of isolated loco-regional or contra-lateral breast cancer recurrences in patients without symptoms has beneficial impact on survival of breast cancer patients when compared to late symptomatic detection[33]; however, it was shown that only 40% of the isolated loco-regional recurrences in asymptomatic patients were detected during routine examination.[34] But, the vast majority of the patients took advantage of the follow-up and one of the important goals of the follow-up care is to offer psychological support and reassurance by their physician.[35, 36]

The type of treatment in patients who were metastatic at first presentation was recorded in the same way as for patients with localised or regional cancer. In case of disease relapse after primary treatment, data about the date of relapse, site of relapse and treatment of relapse were recorded in the section General data. Detailed data about systemic treatment of relapse were recorded also in the Treatment scheme section.

## Conclusions

The clinical cancer registry plays an important role in the evaluation of clinical practice with the purpose to improve organisation in daily clinical work and treatment of the disease. It allows us to continuously compare treatment results with national and international standards. The data can also be used for research projects and studies on cancer survivorship.

The computer program *Onko-Online* allows quick and reliable processing and analysis of 167 different data obtained from breast cancer patients, i.e. general information, medical history, diagnostics, treatment and follow-up. The computer program allows us to follow the timing of different treatments procedures to assure optimal treatment for all breast cancer patients.

A potential limitation of the registry is the incomplete or incorrect data input. With this amount of data collected by different healthcare providers there is a risk that a mistake will occur, but not in the extent to which it could influence the reliability of the data.

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*research article*

# Idiopathic pulmonary fibrosis in patients with early-stage non-small-cell lung cancer after surgical resection

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**Background.** The outcomes of patients with both lung cancer and idiopathic pulmonary fibrosis (IPF) are unfavorable. Therapeutic interventions for lung cancer such as surgery can cause acute exacerbation of IPF (aeIPF). This study aimed to assess the frequency of IPF in a group of patients with early-stage non-small-cell lung cancer (NSCLC) and to report clinical characteristics and outcomes of this cohort of patients.

**Patients and methods.** This observational cohort retrospective study analyzed 641 pathological records of patients after surgical resection of early-stage non-small-cell lung cancer (NSCLC) at University Clinic Golnik from May 2010 to April 2017. Pathological records of NSCLC with coexisting IPF were reviewed. CT scans and biopsy specimens for this group of patients were analyzed by a thoracic radiologist and pathologist, independently. We searched radiological and pathological features of usual interstitial pneumonia (UIP) pattern in this group of patients. We report the clinical characteristics and outcome of this cohort of patients.

**Results.** Out of 641 patients with early-stage NSCLC, only 13 (2.0%) had histologically and radiologically proven coexisting UIP/IPF. Squamous cell carcinoma was the most common type of lung cancer (7/13 patients). The majority of tumors were small size (all being pT1 or pT2), stage I–II (11/13 patients), located in the lower lung lobes (11/13 patients). Almost all patients were current or ex-smokers (11/13 patients). There were two pathologically confirmed fatal cases (15.4%) due to aeIPF in the first two months after radical treatment, one after adjuvant radiotherapy and the other after surgery. Out of 13 patients, one patient had a lung cancer relapse.

**Conclusions.** Frequency of UIP/IPF in surgically treated early stage NSCLC is rather low. Our observational study shows that radical treatment of lung cancer can cause aeIPF with dismal outcome in this group of patients. The standard of care in these mostly elderly patients still remains unresolved.

Key words: early-stage non-small-cell lung cancer; idiopathic pulmonary fibrosis; acute exacerbation; surgery; radiotherapy

## Introduction

In Slovenia, lung cancer is still the leading cause of cancer mortality in men and the third cause of cancer mortality in women.[1] Non-small-cell lung cancer (NSCLC) represents about 85% of all lung cancers.[2] For 20–25% of all lung cancer patients, who are diagnosed at an early stage (stage IA–IIIA), surgery remains the best chance of cure.[3] Patients at very high risk for surgery-related complications can be treated with curative radiotherapy (RT), either hypofractioned high-dose RT or stereotactic body radiation therapy (SBRT) in stage I NSCLC.[3] Reported local control after SBRT reaches around 80%–90% at 5 years.[4,5]

Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease that affects mostly smokers. It is a specific form of a progressive fibrotic interstitial

lung disease of unknown cause, occurring primarily in older adults. It shows histopathologic and radiologic pattern of usual interstitial pneumonia (UIP).[6] Long-term survival of IPF patients still remains poor with median survival rate from 2 to 3 years from time of diagnosis.[7]

Data suggest an increased risk of lung cancer among patients with IPF.[8] Treating patients with early-stage lung cancer and coexisting IPF can be demanding as IPF is independently associated with poorer overall survival in patients with lung cancer who undergo pulmonary resection.[9-11] Surgical procedures can lead to an acute exacerbation of IPF or other respiratory complications.[12] And as reported, pre-existing interstitial lung disease, such as IPF, is a significant risk factor for developing severe radiation pneumonitis after RT.[13-16]

This study aimed to assess the frequency of IPF in a group of patients who had radical surgical resection for an early-stage NSCLC, to describe morphological and clinical characteristics and report outcomes of this cohort of patients.

**TABLE 1.** Baseline clinical, radiological and pathological characteristics of patients with early-stage non-small cell lung cancer and idiopathic interstitial pneumonia

| Characteristics | | N = 13 (%) |
|---|---|---|
| **Gender** | Male | 11 (84.6) |
| | Female | 2 (15.4) |
| **Age, years** | | 73.3 ± 4 |
| **Smoking status** | Current smoker | 3 (23.1) |
| | Former-smoker | 8 (61.5) |
| | Never-smoker | 2 (15.4) |
| **$FEV_1$/FVC before operation** | | 0.71 ± 0.08 |
| **$FEV_1$ before operation** | ml | 2769 ± 690 |
| | % | 101.6 ± 20 |
| **FVC before operation** | ml | 3910 ± 818 |
| | % | 105 ±19 |
| **DLCO before operation** | % | 74.2 ±21 |
| **Histological subtype** | Squamous cell carcinoma | 7 (53.8) |
| | Adenocarcinoma | 5 (38.5) |
| | Adenosquamous cell carcinoma | 1 (7.7) |
| **Pathologic TNM stage (8th edition)** | pT1a | 3 (23.0) |
| | pT2a | 6 (46.2) |
| | pT2b | 4 (30.8) |
| | IA | 2 (15.4) |
| | IB | 2 (15.4) |
| | IIA | 3 (23.0) |
| | IIB | 4 (30.8) |
| | IIIA | 2 (15.4) |
| **Lobe distribution** | Left lower lobe | 7 (53.8) |
| | Right lower lobe | 4 (30.8) |
| | Right middle lobe | 2 (15.4) |
| **Type of operation** | Lobectomy | 11 (84.6) |
| | Bilobectomy | 2 (15.4) |
| **Adjuvant therapy** | Adjuvant chemotherapy | 4 (30.8) |
| | Adjuvant radiotherapy | 1 (7.7) |

Data are presented by mean ± SD or number (N, %).

DLCO = diffusing capacity of the lung for carbon monoxide; $FEV_1$ = forced expiratory volume in 1 s; FVC = forced vital capacity

## Patients and methods

We conducted an observational cohort retrospective study analyzing 641 consecutive pathological records of patients who underwent lung surgery because of an early-stage NSCLC at University Clinic Golnik from May 2010 to April 2017. Patients with metastatic NSCLC or neuroendocrine carcinoma were excluded from the analysis.

The study was conducted in accordance with the ethical standards laid down in an appropriate version of the 1964 Declaration of Helsinki. The study was conducted with the understanding and the consent of the subjects. Prior to intervention for the acquisition of bioptic material, patients have signed an informed consent for intervention and that their data can be used for scientific purposes.

All pathological records with an early-stage NSCLC and interstitial fibrosis with UIP pattern were selected for more detailed analysis. CT scans and pathological specimens for this group of patients were reviewed again by a thoracic radiologist and pathologist, independently. The UIP/IPF patient cohort included patients who were given a diagnosis of definite UIP/IPF, probable UIP/IPF, or possible UIP/IPF in according with the American Thoracic Society, European Respiratory Society, Japanese Respiratory society, and Latin American Thoracic association (ATS/ESR/JRS/ALAT) 2011 statement.[6] All other patients were excluded from further analysis.

The clinical data of patients with coexisting early-stage NSCLC and UIP/IPF were thoroughly reviewed. Demographic data (age, gender, smoking status), pulmonary function test results (forced expiratory volume in 1 s ($FEV_1$), forced vital capacity (FVC), diffusing capacity of the lung for carbon monoxide (DLCO)), as well as data on postoperative morbidity, mortality and adjuvant therapy

**TABLE 2.** Baseline clinical, radiological and pathological characteristics of the two patients who died in two months time after radical treatment of early-stage non-small cell lung cancer due to acute exacerbation of idiopathic pulmonary fibrosis (IPF)

| Characteristics | Patient No. 1 | Patient No. 2 |
|---|---|---|
| **Gender** | Male | Male |
| **Age, years** | 73 | 81 |
| **Smoking status** | Former-smoker | Former-smoker |
| **$FEV_1$/FVC before operation** | 72% | 70% |
| **$FEV_1$ before operation** | 2800 ml (93%) | 3110 ml (121%) |
| **FVC before operation** | 3900 ml (94%) | 4570 ml (122%) |
| **DLCO before operation** | 57% | 47% |
| **Histological subtype** | Squamous cell carcinoma | Squamous cell carcinoma |
| **Pathologic TNM stage ($8^{th}$ edition)** | IIIA | IA |
| **Lobe distribution** | Left lower lobe | Left lower lobe |
| **Type of operation** | Lobectomy | Lobectomy |
| **Adjuvant therapy** | Chemotherapy and radiotherapy | No |
| **Time from the end of radical treatment till death** | 54 days after finishing radiotherapy | 14 days after surgical resection |

DLCO = diffusing capacity of the lung for carbon monoxide; $FEV_1$ = forced expiratory volume in 1 s; FVC = forced vital capacity

(chemotherapy or RT) for lung cancer or further therapy for UIP/IPF were collected from our hospital data base. Most of the patients were followed-up in other institutions, therefore data on pulmonary function tests after radical treatment are missing and not presented in this paper.

The period of data collection was from May 2010 to April 2018.

## Results

Out of 641 patients with an early-stage NSCLC only 13 (2.0%) had histologically and radiologically proven coexisting UIP/IPF.

In this group of patients, 11/13 (84.6%) were men, 11/13 (84.6%) were current or former smokers. Mean age at time of diagnosis was 73.3 years. Pulmonary function test results before resection ($FEV_1$, FVC, DLCO) are described in Table 1.

Squamous cell carcinoma was diagnosed in 7/13 (53.8%) patients and adenocarcinoma in 5/13 (38.5%) patients. Pathological stage I was present in 4/13 (30.8%) patients, stage II in 7/13 (53.8%) patients and stage III in 2/13 (15.4%) patients. In 11/13 (84.6%) of patients the tumor was located in the lower lobes. Lobectomy was performed in 11/13 (84.6%) of patients, other 2/13 (15.4%) patients had bilobectomy. After radical surgery, 4/13 (30.8%) patients received adjuvant chemotherapy and 1/13 (7.7%) patient received adjuvant radiotherapy because of pN2 stage.

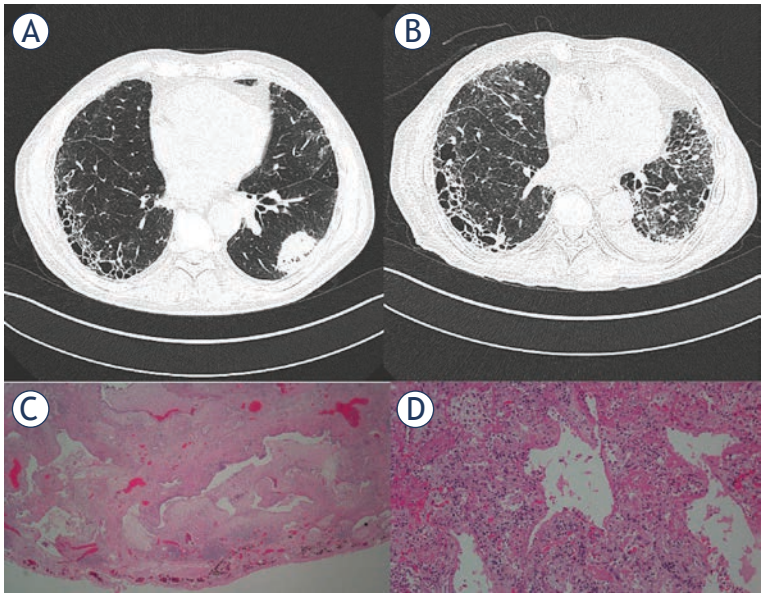


**FIGURE 1.** Radiological images and images of pathological specimens for Patient No.1. **(A)** CT scan before lung resection demonstrating honeycomb cystic changes in the subpleural regions of the lung characteristic for typical UIP pattern and tumor in left lower lobe. **(B)** CT scan after (radical treatment) lobectomy showing extensive bilateral ground glass opacities which are consistant with acute exacerbation of UIP and progressive reticular fibrotic changes. **(C)** Surgical specimen showing subpleural honeycombing with fibroblastic focus. **(D)** Autopsy specimen showing acute lung injury with hyaline membranes.

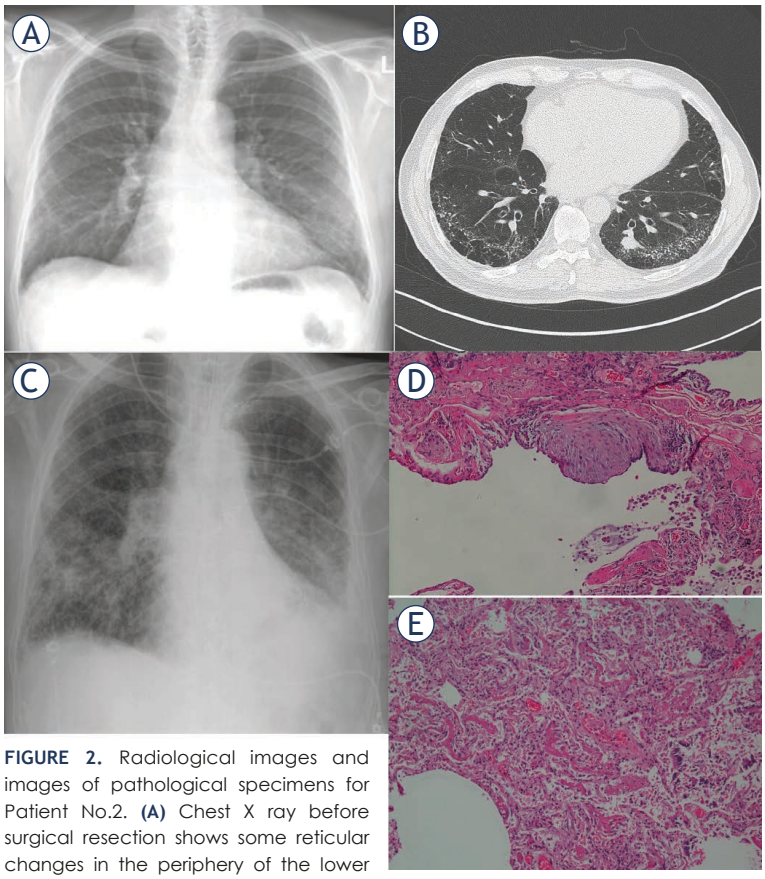


**FIGURE 2.** Radiological images and images of pathological specimens for Patient No.2. **(A)** Chest X ray before surgical resection shows some reticular changes in the periphery of the lower lobes. CT shows reticular fibrotic changes with multiple calcifications in the periphery of the lung in combination with paraseptal emphsema and small tumor in the left lower lobe. **(B)** Chest X ray after left sided lobectomy shows new bilateral alveolar opacities. **(C)** Surgical resection specimen demonstrating microcystic changes with fibroblastic focus. **(D)** Autopsy specimen showing acute lung injury with hyaline membranes

Baseline clinical, pathological and radiological characteristics of patients are shown in Table 1.

There were 2/13 pathologically confirmed fatal cases (15.4%) due to acute exacerbation of IPF (aeIPF). Characteristics of these two patients are presented in Table 2. Autopsy was performed in both cases to determine the cause of death. Patient No. 1 started to clinically deteriorate with respiratory insufficiency and dry cough four weeks after finishing postoperative adjuvant RT, and died 54 days after finishing it. In patient No. 2 acute respiratory deterioration developed on the second postoperative day, leading to death on day 14 after surgery. Both patients were treated with high dose corticosteroids and oxygen. Figure 1 and 2 are showing radiological images and images of pathological specimens for these two patients.

Another patient died in the first month after surgery, data on the cause of death is missing. Three patients (23.1%) had documented UIP/IPF progression during follow-up in the period of the data collection. They were all regularly seen by an interstitial lung diseases specialist and received pharmacologic therapy for UIP/IPF, one patient was treated with corticosteroid monotherapy and two were treated with pirfenidone.

Out of 13 patients only one (7.7%) had a lung cancer relapse during data collection period and died because of this.

## Discussion

Based on our one-center retrospective analysis, only 13/641 (2.0%) of patents with early NSCLC had concomitant IUP/IPF. To our knowledge, this is the lowest percentage to be reported till now, as earlier reports found incidence from 2.4–24.3%.[17-19] The reason is probably the use of more strict inclusion criteria in our study. We followed both histological and radiological criteria for UIP/IPF and not only histological analysis. Another reason could be that surgical resection was performed in fewer patients compared to other studies due to more strict criteria for surgery. Anyhow, looking closely only at the characteristics of the two patients that died because of aeUIP, our criteria for surgery were not strict. They were elderly and had quiet low DLCO. Due to a small number of patients it is difficult to make a general conclusion.

Tumors were mostly small, located in the lower lobes, with squamous histology being more frequent. We noticed male predominance and highly positive smoking status. All this is in accordance with earlier reports.[19,20]

Two patients (15.4%) had an aeIPF with fatal outcome after radical therapy, one after surgery and one after postoperative radiotherapy. It is known, that patients with IPF are at very high risk for adverse pulmonary events after lung resection surgery and that the greater the extent of lung resection, the higher incidence of not only aeIPF, but also pneumonia, prolonged air leakage, bronchopleural fistula, empyema in the postoperative period.[21,22] There are now reports that limited resections are acceptable if the resection can be achieved with an adequate margin.[23] New treatment options such as SBRT, which deliver highly conformal, high doses of radiotherapy to clinical target and spare the surrounding tissue, were tested as an alternative to surgery. Yet, also after SBRT there are cases of severe radiation pneumonitis published in the literature.[20] Regarding which is the standard of

care in these mostly elderly patients still remains unresolved. There is a great need to conduct a randomized controlled trial to compare survival and quality of life after surgical treatment, either radical or limited resection, versus nonsurgical treatment and to determine predictive factors that would help to guide the decision. Clinical registries could also prospectively collect data on this group of patients, helping to gain more real-life data.

Until we get more of this hard data on which oncological-surgical treatment is most appropriate, it is worthwhile to discuss such clinical cases in an individual manner at a multidisciplinary tumor board, with an interstitial lung disease specialist being part of it. At the same time, these patients should be followed by interstitial lung diseases specialist after completing radical treatment for lung cancer, receiving the appropriate therapy for IPF, if needed. Intensive surveillance is a must in this group of patients.

Our retrospective observational study has many limitations. Our major limitation is a retrospective design of the study. This limited the amount of data available for analysis. As stated before, there are missing data on lung function tests after radical treatment, which would enrich our analysis. Another limitation is that our patients had only performed a CT scan preoperatively, not a high-resolution CT, which is an essential component of the diagnostic pathway in IPF.[24]

## Conclusions

The decision on optimal treatment of patients with early-stage NSCLC with concurrent IPF should be made in a multidisciplinary team with close cooperation with specialist for interstitial lung diseases. Not depending on the type of radical treatment, patients with IPF should be then regularly followed by specialist for interstitial lung diseases.

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*research article*

# Dosimetric study for spine stereotactic body radiation therapy: magnetic resonance guided linear accelerator versus volumetric modulated arc therapy

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**Background.** Stereotactic body radiation therapy (SBRT) given in 1-5 fractions is an effective treatment for vertebral metastases. Real-time magnetic resonance-guided radiotherapy (MRgRT) improves soft tissue contrast, which translates into accurate delivery of spine SBRT. Here we report on clinical implementation of MRgRT for spine SBRT, the quality of MRgRT plans compared to TrueBeam based volumetric modulated arc therapy (VMAT) plans in the treatment of spine metastases and benefits of MRgRT MR scan.

**Patients and methods.** Ten metastatic lesions were included in this study for plan comparison. Lesions were spread across thoracic spine and lumbosacral spine. Three fraction spine SBRT plans: 27Gy to planning target volume (PTV) and 30Gy to gross tumor volume (GTV) were generated on the ViewRay MRIdian Linac system and compared to TrueBeam™ STx based VMAT plans. Plans were compared using metrics such as minimum dose, maximum dose, mean dose, ratio of the dose to 50% of the volume (R50), conformity index, homogeneity index and dose to the spinal cord.

**Results.** MRIdian plans achieved equivalent target coverage and spinal cord dose compared to VMAT plans. The maximum and minimum PTV doses and homogeneity index were equivalent for both planning systems. R50 was lower for MRIdian plans compared to VMAT plans, indicating a lower spread of intermediate doses with MRIdian system (5.16 *vs.* 6.11, p = 0.03).

**Conclusions.** MRgRT can deliver high-quality spine SBRT plans comparable to TrueBeam volumetric modulated arc therapy (VMAT) plans.

Key words: MR guided radiotherapy; spine radiotherapy; SBRT; treatment planning; VMAT

## Introduction

Stereotactic body radiation therapy (SBRT) given in 1–5 fractions is an effective treatment for spinal metastases.[1] Spine SBRT involves tight planning margins and steep dose gradients to the surrounding organs at risk (OAR). Spinal cord, which is a serial organ, is the most important dose-limiting structure in spine SBRT planning. The risk of radiation myelopathy can be kept to ≤ 1% with meticulous radiotherapy planning and delivery.[2] Multiple studies have demonstrated the safety and feasibility of using stereotactic radiotherapy for spinal metastases.[3,4] In many published de novo and adjuvant studies, spine SBRT has led to one year local control rates of 80–90%.[5,6] Given its safety and

efficacy, use of spine SBRT in the United States has increased from 2% to 20% over the last decade.[7]

Spine SBRT is often delivered with dynamic arc, static intensity modulated radiotherapy (IMRT) and volumetric modulated arc therapy (VMAT) on linear accelerator (Linac), non-isocentric robotic delivery (CyberKnife) and Tomotherapy using computed tomography (CT)-based platforms.[8,9] At the time of planning, spinal cord volume is usually defined on diagnostic magnetic resonance (MR) images fused with planning kilovoltage computed tomography (kVCT). This approach can result in fusion errors on the order of 2 mm.[10] Despite near-rigid full body immobilization and on-board CT-based imaging, inter-fraction and intra-fraction motion of the spinal cord necessitates a planning risk volume (PRV) margin of 1–2 mm to ensure safe treatment delivery.[11]

In a study that evaluated the effect of setup errors on dose distribution for spine SBRT[12], investigators used cone beam computed tomography (CBCT) scans to assess the actual dose to the spinal cord PRV generated by expanding spinal cord by 2 mm. The difference in minimum dose to the upper 10% of the PRV (*i.e.*, D10) was 0.03 ± 0.3 Gy (maximum, 0.9 Gy). Thus, although setup errors using CT-based image-guidance are often small and could result in non-significant change to the planned OAR dose, they could very easily become clinically significant given the steep dose gradient associated with these highly specialized treatments, especially if the error was found to be in the direction of the spinal cord. Compared to CT-guided radiotherapy, magnetic resonance (MRI)-guided platform improves soft tissue contrast, which can translate into accurate contouring of target and/or OARs.[12]

Magnetic resonance-guided radiotherapy (MRgRT) delivery systems have now entered clinical practice at several major treatment centers. One such system is the ViewRay MRIdian Linac™ (ViewRay, Inc., Oakwood Village, Ohio, USA), which combines a 0.345T field strength split-bore magnet MRI with a 28 cm gap that contains 6 MV flattening filter free linear accelerator (Linac).[13] The imaging field of view is 50 cm wide with 70 cm diameter of bore body coil, with a capability to acquire scans as fast as 17 sec or 25 sec using the true FISP (TRUFI) imaging sequence. The TRUFI imaging sequence on the ViewRay MRIdian Linac™ platform enables real-time visualization of the spinal cord and surrounding cerebrospinal fluid, thereby making MRIdian an optimal modality for image guided radiotherapy.[14] This novel Linac system is equipped with a slightly de-focused double-stack multi-leaf collimator (MLC). This system is designed so that the beams have sharp penumbra with minimal leakage through the leaves. MLCs are designed to project field sizes from 0.2 x 0.4 $cm^2$ up to 27.4 x 24.1 $cm^2$. The MRIdian system uses step-and-shoot intensity modulated radiation therapy (IMRT) technique to deliver dose that is calculated with a Monte Carlo algorithm.

Previous studies have compared dosimetric data for normal tissues and target for different treatment planning stations and delivery techniques.[9,15] In this study, we report on the quality of ViewRay MRIdian Linac treatment plans compared to TrueBeam™ STx (Varian Medical Systems, Palo Alto, CA) volumetric modulated arc therapy (VMAT) plans and clinical implementation of MRgRT for spine SBRT along with benefits of using MRgRT for spine SBRT.

## Patients and methods

Patients previously treated with vertebral body metastases between 2015 and 2018 were included in this retrospective study. This study was approved by the local institutional review board. For simulation, patients were immobilized in a BodyFIX bluebag (Elekta, Stockholm, Sweden) with vacuum wrap.[16] They were scanned in supine position with arms elevated above the head. All scans were acquired on a Siemens SOMATOM Definition Edge scanner, with a slice thickness of 1 to 2 mm.

Planning CT and diagnostic MR scans were exported to MIM Maestro (MIM Software, Cleveland, OH, USA) for segmentation of target and OARs. Rigid registration was performed between CT simulation scan and the diagnostic MRI scan (MRI Dx) using the MIM optimization algorithm. Segmentation was done by radiation oncologists with expertise in spine SBRT. Radiographically visible tumor was contoured as gross tumor volume (GTV). Clinical target volume (CTV) was contoured using the international consensus guidelines.[17] A geometric margin of 3 mm excluding the spinal cord was used to generate planning target volume (PTV), and a 2 mm margin was used for spinal cord planning risk volume (PRV). Similar principles were used to contour cauda equina.

Common practice for spine SBRT at our institution involves a prescription dose of 27 Gy to PTV in 3 fractions with a simultaneous integrated boost (SIB) of 30 Gy to GTV, which is based on published prospective studies.[3,5] For dosimetric comparison,

**TABLE 1.** Dose volume parameters for PTV, GTV, Spinal Cord and Cauda Equina for MRIdian Linac IMRT plans Vs TrueBeam™ STx VMAT plans

| Structures | TrueBeam™ STx VMAT | MRIdian Linac IMRT | p-value |
|---|---|---|---|
| **PTV** | **Median(range)** | **Median(range)** | |
| D98% (Gy) | 25.7 (15.6–29.5) | 26.5 (17.7–29.7) | 0.20 |
| D50% (Gy) | 29.0 (27.9–39.1) | 30.0 (26.4–33.2) | 0.77 |
| Conformity Index | 0.97 (0.93–1.0) | 0.97 (0.90–1.0) | 0.13 |
| Dose Homogeneity Index | 0.22 (0.05–0.6) | 0.19 (0.1–0.57) | 0.49 |
| R50 | 6.1 (2.9–16.7) | 5.2 (2.8–11.9) | 0.05 |
| **GTV** | | | |
| D50% (Gy) | 31.6 (30.4–34.2) | 31.7 (31.0–34.6) | 0.36 |
| D98% (Gy) | 30.12 (25–33.63) | 30.35 (29.17–32.89) | 0.23 |
| Conformity Index | 0.99 (0.95–1.0) | 0.99 (0.96–1.0) | 0.58 |
| Dose Homogeneity Index | 0.09 (0.04–0.33) | 0.08 (0.01–0.18) | 0.30 |
| R50 | 32 (6.08–69.0) | 29 (5.8–66.0) | 0.01 |
| **Spinal Cord** | | | |
| Max dose (Gy) | 12.6 (9.1–15.6) | 13.3 (10.35–17.3) | 0.13 |
| D0.03 (Gy) | 12 (9.0–15.3) | 12.8 (9.5–16.5) | 0.25 |
| **Cauda Equina** | | | |
| Max dose (Gy) | 16 (0.22–28.0) | 18 (0.3–32) | 0.38 |
| D0.03 (Gy) | 13 (0.2–20.0) | 16 (0.25–31) | 0.07 |

the same set of contours was used to generate both MRIdian and VMAT plans. Treatment planning objectives were: at least 95% of the target volume receives the prescribed dose; hotspots were limited to 110% within 1 cm of the target volume and 105% outside. For spinal cord and spinal cord PVR maximum dose was constrained to 18 Gy and 20 Gy respectively. Mean dose to kidneys was restricted to less than or equal to 10 Gy. For lungs, volume of lungs receiving 5 Gy, 12.5 Gy, 20 Gy and 12 Gy was restricted to 50%, 15%, 10% and 1000 cc respectively.

CT scans and contours were exported to Pinnacle treatment planning system (TPS) to generate VMAT plans for TrueBeam ™ STx. VMAT plans were generated using three co-planar 6 MV arcs with gantry angles varying from 178° to 182° (CCW), 183° to 178° (CW), 178° to 182° (CCW) and collimator set at 330°, 25° and 320°. TrueBeam™ STx is equipped with a six degree of freedom (DOF) couch, which allows for more variable beam arrangement. Final dose distribution was calculated with the anisotropic analytic algorithm with dose grid of 2 mm.

For the MRIdian plans, CT images and contours that were used for VMAT plans were imported into MRIdian TPS. On average, 10 to 15 beams spaced 20° to 28° apart (110° to 221°) were used to generate a step-and-shoot IMRT plan. Beams entering through the corners of the couch were removed to avoid dosimetric uncertainty. The isocenter was placed in the PTV. Final dose calculation was done using grid size of 2 mm with Monte Carlo. Final dose distribution from MRIdian and Pinnacle were exported to MIM to tabulate and compare clinically relevant DVH parameters.

For dosimetric analysis, VMAT and MRIdian Linac plans were compared using plan metrics such as near minimum dose (D98%-Dose to 98% of PTV), near maximum dose (D2%), median dose (D50%), conformity index (CI) and dose homogeneity index (HI) for PTV. CI and HI were calculated as shown below[18]:

$$CI = \frac{\text{Volume of the prescription isodose (27 Gy)}}{\text{Volume of PTV}}$$

$$DHI = \frac{D2\% - D98\%}{D50\%}$$

To evaluate the impact of intermediate dose on the normal tissue R50, the ratio of volume inside 50% isodose line to the PTV volume was calculated.

Dosimetric data for cord was compared between the two plans. Wilcoxon matched pairs signed-rank test, a non-parametric equivalent of paired t-test, was used to compare dosimetric parameters between VMAT and MRIdian Linac plans. For all statistical analysis SPPS version 25 was used.

## Results

Ten metastatic lesions from nine patients were included in this study for plan comparison. Lesions were spread across thoracic spine (T6-T12) and also lumbosacral spine (L2-S1). Dose-volume histogram (DVH) parameters for both plans are shown in Table 1. Detailed dosimetric results for all cases are summarized in Table 2. All plans were able to meet the planning parameters. R50 was lower for MRIdian Linac plans when compared to VMAT plans, indicating a lower spread of intermediate doses with MRIdian (5.16 *vs.* 6.11, p = 0.056) for PTV. Average D98% (Near Minimum), D2% (Near Maximum) and D50% (Median dose) were similar between the two plans. HI and CI were also similar between VMAT and MRIdian Linac plans. The percentage difference for D98%, D2%, and D50% were

**TABLE 2.** Detailed Dose volume histogram parameters for PTV, GTV, Spinal Cord and Cauda Equina for MRIdian Linac IMRT plans Vs TrueBeam™ STx VMAT plans

| Case # | PTV | | | | | GTV | | | | | Spinal Cord | | | | Cauda Equina | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Volume (cc) | Maximum Dose (Gy) | | D95 (Gy) | | Volume (cc) | Maximum Dose (Gy) | | D95 (Gy) | | Maximum Dose (Gy) | | D0.03 cc (Gy) | | Maximum Dose (Gy) | | D0.03 cc (Gy) | |
| | | MRIdian Linac IMRT | True Beam VMAT | MRIdian Linac IMRT | True Beam VMAT | | MRIdian Linac IMRT | True Beam VMAT | MRIdian Linac IMRT | True Beam VMAT | MRIdian Linac IMRT | True Beam VMAT | MRIdian Linac IMRT | True Beam VMAT | MRIdian Linac IMRT | True Beam VMAT | MRIdian Linac IMRT | True Beam VMAT |
| 1 | 60.37 | 34.69 | 35.44 | 27.43 | 27.50 | 7.02 | 34.69 | 35.44 | 32.46 | 33.76 | 14.82 | 14.77 | 14.67 | 14.47 | 19.70 | 21.72 | 19.67 | 20.19 |
| 2 | 50.63 | 32.45 | 32.27 | 27.00 | 27.31 | 6.82 | 32.94 | 32.41 | 30.11 | 30.05 | 13.33 | 13.45 | 13.02 | 13.10 | 20.00 | 21.15 | 19.92 | 18.95 |
| 3 | 66.3 | 36.41 | 37.00 | 27.75 | 27.12 | 27.67 | 36.41 | 37.00 | 32.58 | 29.61 | 17.01 | 11.69 | 16.56 | 10.90 | 21.07 | 14.12 | 20.23 | 13.58 |
| 4 | 47.63 | 31.68 | 30.85 | 27.19 | 27.94 | 18.8 | 34.02 | 33.45 | 30.06 | 30.52 | 13.25 | 15.65 | 12.62 | 15.34 | 24.08 | 21.19 | 22.48 | 19.79 |
| 5 | 2.6 | 33.87 | 30.71 | 27.73 | 27.26 | 0.9 | 33.87 | 32.02 | 30.24 | 30.21 | 12.58 | 13.81 | 11.92 | 12.90 | 9.31 | 12.37 | 7.73 | 7.56 |
| 6 | 3.93 | 33.52 | 31.40 | 27.67 | 27.89 | 1.65 | 33.52 | 34.96 | 32.17 | 27.39 | 10.35 | 9.10 | 10.32 | 9.09 | 16.44 | 18.01 | 12.58 | 12.32 |
| 7 | 3.05 | 32.64 | 30.88 | 27.50 | 27.82 | 1.07 | 32.65 | 33.70 | 30.44 | 29.02 | 11.17 | 10.10 | 11.15 | 10.09 | 32.13 | 28.01 | 31.16 | 19.32 |
| 8 | 25.43 | 32.73 | 34.07 | 27.92 | 27.99 | 2.31 | 32.73 | 34.07 | 30.31 | 31.43 | 13.72 | 10.98 | 13.22 | 10.36 | 13.72 | 10.6 | 13.19 | 10.25 |
| 9 | 29.02 | 33.95 | 34.14 | 27.50 | 27.60 | 1.89 | 35.70 | 34.14 | 30.05 | 30.52 | 11.04 | 9.25 | 9.53 | 8.96 | 0.32 | 0.22 | 0.25 | 0.21 |
| 10 | 38.29 | 33.02 | 33.95 | 27.88 | 27.02 | 2.51 | 33.02 | 33.95 | 30.45 | 30.51 | 17.30 | 14.11 | 14.51 | 13.75 | 3.54 | 3.67 | 3.20 | 3.40 |

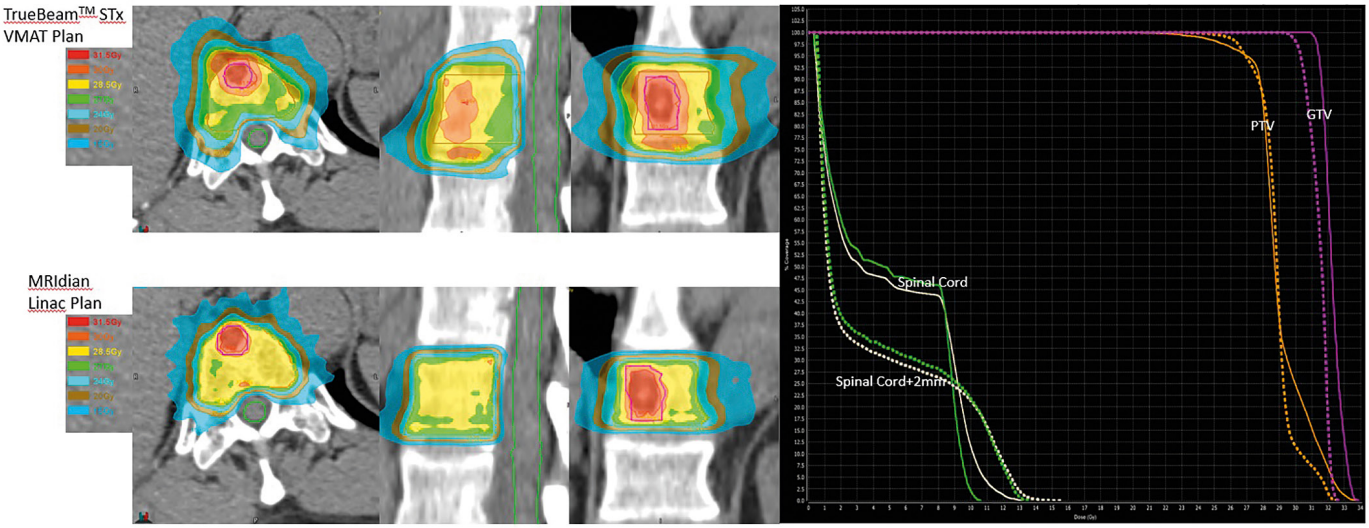


**FIGURE 1.** Isodose distribution (right) for TrueBeam™ STx VMAT and MRIdian Linac Plans: 30 Gy to gross tumor volume (GTV) and 27 Gy to planning target volume (PTV) in 3 fractions. On left, solid and dashed lines represents dose volume histogram for TrueBeam™ STx VMAT and MRIdian Linac Plans for GTV, PTV, Spinal Cord and Spinal Cord+2mm.

3%, 4.5% and 3.3%, respectively, between both the plans for PTV and D50% for GTV was within 0.3% and spinal cord maximum dose was within 5.5% for both the plans. Dose to other OARs were within acceptable limits for plans and there no significant difference between the plans.

The average beam on time for the VMAT plans was 7 minutes compared to 12 minutes for the MRIdian Linac plans. Dose to the spinal cord and Cauda equina was also calculated and shown to be comparable between MRIdian Linac and VMAT plans (Table 1). Isodose distribution and DVH for MRIdian Linac *vs.* VMAT are shown in Figure 1. MRIdian Linac setup image quality was superior to isolate the target and spinal cord for each fraction compared to kVCT and megavoltage computed tomography (MVCT) (Figure 3, 4) thus minimizing setup errors.

## Discussion

Increased global incidence of cancer in combination with improved systemic therapies has led to an increase in the prevalence of oligometastatic disease involving bone.[19] Synchronous or me-

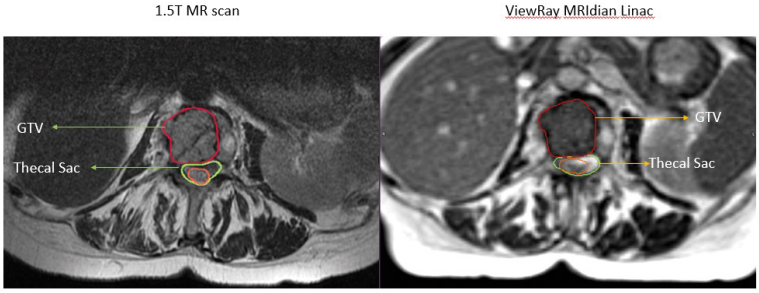


**FIGURE 2.** A metastatic tumor in the vertebral body of lumbar spine and adjacent spinal cord scan acquired on 1.5T diagnostic magnetic resonance (MR) and 0.345T ViewRay MRIdian Linac system. MRIdian scan allows for accurate delineation of the tumor and the spinal cord without having to rely on the fused diagnostic MR scan.

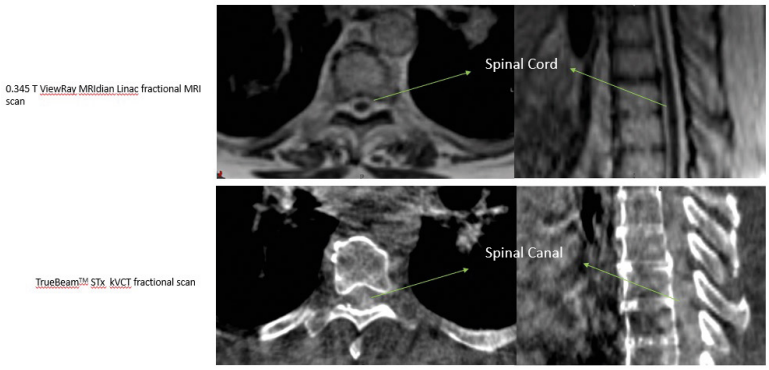


**FIGURE 3.** Fractional 0.345T MRIdian Linac MR scan (Top) and Kilo Voltage Cone Beam (Bottom) used for daily setup verification before radiotherapy treatment. Visibility of spinal cord on 0.345T is demonstrated increasing the accuracy on treatment delivery.

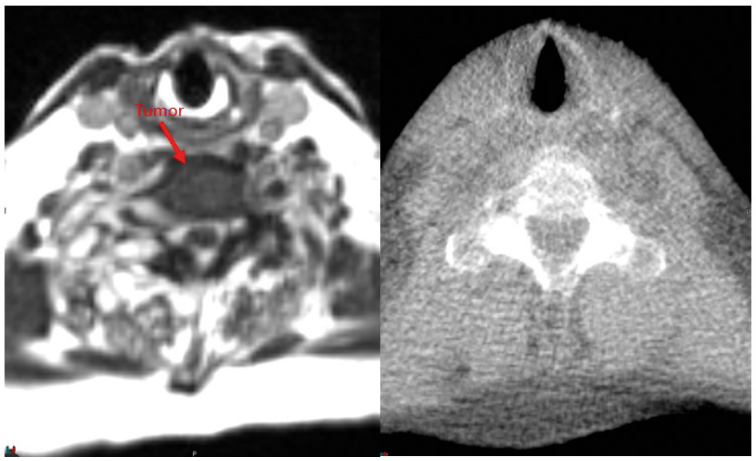


**FIGURE 4.** Fractional 0.345T MRIdian Linac MR scan (Left) and TomoTherapy® Mega Voltage CT (Right) used for daily setup verification before radiotherapy treatment. Visibility of target (red) on 0.345T is demonstrated.

tachronous bony vertebral/spinal metastases are diagnosed in approximately 40–70% of patients with cancer, mainly secondary to breast, lung, or prostate adenocarcinoma.[20,21] Aggressive treatment of isolated metastases in select patients may lead to improved outcomes.[22,23]

Multiple studies have compared treatment planning quality among dynamic conformal arcs, static IMRT, VMAT and tomotherapy for spine.[24-26] Matuszak *et al.* study concludes that VMAT improved the isodose conformality and reduced the treatment time by 37% compared to IMRT.[27] In this study, we have shown that MRIdian Linac plans are comparable to TrueBeam™ STx VMAT plans with respect to target metrics and spinal cord dosimetry. MRIdian Linac plans generated with manual beam angle selection helped to limit the beam angles entering through the critical structures. Beam-on times were higher for MRIdian Linac compared to TrueBeam™ STx given the lack of dynamic treatment delivery with ViewRay MRIdian Linac system. Dosimetric results of this study have helped us to clinically start treating spine SBRT on the MRIdian Linac system. Although MRIdian Linac plans resulted in comparable dose to spine yet clinical relevance of these dosimetric differences is unknown.

In our experience, the ability to accurately visualize the spinal cord is a significant advantage with the ViewRay MRgRT for several reasons. MRI-guided radiotherapy allows physician contouring to rely on the optimally visualized spinal cord on MR simulation images and not on CT-MRI fusion with accompanying errors associated with registration.[10] Figure 2 shows a representative image of a T1 weighted image obtained on a 1.5T diagnostic MRI compared to TRUFI sequence on 0.345T ViewRay MRIdian system demonstrating excellent tumor demarcation on both images sets. At several clinics, including our institution spine SBRT treatments are delivered not only on TrueBeam but also Tomotherapy. Figures 3 and 4 show examples of 0.345T MRI images compared to kV CBCT from TrueBeam™ STx and to MVCT acquired on TomoTherapy®. As can be seen, MRI provides images with superior soft tissue contrast, allowing better visualization of the spinal cord and spinal canal on the volumetric image acquired for setup verification. This enables accurate patient setup for treatment delivery and could potentially minimize set up errors.

Spine SBRT plans usually have a steep dose gradient at the spinal cord/PTV interface. In a systematic review by Chang *et al.*, crude risk of local failure at 1-year was 21.4%. Around 67% of these failures occurred within the epidural space.[28] Sahgal *et al.* have reported that the majority of their local failures occurred at the spinal cord PRV-PTV

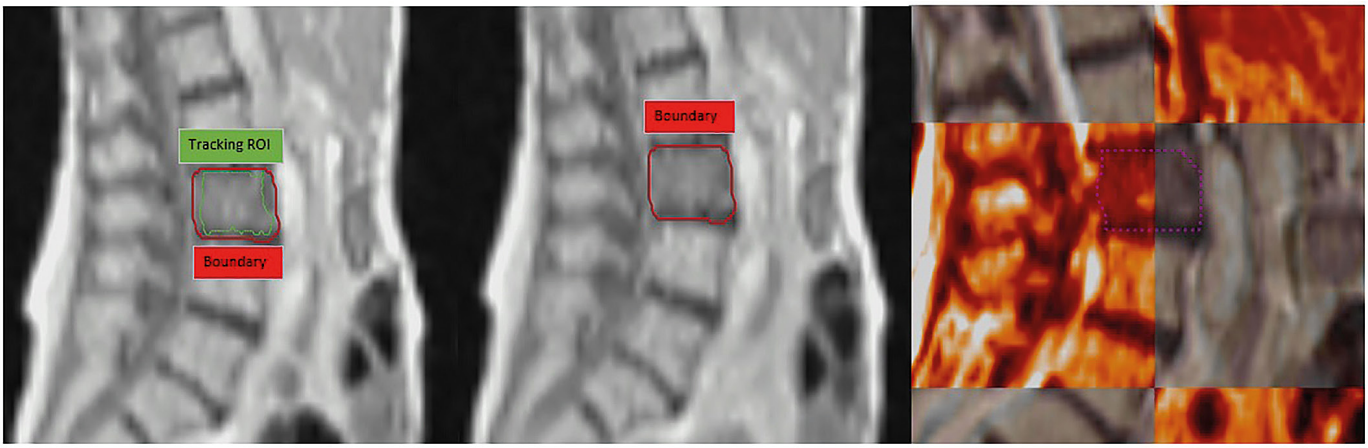


**FIGURE 5.** 0.345T MRIdian Linac cine acquired in sagittal plane. Any changes in reference setup results in shutting the radiation beam off as tracking structure (green) moves outside the boundary (red).

interface. Their data suggest that as the cord PRV to PTV distance decreases, the risk of local failure increases.[29] This is another area where MRIdian treatment delivery system could have advantages. Spinal cord PRV margin comprises patient set up uncertainty, organ motion, intrafraction patient motion and, contouring uncertainty. MRIdian can help minimize the margin required for contouring uncertainty, organ motion and intrafraction motion, allowing us to treat without having to add a separate PRV or a minimal PRV margin to the spinal cord. This thereby increases the distance between PTV and dose-limiting OAR and limits underdosing of epidural component of the PTV, where significant local failures tend to occur. By using "tracking region of interest (ROI)", accurate online tracking of the target can be performed (Figure 5). This in turn has significant impact on dose deposition, given the close proximity of this tracking ROI to spinal cord, where a steep dose gradient exists. Additionally, the online adaptive workflow is a great advantage of the MRIdian system that can be utilized for challenging patients with minimal separation between the tumor and spinal cord.[30]

MRI-guided therapy with MRIdian does have a few limitations. The MRIdian couch does not allow for six degrees of freedom or non-coplanar beam angles. It also does not permit modification of collimator angle or allow dynamic treatment delivery. Beam entry from couch edges for treatment plans are restricted due to high couch attenuation. Also, the number of beams used for planning are restricted to keep the total treatment time reasonable. Another limitation of the system is the lowest monitor units (MU) that can be delivered with the MRIdian Linac system is 1. Additionally, MRgRT is contraindicated in the post-operative setting if patients have magnetic resonance imaging (MRI)-incompatible metal implants. In many cases, however, the benefits of substantially improving soft tissue contrast, ability of real-time tracking and online adaptation may outweigh any planning and delivery difficulties encountered with MRgRT.

## Conclusions

Here, we have shown that 3-fraction spine SBRT plans are dosimetrically comparable between MRIdian and TrueBeam™ STx VMAT plans and MRgRT can be successfully used for SBRT spine with reasonable delivery time.

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# Comment on "State of the art in magnetic resonance imaging of hepatocellular carcinoma": the role of DWI

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We read with interest the article from Dr Horvat and colleagues in Radiology and Oncology in which they assessed the State of the art in magnetic resonance imaging (MRI) of hepatocellular carcinoma (HCC).[1] The main strength is due to the good update on the role of this technique for HCC. We congratulate the authors on their accuracy in data presentation. Nevertheless, we would like to point out several features that the Radiology and Oncology readers should know about the Diffusion Weighted Imaging (DWI) and the possibility to use quantitative parameters extracted by DWI to characterize HCC, data not assessed by authors.[1] DWI has been applied to liver imaging as an excellent tool for detection and characterization of focal liver lesions. The assessment of DW images can be done qualitatively and quantitatively, through the apparent diffusion coefficient (ADC) map. Le Bihan *et al*. as a first described the intravoxel incoherent motion (IVIM). IVIM data can be assessed qualitatively and quantitatively. IVIM data enable improved detection and characterization of HCC.[2] Also, traditionally DWI approach to analyze data is founded on the hypothesis that water molecules diffuse within a voxel following a single direction with a Gaussian behavior without any restriction. However, water molecules within biologic tissues exhibits a non-Gaussian phenomena proposed by Jensen in 2005 called Diffusion Kurtosis Imaging (DKI).[3] DKI is more accurate than traditional ADC in tumor assessment.[3] The role of DWI and functional parameters extracted by DWI in HCC patient has been evaluated by different studies, showed that the DWI could be used as a helpful diagnostic tool for HCC in patients with chronic liver disease, since DWI can accurately detect HCC in patients with chronic liver disease regardless of the lesion size. The major limits of DWI are the different parameters used in DWI sequences, which may affect the results of ADC calculation. Several researches have evaluated the relationship between functional data by DWI and histological grade of HCC. Granata *et al*.[4] found that DWI could be used to predict the histological grade of HCC, showing a good correlation between ADC and grading, between perfusion fraction (fp) and grading, and between tissue pure diffusivity (Dt) and grading. The mayor limit of DWI and IVIM parameters to discriminate the histological grade of HCC is depending on the fitting methods used to obtained functional parameters.

Goshima *et al* compared DKI with conventional DWI for assessing the response to treatment in hypervascular HCC.[5] The sensitivity, specificity, and AUC of the ROC curve for the assessment of HCC viability were greater using MK than using ADC.[5]

In conclusion, DWI should be an integral part of study protocol for HCC patients, considering the

great advantages due to DWI and DWI-based approaches in detection and characterization of HCC.

## Acknowledgement

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# Reply to comments on "State of the art in magnetic resonance imaging of hepatocellular carcinoma": the role of DWI

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## Author's reply

We were very pleased to read the comments from Dr Granata and colleagues in Radiology and Oncology about our article "State of the art in magnetic resonance imaging of hepatocellular carcinoma".[1] As highlighted, the diffusion weighted imaging (DWI) plays a key role as a qualitative and quantitative method in the detection of hepatocellular carcinoma (HCC), mostly for small (< 20 mm) or well-differentiated HCC with atypical post-contrast imaging patterns[2], helping differentiate benign from malignant focal hepatic lesions, and also allowing the evaluation of treatment response to systemic and locoregional therapies in hepatic malignancies.

DWI is an MRI sequence which provides useful information especially in the absence of intravenous contrast media.[3] In this context, the intravoxel incoherent motion (IVIM) model is based on the fact that perfusion exists inherently in DWI voxels and influences the measurement of the apparent diffusion coefficient (ADC), allowing qualitative and quantitative assessment. It can be used in the differentiation between benign and malignant hepatic nodule, such as focal nodular hyperplasia and HCC.[4] Furthermore, a few recent studies showed that there is a correlation between the histopathological grade and prognosis of HCC and DWI, demonstrating that significantly lower ADC values in poorly differentiated tumors with a shorter recurrence-free survival and a cut-off value of 1.175 x $10^{-3}$ $mm^2/s$ to predict microvascular invasion.[5,6]

Regarding Diffusion kurtosis imaging (DKI), we also recognize its current role in the evaluation of non-Gaussian water diffusion, providing better information in heterogeneous tissues such as large HCCs, even in the post-treatment studies. Wang *et al*. suggested that a mean Kurtosis value cut-off of 0.917 has a good sensitivity, specificity and diagnostic accuracy in the prediction of microvascular invasion, as the ADC value commented above.[7] However, to our knowledge there is not enough evidence to correlate the quantitative parameters obtained with DWI and IVIM with HCC physiopathology or pathologic subtypes. This is possibly due to the discrepancy in the current spatial resolution of DWI/IVIM and pathologic findings, the currently described correlations are significant but underline a gap of knowledge between what is observed at MRI and what is observed at pathology. The hope is that IVIM may be able to describe subcategories of HCCs of among the pathologic descriptors allowing for better treatment tailoring and prognostic assessment.

Finally, it is important to emphasize that all the information obtained from DWI is complementary and does not replace the use of intravenous contrast agents, Hectors *et al*. proved that these

sequences offer non-redundant data on flow and perfusion inside the tumor, probably due to the change in the microvascular supply, although they presented moderate to strong correlation in the surrounding liver.[8]

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doi: 10.2478/raon-2019-0033

# KRAS, NRAS, BRAF, HER2 in mikrosatelitska nestabilnost pri metastatskem kolorektalnem raku. Klinični pomen

Afrăsânie VA, Marinca MV, Alexa-Stratulat T, Gafton B, Păduraru M, Adavidoaiei AM, Miron L, Rusu C

**Izhodišča.** Kolorektalni rak lahko v onkologiji uspešno izkoristimo kot model razvoja genetskih bioloških označevalcev. Poznamo več prediktivnih in napovednih genetskih sprememb, ki jih uporabljamo v klinični praksi. Genska družina RAS, ki vključuje KRAS in NRAS, služi kot napovedovalec uspešnosti zdravljenja usmerjenega proti receptorju za epitelijski rastni faktor (EGFR). Tudi mutacije NRAS imajo lahko napovedni pomen: bolniki z alteracijami NRAS imajo pomembno krajše preživetje kot bolniki z divjim tipom tumorja. Mutacije BRAF V600E so redke in se pojavljajo predvsem v tumorjih ascendentnega kolona pri starejših bolnicah. BRAF je ključnega pomena pri napovedovanju poteka bolezni: bolniki z mutacijo BRAF imajo za 10–16 mesecev krajše preživetje in BRAF je morda tudi negativni napovedni kazalec pri bolnikih, ki so kandidati za jetrno ali pljučno metastazektomijo. Poleg tega to mutacijo uporabljamo kot negativni prediktivni dejavnik za zdravljenja, ki so usmerjena proti EGFR. V zadnjem času smo pri kolorektalnem raku prepoznali dva nova tumorska označevalca: HER2 in mikrosatelitska nestabilnost. Medtem ko se napovedna vloga HER2 še vedno preučuje v različnih prospektivnih raziskavah, pa mikrosatelitska nestabilnost že usmerja klinične odločitve pri lokalno napredovalem kolorektalnem raku.

**Zaključki.** Izsledki so pokazali, da zgoraj omenjeni genetski tumorski označevalci omogočajo izbiro pravega zdravila, ki ga uporabljamo pri pravem bolniku. Tak pristop prispeva k bolj individualnemu, k bolniku usmerjenemu zdravljenju v dnevni klinični praksi.

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# Heterotopne osifikacije. Radiološki in patološki vidiki

Mujtaba B, Taher A, Madewell JE, Aslam R, Hanafy AK, Fiala MJ

**Izhodišča.** Heterotopna osifikacija je pogosto stanje povezano s tvorbo ektopične kostnine v mehkih tkivih. Etiološko gledano je lahko pridobljena (pogosteje) ali prirojena. Pridobljena oblika je neredko povezana s poškodbami. Patogeneza te bolezni še vedno ni dokončno razjasnjena, trenutno pa potekajo številne obetajoče raziskave na temo preprečevanja in zdravljenja tega stanja.

**Zaključki.** Ker lahko heterotopna osifikacija vodi v prizadetost bolnikov, je pomembna prepoznava te bolezni z namenom nudenja optimalnega zdravljenja.

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# Večgenski podpisi pri zgodnjem, hormonsko odvisnem in Her2 negativnem raku dojke

Ovčariček T, Takač I, Matos E

**Izhodišča.** Standardno zdravljenje hormonsko odvisnega in HER2 negativnega zgodnjega raka dojke je operacija, ki ji sledi dopolnilno sistemsko zdravljenje, to je zdravljenje samo s hormonsko terapijo ali zdravljenje s kemoterapijo in nato hormonsko terapijo. Dopolnilno sistemsko zdravljenje zmanjša tveganje za ponovitev bolezni ali smrt zaradi raka dojke. Ali bo posamezna bolnica imela dobrobit od dopolnilne kemoterapije, je pomembno klinično vprašanje. Odločitev, ki sloni zgolj na klinično-patoloških značilnostih bolezni, pogosto vodi v prekomerno zdravljenje. Večgenski podpisi predstavljajo pomemben napredek pri izboru bolnic z večjim tveganjem za ponovitev bolezni, ki najverjetneje imajo korist od dodane kemoterapije k hormonski terapiji.

**Zaključki.** Komercialno je na voljo več podpisov, nekateri so že vključeno v mednarodna priporočila. Najobsežneje so proučevali *Oncotype DX in MammaPrint*, podprta sta z dokazi stopnje IA.

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# Razmerje med nivojem spolnih hormonov in absorpcijo $^{18}$F-FDG v jajčnikih pri ženskah pred menopavzo

Kim TH, Kim MR, Jung Y, An YS

**Izhodišča.** Namen raziskave je bil oceniti učinek spolnih hormonov na absorpcijo F-18 fluorodeoksiglukoze ($^{18}$F-FDG) normalnih jajčnikov.

**Bolniki in metode.** V študijo je bilo vključenih 197 žensk pred menopavzo pri katerih smo s pomočjo $^{18}$F-FDG pozitronske emisijske tomografije/slikane s kompjutersko tomografijo (PET/CT), ugotavljali povezavo med privzema $^{18}$F-FDG v jajčnikih in nivojem spolnih hormonov, estradiolom, progesteronom, testosteronom, folikle stimulirajočim hormonom in luteinizirajočim hormonom. Analizirali smo povezavo med fazo menstruacijskega cikla in privzemom $^{18}$F-FDG v jajčnikih.

**Rezultati.** Najvišji privzem $^{18}$F-FDG v jajčnikih je bil 2 tedna po nastopu menstruacije (mediana največje standardizirane vrednosti privzema [SUVmax] = 3,40, srednja vrednost SUV [SUV] = 2,20), najnižji privzem $^{18}$F-FDG v jajčnikih pa med prvim tednom menstruacijskega cikla (mediana SUVmax = 1,60, mediana SUV = 1,20). Privzem $^{18}$F-FDG v jajčnikih je bil šibko pozitivno povezan s koncentracijo progesterona (rho = 0,28, p <0,001 za SUVmax, rho = 0,30, p <0,001 za SUVmean) pri bolnicah, ki so bile v folikularni fazi mesečnega cikla (rho = 0,28). = 0,29, p = 0,003 za obe vrednosti SUVmax in SUV). Pri bolnicah v luteinski fazi mesečnega cikla te povezave ni bilo.

**Zaključki.** Na osnovi snovi slikanja s pomočjo PET/CT smo ugotovili, da se presnova glukoze v jajčnikih pri ženskah pred menopavzo povečuje s povečanjem koncentracije progesterona.

Radiol Oncol 2019; 52(3): 300-306.
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# Lokalna ponovitev sarkoma mehkih tkiv: Radiomska analiza

Tagliafico AS, Bignotti B, Rossi F, Valdora F, Martinoli C

**Izhodišča.** Namen raziskave je bil opraviti radiomsko analizo sledenja za lokalno ponovitev sarkoma mehkih tkiv na udih.

**Bolniki.** Raziskava je del prospektivne multicentrične študije, ki jo je odobrila etična komisija s podporo ESSR (European society of Musculoskeletal Radiology). Radiomsko analizo smo opravili na slikah zaporednih bolnikov, pri katerih smo preiskavo 1,5T MR opravili med marcem 2016 in septembrom 2018 v T1 obteženih sekvencah s hitrim spinskim odmevom v aksialni ravnini, T2 obteženih sekvencah z dušenjem maščobe in T1 obteženih sekvencah po injiciranju kontrasta (Gd).

**Rezultati.** Enajstim odraslih bolnikom (6 moških in 5 žensk; povprečna starost 57,8 ± 17,8) smo naredili preiskavo MR, da bi izključili lokalno ponovitev bolezni sarkoma mehkih tkiv. Skupno smo analizirali 33 kontrolnih pregledov. Za vsakega bolnika smo analizirali 198 naborov podatkov tako zdravih kot patološko spremenjenih tkiv. Štiri radiomske značilnost so bile statistično značilno povezane z velikostjo tumorja ($p < 0,02$) in štiri z gradusom ($p < 0,05$). Analiza ROC je pokazala AUC med 0,71 (95 % IZ: 0,55–0,87) za T1 obteženo sekvenco in 0,96 (95 % IZ: 0,87–1,00) za T1 obteženo sekvenco po injiciranju kontrasta.

**Zaključki.** Radiomske značilnosti nam omogočajo razlikovanje med zdravimi in patološkimi tkivi pri sledenju za lokalno ponovitev bolezni sarkoma mehkih tkiv. Radiomike so pri ocenjevanju te bolezni uporabne ne zgolj za odkrivanje, temveč tudi za vrednotenje lezij.

Radiol Oncol 2019; 52(3): 307-315.
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# Dusp6 inhibira epitelijsko-mezenhimsko tranzicijo pri endometrijskem raku preko signalne poti ERK

Fan MJ, Liang SM, He PJ, Zhao XB, Li MJ, Geng F

**Izhodišča.** Endometrijski rak je najbolj pogosta diagnoza ginekološke malignosti pri ženskah v razvitih državah. DUSP6 deluje kot negativni regulator na signalno pot ERK, ki pa je kot molekularno stikalo udeležena v signalni poti MAPK pri razvoju malignih bolezni. Pretekle raziskave so pokazale, da naj bi DUSP6 inhibiral tumorogenezo in z epitelijsko-mezenhimsko tranzicijo povezane lastnosti več vrst raka, vendar pa je njegova natančna vloga pri endometrijskemu raku še vedno nejasna.

**Materiali in metode.** Nivo DUSP6, E-cad in N-cad pri endometrijskem rakavem in zdravem tkivu smo določili s prenosom po Westernu in z imunohistokemijo. Celično rast, invazivnost in sposobnost migracije smo izmerili s pomočjo celične linije endometrijskega raka Ishikawa 3H12, ki so čezmerno izražale ali pa so imele utišano (*knock-down*) izražanje gena DUSP6. Določili smo tudi nivo proteinov E-cadherin, N-cadherin in Vimentin, ki so povezani z označevalci epitelijsko-mezenhimske tranzicije. Vpliv DUSP6 na signalno pot ERK smo ocenili na podlagi ugotavljanja ERK in p-ERK.

**Rezultati.** V primerjavi z zdravimi celicami je bilo pri endometrijskem raku opaziti znižanje izražanja DUSP6. Prekomerno izražanje DUSP6 je pomembno vplivalo na oslabljeno rast, invazijo in migracijsko sposobnost tumorskih celic, obenem pa zmanjšalo nivo označevalcev povezanih z epitelijsko-mezenhimsko tranzicijo. Utišanje DUSP6 se je izkazalo za nasprotno. Celice s prekomernim izražanjem DUSP6 so imele znižano izražanje p-ERK; celice s utišanim izražanjem DUSP6 pa so povečale izražanje p-ERK.

**Zaključki.** Pri celicah Ishikawa 3H12 je DUSP6 inhibiral rast, invazivnost in sposobnost migracije ter obenem zmanjšal lastnosti, povezane z epitelijsko-mezenhimsko tranzicijo. Tumorska supresivna vloga DUSP6 pri endometrijskem raku je dosežena z inhibicijo signalne poti ERK.

Radiol Oncol 2019; 52(3): 316-322.
doi: 10.2478/raon-2019-0044

# Klinični pomen mejnih rezultatov testa Hybrid Capture 2 HPV DNA na vzorcih maternične vratu odvzetih v transportni medij STM

Varl J, Ivanuš U, Pohar Marinšek Ž, Jerman T, Oštrbenk Valenčak A, Poljak M, Kloboves Prevodnik V

**Izhodišča.** V slovenskem državnem presejalnem programu zgodnjega odkrivanja predrakavih sprememb materničnega vratu ZORA kot triažni test uporabljamo test Hybrid Capture 2 (HC2). Ker želimo izboljšati slabšo analitično natančnost rezultatov testa HC2 blizu meje pozitivnosti 1 RLU/CO (angl. *relative light unit/cut-off*), sledimo internim navodilom in vse mejne vzorce z rezultatom znotraj območja 0,7–2,0 RLU/CO (siva cona) ponovno testiramo s testom HC2. Cilja naše raziskave sta bila: (i) določiti klinični pomen rezultatov testa HC2 znotraj treh različnih sivih con na vzorcih odvzetih v transportni medij STM (angl. *specimen transport medium*) in (ii) ugotoviti, ali je trenutni algoritem ponavljanja vzorcev znotraj sive cone klinično pomemben.

**Bolniki in metode.** V raziskavo smo vključili 594 žensk, starih od 20 do 65 let. Vse sodelujoče ženske smo napotili na kolposkopijo in v primeru nenormalnega rezultata je ginekolog izvedel tudi biopsijo. Določili smo porazdelitev rezultatov testa HC2 in delež žensk s CIN2+ lezijami znotraj treh različnih sivih con: 1,0–2,5, 0,4–4,0 in 0,7–2,0 RLU/CO, ponovno smo testirali vzorce z rezultati znotraj območja 0,4–4,0 RLU/CO in izračunali občutljivost ter specifičnost testa HC2 pri različnih vrednostih RLU/CO.

**Rezultati.** Delež žensk z rezultati testa HC2 znotraj 1,0–2,5, 0,4–4,0 in 0,7–2,0 območij RLU/CO je znašal 3,9 %, 10,8 % in 4,5 %. Delež žensk s CIN2+ lezijami v teh treh sivih conah je znašal 2,5 %, 5,6 % in 1,2 %. S ponovnim testiranjem mejnih vzorcev nismo odkrili dodatnih CIN2+ lezij. Znotraj 1,0-2,5 območja RLU/CO se je občutljivost zmanjšala iz 93,8 % na 91,4 %, hkrati se je specifičnost povečala iz 63,3 % na 67,5 %; v 0,4–4,0 območju RLU/CO se je občutljivost zmanjšala iz 95,1 % na 89,5 %, specifičnost pa je narasla iz 56,8 % na 69,4 %; in nazadnje v 0,7-2,0 območju RLU/CO je občutljivost ostala skoraj nespremenjena (94,4 % proti 93,2 %), specifičnost pa se je povečala iz 60,6 % na 66,4 %.

**Zaključki.** Rezultati nakazujejo, da ponovno testiranje vzorcev STM v sivih conah ni potrebno. Ponovno testiranje vzorcev v negativni sivi coni ne poveča občutljivosti, medtem ko ponovnem testiranju v pozitivni sivi coni ne sledi manj intenzivno vodenje žensk, saj so te ženske povabljene na nadaljnjo obravnavo ne glede na rezultat ponovnega testiranja. Poleg tega večina vzorcev po ponovnem testiranju ne spremeni rezultata testa HC2. In nazadnje, število žensk s CIN2+ lezijami in rezultati testa HC2 znotraj sivih con je majhno.

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# Genska ekspresija transkripcijskih faktorjev pri kroničnem rinosinuzitisu z nosnimi polipi in brez nosnih polipov

Košak Soklič T, Rijavec M, Šilar M, Koren A, Kern I, Hočevar-Boltežar I, Korošec P

**Izhodišča.** Nekateri bolniki s kroničnim rinosinuzitisom imajo kljub ustreznemu zdravljenju težke stalne simptome in ponovitve po operacijah. Želeli smo opredeliti gensko ekspresijo glavnih transkripcijskih faktorjev podtipov limfocitov T pri kroničnem rinosinuzitisu z nosnimi polipi (KRSwNP) in kroničnem rinosinuzitisu brez nosnih polipov (KRSsNP), ki bi lahko predstavljali potencialne nove tarče topikalnega zdravljenja z DNAcimi.

**Bolniki in metode.** Prospektivno smo vključili 22 bolnikov z novoodkritim kroničnim rinosinuzitisom (14 KRSwNP in 8 KRSsNP), jim odvzeli biopsijo sluznice in jo histopatološko analizirali. Gensko ekspresijo transkripcijskih faktorjev T-box transcription factor *(*T-bet, *TBX21),* GATA binding protein 3 *(GATA3),* Retinoic acid-related orphan receptor C *(RORC)* in Forkhead box P3 (*FOXP3)* smo analizirali s kvantitativno verižno reakcijo s polimerazo v realnem času (RT-qPCR).

**Rezultati.** Eozinofilni KRSwNP je izražal večjo gensko ekspresijo *GATA3* v primerjavi z neeozinofilnim KRSwNP, medtem ko ni bilo razlik v ekspresiji T-bet, *RORC* in *FOXP3* med eozinofilnim in neeozinofilnim KRSwNP. Pri KRSsNP smo ugotovili simultano zvišane nivoje ekspresij T-bet, *GATA3* in *RORC* v primerjavi s KRSwNP; ni pa bilo razlike v genski ekspresiji *FOXP3* med KRSwNP in KRSsNP.

**Zaključki.** Pri eozinofilnem KRSwNP smo potrdili vnetje tipa 2 s povečano gensko ekspresijo *GATA3.* Pri KRSsNP smo nepričakovano ugotovili simultano zvišani ekspresiji T-bet in *GATA3,* ki sta ostali nepojasnjeni; lahko bi izvirali iz aktiviranih CD8+ celic, pomnoženih v sluznici bolnikov s KRSsNP. Povečana ekspresija *RORC* pri KRSsNP bi bila lahko del normalnega homeostatskega imunskega odziva nosne sluznice, ki je verjetno bolje ohranjenega pri bolnikih s KRSsNP kot pri bolnikih s KRSwNP. Potrebne bi bile dodatne analize ekspresije transkripcijskih faktorjev pri fenotipih kroničnega rinosinuzitisa.

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# Dejavniki, ki vplivajo na obolevnost in smrtnost bolnikov po zapiranju protektivnih stom: Retrospektivna kohortna raziskava dvanajstletnega obdobja

Krebs B, Ivanecz A, Potrč S, Horvat M

**Izhodišča.** Izpeljava protektivne ali zaščitne stome je pogost postopek pri kirurgiji raka danke, kjer poskušamo zmanjšati učinek morebitnega puščanja na anastomozi. Nato z manjšim kirurškim posegom stomo čez nekaj mesecev zapremo. Cilj naše raziskave je bila ocena obolevnosti in smrtnosti postopka zapiranja stome in identificirati dejavnike, ki vplivajo na zaplete.

**Bolniki in metode.** Narejena je bila retrospektivna kohortna raziskava vseh bolnikov, pri katerih smo med leti 2003 in 2014 opravili operacijo zapiranja stome. Natančneje smo preučili nekatere dejavnike, ki bi lahko vplivali na stopnjo zapletov po tej operaciji: spol, starost, ASA ocena, kirurška tehnika in čas od prvotne operacije do zapiranja stome.

**Rezultati.** V raziskavo smo vključili 218 bolnikov. Do pooperativnih zapletov je prišlo pri 54 bolnikih (24,8 %). Najpogostejši zaplet je bil pooperativni ileus (10 %) in vnetje kirurške rane (5 %). Štirje bolniki so zaradi zapletov umrli (1,8 %). Na stopnjo zapletov niso vplivali bolnikov spol, starost, ASA ocena in tehnika zapiranja. Edini dejavnik, ki je statistično značilno povečal možnost zapleta, je bil čas do zapiranja. Bolniki, pri katerih smo stomo zaprli prej kot v 8 mesecih po prvotni operaciji, so imeli manj zapletov ($p<0,05$).

**Zaključki.** Glede na rezultate naše raziskave je za zmanjšanje skupne stopnje zapletov priporočljivo zapreti zaščitno stomo prej kot v osmih mesecih po prvotni operaciji. Ker je dokazano, da zapiranje zaščitne stome ni enostaven poseg, je potrebno napraviti vse, da se izognemo relativni visoki obolevnosti in celo smrtnosti tega posega.

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doi: 10.2478/raon-2019-0047

# Z zdravjem povezana kakovost življenja v hrvaški splošni populaciji in pri bolnikih s plazmocitomom ocenjena z vprašalnikoma EORTC QLQ-C30 in EORTC QLQ-MY20

Ledinski Fičko S, Pejša V, Zadnik V

**Izhodišča.** Vpliv bolezni in zdravljenja na splošno počutje bolnika ter zmožnost opravljanja vsakdanjih opravil postaja eno ključnih zanimanj klinične prakse in raziskav. Z zdravjem povezana kakovost življenja (angl. *health related quality of life, HrQoL*) vedno pogosteje uporabljamo kot ključni kazalnik rezultatov kliničnih raziskav. Evropska organizacija za raziskave in zdravljenje raka (EORTC) razvija pristope k ocenjevanju kakovosti življenja bolnikov z rakom. Zasnovali so osrednji vprašalnik EORTC QoL za bolnike s katerokoli rakavo boleznijo in različne dopolnilne, bolezensko specifične module. Dosedanje raziskave so pokazale, da so vrsta raka, obseg bolezni, klinični simptomi in način zdravljenja pomembni dejavniki HrQoL pri bolnikih z diseminiranim plazmocitomom (MM). Cilja raziskave sta bila: (1) pridobiti referenčne podatke o kakovosti življenja na reprezentativnem vzorcu hrvaške splošne populacije s pomočjo vprašalnika EORTC QLQ-C30 ter (2) z vprašalnikoma EORTC QLQ-C30 in EORTC QLQ-MY20 oceniti vpliv bolezni in primarnega sistemskega zdravljenja na HrQoL pri skupini hrvaških bolnikov z novo diagnosticiranim MM.

**Bolniki in metode.** V prvem delu raziskave smo prospektivno longitudinalno analizirali HrQoL v hrvaški splošni populaciji. V drugem delu pa smo HrQoL ocenili pri bolnikih z MM pred in po zdravljenju. Glede na način primarnega sistemskega zdravljenja smo bolnike razdelili v dve skupini: skupino zdravljenih z visokim odmerkom melfalana in presaditvijo krvotvornih matičnih celic ter skupino zdravljenih samo s kemoterapijo. Uporabili smo vprašalnika EORTC QLQ-C30 in EORTC QLQ-MY20. Odgovore, ki smo jih pridobljeni s pomočjo vprašalnikov, smo pretvorili v ustrezne dimenzije za ocenjevanje z zdravjem povezane kakovosti življenja.

**Rezultati.** Spol, starost in prebivališče ob diagnozi so značilno vplivali na kakovost življenja hrvaškega prebivalstva. Pri bolnikih z MM so bili splošna kakovost življenja, specifične dimenzije kakovosti življenja, kot tudi simptomi in lastnosti že pred začetkom zdravljenja slabši kot pri splošni populaciji. Vrsta bolezni, ki ji je sledila izbira možnosti terapije, so bili pomembni dejavniki HrQoL. V kakovosti življenja bolnikov z MM je imela ključno vlogo izbira terapije.

**Zaključki.** Referenčne populacijske vrednosti HrQoL, ki so zdaj na voljo za hrvaško prebivalstvo, bodo omogočale raziskovalcem in zdravnikom, da izračunajo pričakovane vrednosti HrQoL za posamezne hrvaške bolnike z rakom glede na njihovo starost, spol in kraj bivanja. Pričakovati je, da bo ocenjevanje in spremljanje sprememb v kakovosti življenja bolnikov pripomoglo k boljši obravnavi bolnikov in s tem izboljšalo rezultate zdravljen. Rezultati naše raziskave potrjujejo dosedanje raziskave in dajejo nove vpoglede v učinke dveh vrst zdravljenja pri bolnikih z MM.

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doi: 10.2478/raon-2019-0043

# Vprašalnik in računalniški program Onko-Online. 25 let kliničnega registra raka dojk v Univerzitetnem kliničnem centru Maribor

Arko D, Takač I

**Izhodišča.** Visokokakovostni podatki o vsakodnevni oskrbi, ki jih zbiramo v kliničnih registrih, pomembno prispevajo k izboljšanju obravnave bolnikov z rakom. Klinični registri vsebujejo vse pomembne podatke, ki jih zabeležimo med diagnostiko in zdravljenjem raka, spremljanjem po zdravljenju ter podatke o preživetju. Analize teh zelo obsežnih podatkov omogočajo izboljšanje kakovosti oskrbe bolnikov in primerjavo z drugimi ponudniki zdravstvenih storitev.

**Metode.** Prvi vprašalnik za spremljanje bolnic z rakom dojk na Oddelku za ginekološko onkologijo in onkologijo dojk v Splošni bolnišnici Maribor smo vpeljali leta 1994. Na podlagi svojih izkušenj in novih pristopov pri zdravljenju raka dojk smo vsebino vprašalnika spremenili in razširili v obliko, ki je trenutno v uporabi in ki je predstavljala temelj za razvoj ustreznega računalniškega programa z imenom *Onko-Online* leta 2014.

**Rezultati.** V obdobju 25 let smo zbrali podatke približno 3600 bolnic z rakom dojk. Računalniški program *Onko-Online* je omogočil hitro in zanesljivo zbiranje podatkov, njihovo obdelavo in analizo 167 različnih podatkov o bolnicah z rakom dojk, vključno s splošnimi podatki, anamnezo, diagnostičnimi postopki, zdravljenjem in spremljanjem po končanem zdravljenju.

**Zaključki.** Klinični register raka dojk *Onko-Online* omogoča zbiranje podatkov, ki nam pomagajo izboljšati diagnostiko in zdravljenje bolnic z rakom dojk, organizirati vsakodnevno prakso in primerjati naše rezultate zdravljenja z nacionalnimi in mednarodnimi standardi. Pomanjkljivost registra je morebitno nepopolno ali napačno vnašanje podatkov različnih zdravstvenih izvajalcev, ki so vključeni v zdravljenje bolnic z rakom dojk.

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# Idiopatska pljučna fibroza pri bolnikih po kirurški resekciji zaradi operabilnega nedrobnoceličnega pljučnega raka

Hribernik N, Požek I, Kern I

**Izhodišča.** Napoved poteka bolezni pri bolnikih s sočasnim pljučnim rakom in idiopatsko pljučno fibrozo je izjemno slaba. Radikalno zdravljenje pljučnega raka, kot je npr. kirurška resekcija, lahko povzroči akutno eksacerbacijo idiopatske pljučne fibroze. Z raziskavo smo želeli oceniti pogostnost idiopatske pljučne fibroze v skupini bolnikov z operabilnim nedrobnoceličnim pljučnim rakom ter analizirati klinične značilnosti in izid zdravljenja te kohorte bolnikov.

**Bolniki in metode.** Z observacijsko kohortno retrospektivno raziskavo smo analizirali 641 patoloških izvidov bolnikov po kirurški resekciji operabilnega nedrobnoceličnega pljučnega raka na Univerzitetni kliniki Golnik od maja 2010 do aprila 2017. Patološke izvide s sočasnim nedrobnoceličnim pljučnim rakom in idiopatsko pljučno fibrozo smo ponovno pregledali. Neodvisno sta ponovno pregledala slike CT in histološke vzorce torakalni radiolog in patolog. Iskali smo radiološki in patološki vzorec običajne intersticijske pljučnice. Zanimale so nas klinične značilnosti in izid zdravljenja v tej skupini bolnikov.

**Rezultati.** Od 641 bolnikov po kirurški resekciji zaradi nedrobnoceličnega pljučnega raka smo le pri 13 (2%) bolnikih histološko in radiološko potrdili sočasno običajno interticijsko pljučnico/idiopatsko pljučno fibrozo. Večina tumorjev je bilo majhnih (vsi so bili ali pT1 ali pT2), stadija I–II (11/13 bolnikov), lociranih v spodnjih pljučnih režnjih (11/13). Skoraj vsi bolniki so bili kadilci ali bivši kadilci (11/13). Dva bolnika (15,4 %) sta po radikalnem zdravljenju doživela eksacerbacijo idiopatske pljučne fibroze ter posledično umrla, prvi takoj po operaciji, drugi pa po zaključeni dopolnilni radioterapiji. Izmed vseh 13 bolnikov je le en bolnik imel ponovitev pljučnega raka.

**Zaključki.** Pogostnost sočasne običajne intersticijske pljučnice/idiopatske pljučne fibroze pri kirurško zdravljenih operabilnih nedrobnoceličnih pljučnih rakih je nizka. Naša observacijska raziskava potrjuje možnost razvoja eksacerbacije idiopatske pljučne fibroze s slabim izidom ob radikalnem zdravljenju. Zaenkrat še ni jasno opredeljeno, katero je najbolj optimalno radikalno zdravljenje pri teh bolnikih.

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# Dozimetrična študija stereotaktične telesne radioterapije hrbtenice: primerjava med linearnim pospeševalnikom sklopljenim z magnetno resonanco in volumetrično modulirno ločno terapijo

Yadav P, Musunuru HB, Witt JS, Bassetti M, Bayouth J, Baschnagel AM

**Izhodišča.** Stereotaktična telesna radioterapija (SBRT) z 1–5 frakcijami je učinkovito zdravljenje zasevkov v vretencih. Z magnetno resonanco vodeno obsevanje v realnem času (MRgRT) izboljša kontrastnost mehkih tkiv, ki se odraža v natančnosti izvedbe SBRT hrbtenice. Na tem mestu poročamo o klinični uporabi MRgRT pri SBRT hrbtenice, kakovosti obsevalnih načrtov MRgRT v primerjavi s načrti volumetrične modulirane ločne terapije z napravo TrueBeam pri zdravljenju zasevkov v vretencih in prednostih MRgRT slikanja.

**Bolniki in metode.** Za primerjavo načrtov obsevanja je bilo v raziskavo vključenih 10 metastatičnih lezij. Te so se nahajale v torakalni in lumbosakralni hrbtenici. SBRT načrti s tremi frakcijami (27 Gy na planirni tarčni volumen [PTV] in 30 Gy na tumorski volumen [GTV]) so bili izdelani s sistemom ViewRay MRIdian Linac in primerjani z načrti VMAT, izdelanimi za TrueBeamTM STx. Plani so bili primerjani z različnimi merili, kot so minimalna doza, maksimalna doza, srednja doza, razmerje med volumnom znotraj 50 % izodoze in volumnom PTV (R50), indeks konformnosti, indeks homogenosti in doza na hrbtenjačo.

**Rezultati.** Z obsevalnimi načrti MRIdian sta bila dosežena primerljiva pokritost tarče in doza na hrbtenjačo, kot z načrti VMAT. Maksimalne in minimalne doze v PTV ter indeks homogenosti so bili primerljivi pri obeh načrtovalnih sistemih. Pri načrtih MRIdina je bil R50 nižji, kot pri načrtih VMAT, kar kaže na manjšo razpršenost vmesnih doz pri sistemu MTIdian (5.16 oz. 6.11, p = 0.03).

**Zaključki.** MRgRT lahko izseva visoko kakovostne načrte pri SBRT hrbtenjače, primerljive z načrti VMAT naprave TrueBeam.


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je neprofitno, neinstitucionalno in nestrankarsko
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# Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the third quarter of 2019

Dr. Josip Cholewa Foundation for cancer research and education continues with its planned activities in the third quarter of 2019. Its primary focus remains the provision of grants, scholarships, and other forms of financial assistance for basic, clinical and public health research in the field of oncology. In parallel, it also makes efforts to provide financial and other support for the organisation of congresses, symposia and other forms of meetings to spread the knowledge about prevention and treatment of cancer, and finally about rehabilitation for cancer patients. In Foundation's strategy, the spread of knowledge should not be restricted only to the professionals that treat cancer patients, but also to the patients themselves and to the general public.

The Foundation continues to provide support for »Radiology and Oncology«, a quarterly scientific magazine with a respectable impact factor that publishes research and review articles about all aspects of cancer. The magazine is edited and published in Ljubljana, Slovenia. »Radiology and Oncology« is an open access journal available to everyone free of charge. Its long tradition represents a guarantee for the continuity of international exchange of ideas and research results in the field of oncology for all in Slovenia that are interested and involved in helping people affected by many different aspects of cancer.

The Foundation will continue with its activities in the future, especially since the problems associated with cancer affect more and more people in Slovenia and elsewhere. Ever more treatment that is successful reflects in results with longer survival in many patients with previously incurable cancer conditions. Thus adding many new dimensions in life of cancer survivors and their families.

Borut Štabuc, M.D., Ph.D.
Tomaž Benulič, M.D.
Andrej Plesničar, M.D., M.Sc.
Viljem Kovač M.D., Ph.D.

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**Datum zadnje revizije besedila:** za 1,5 mg/ml: 24.05.2017, za 3 mg/ml: 23.08.2018

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**SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA**
▼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.
**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 abemaciklība. Ena filmsko obložena tableta vsebuje 14 mg/28 mg/42 mg laktoze (v obliki monohidrata). **Terapevtske indikacije** Zdravilo Verzenios je indicirano za zdravljenje žensk z lokalno napredovalim ali metastatskim, na hormonske receptorje (HR – *Hormone Receptor*) pozitivnim in na receptorje humanega epidermalnega rastnega faktorja 2 (HER2 – *Human Epidermal Growth Factor Receptor 2*) 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- in perimenopavzi je treba endokrino zdravljenje kombinirati z agonistom gonadoliberina (LHRH – *Luteinizing Hormone–Releasing Hormone*). **Odmerjanje in način uporabe** Zdravljenje z zdravilom Verzenios mora uvesti in nadzorovati zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje rakavih bolezni. *Zdravilo Verzenios v kombinaciji z endokrinim zdravljenjem* Priporočeni odmerek abemaciklība je 150 mg dvakrat na dan, kadar se uporablja v kombinaciji z endokrinim zdravljenjem. Zdravilo Verzenios je treba jemati, dokler ima bolnica od zdravljenja klinično korist ali dokler se ne pojavi nesprejemljiva toksičnost. 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. Sočasni uporabi močnih zaviralcev CYP3A4 se je treba izogibati. Če se uporabi močnih zaviralcev CYP3A4 ni mogoče izogniti, je treba odmerek abemaciklība zmanjšati na 100 mg dvakrat na dan. Pri bolnicah, pri katerih je bil odmerek zmanjšan na 100 mg abemaciklība dvakrat na dan in pri katerih se sočasnemu dajanju močnega zaviralca CYP3A4 ni mogoče izogniti, je treba odmerek abemaciklība dodatno zmanjšati na 50 mg dvakrat na dan. Pri bolnicah, pri katerih je bil odmerek zmanjšan na 50 mg abemaciklība dvakrat na dan in pri katerih se sočasnemu dajanju močnega zaviralca CYP3A4 ni mogoče izogniti, je mogoče z odmerkom abemaciklība nadaljevati ob natančnem spremljanju znakov toksičnosti. Alternativno je mogoče odmerek abemaciklība zmanjšati na 50 mg enkrat na dan ali prekiniti dajanje abemaciklība. Če je uporaba zaviralca CYP3A4 prekinjena, je treba odmerek abemaciklība 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 abemaciklība 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. Zdravilo se ne sme jemati z grenivko ali grenivkinim sokom. Bolnice naj odmerke vzamejo vsak dan ob približno istem času. Tableto je treba zaužiti celo (bolnice je 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. Bolnice je treba spremljati za znake in simptome globoke venske tromboze in pljučne embolije ter jih zdraviti, kot je medicinsko utemeljeno. 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 abemaciklība. Bolnice z redkimi dednimi motnjami, kot so intoleranca za galaktozo, popolno pomanjkanje laktaze ali malapsorpcija glukoze/galaktoze, tega zdravila ne smejo jemati. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij** Abemaciklib se primarno presnavlja s CYP3A4. Sočasna uporaba abemaciklība in zaviralcev CYP3A4 lahko poveča plazemsko koncentracijo abemaciklība. Uporabi močnih zaviralcev CYP3A4 sočasno z abemaciklibom se je treba izogibati. Če je močne zaviralce CYP3A4 treba dajati sočasno, je treba odmerek abemaciklība 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 abemaciklība. 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 abemaciklība 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, anemija, utrujenost, navzea, bruhanje in zmanjšanje apetita. *Zelo pogosti:* okužbe, nevtropenija, levkopenija, anemija, trombocitopenija, driska, bruhanje, navzea, zmanjšanje apetita, disgevzija, omotica, alopecija, pruritus, izpuščaj, utrujenost, pireksija, povečana vrednost alanin-aminotransferaze, povečana vrednost aspartat-aminotransferaze *Pogosti:* limfopenija, povečano solzenje, venska trombembolija, suha koža, mišična šibkost *Občasni:* febrilna nevtropenija **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 zadnje revizije besedila**: 2.11.2018 **Režim izdaje:** Rp/Spec - Predpisovanje in izdaja zdravila je le na recept zdravnika specialista ustreznega področja medicine ali od njega pooblaščenega zdravnika.

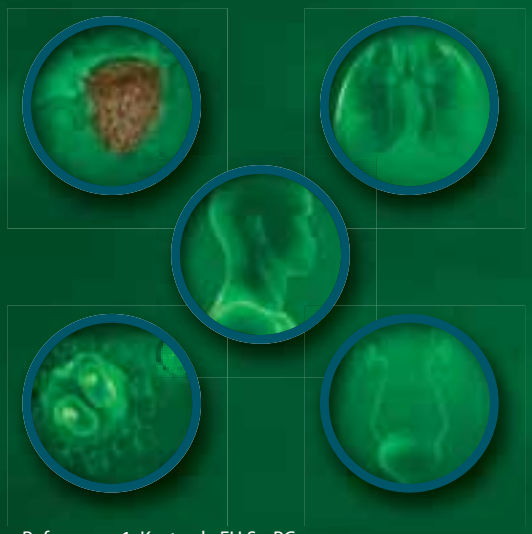

**Reference**
**1.** Povzetek glavnih značilnosti zdravila Verzenios. Datum zadnje revizije besedila: 2.11.2018. **2.** Povzetek glavnih značilnosti zdravila Ibrance. Dostop preverjen 22.11.2018. **3.** Povzetek glavnih značilnosti zdravila Kisqali. Dostop preverjen 22.11.2018.


**Pomembno obvestilo**
Pričujoče gradivo je namenjeno samo za strokovno javnost. Predpisovanje in izdaja zdravila Verzenios je le na recept zdravnika specialista ustreznega področja medicine ali od njega pooblaščenega zdravnika. Pred predpisovanjem zdravila Verzenios vas vljudno prosimo, da preberete celotni Povzetek glavnih značilnosti zdravila. Podrobnejše informacije o zdravilu Verzenios in o zadnji reviziji besedila Povzetka glavnih značilnosti zdravila so na voljo na sedežu podjetja Eli Lilly (naslov podjetja in kontaktni podatki spodaj) in na spletni strani European Medicines Agency (EMA): www.ema.europa.eu in na spletni strani European Commission http://ec.europa.eu/health/documents/community-register/html/alfregister.htm.


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-0001, 23.11.2018, Samo za strokovno javnost.



# KEYTRUDA®
(pembrolizumab, MSD)

- Melanom[1]
- Nedrobnocelični pljučni rak[1]
- Urotelijski karcinom[1]
- Hodgkinov limfom[1]
- Ploščatocelični karcinom glave in vratu[1]

References: 1. Keytruda EU SmPC

**SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA**
**Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila!**

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti.

**Ime zdravila:** KEYTRUDA 25 mg/ml koncentrat za raztopino za infundiranje vsebuje pembrolizumab. **Terapevtske indikacije:** Zdravilo KEYTRUDA je kot samostojno zdravljenje indicirano za zdravljenje: napredovalega (neoperabilnega ali metastatskega) melanoma pri odraslih; za adjuvantno zdravljenje odraslih z melanomom v stadiju III, ki se je razširil na bezgavke, po popolni kirurški odstranitvi; 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 bolnikov s ponovljenim ali neodzivnim klasičnim Hodgkinovim limfomom (cHL), pri katerih avtologna presaditev matičnih celic (ASCT) in zdravljenje z brentuksimabom vedotinom (BV) nista bila uspešna, in odraslih bolnikov, ki za presaditev niso primerni, zdravljenje z BV pa pri njih ni bilo uspešno; lokalno napredovalega ali metastatskega urotelijskega karcinoma pri odraslih, predhodno zdravljenih s kemoterapijo, ki je vključevala platino; lokalno napredovalega ali metastatskega urotelijskega karcinoma 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 karcinoma 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. Zdravilo KEYTRUDA je v kombinaciji s pemetreksedom in kemoterapijo na osnovi platine 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. **Odmerjanje in način uporabe:** Testiranje PD-L1 pri bolnikih z NSCLC, urotelijskim karcinomom ali HNSCC: Pri bolnikih z NSCLC je priporočljivo opraviti testiranje izraženosti PD-L1 tumorja z validirano preiskavo. Bolnike s predhodno nezdravljenim urotelijskim karcinomom ali HNSCC je treba za zdravljenje izbrati na podlagi izraženosti PD-L1, potrjene z validirano preiskavo. Odmerjanje: Priporočeni odmerek zdravila KEYTRUDA za samostojno zdravljenje je bodisi 200 mg na 3 tedne ali 400 mg na 6 tednov, apliciran z intravensko infuzijo v 30 minutah. Priporočeni odmerek za kombinirano zdravljenje je 200 mg na 3 tedne, apliciran z intravensko infuzijo v 30 minutah. Če se uporablja kot del kombiniranega zdravljenja skupaj s kemoterapijo, je treba zdravilo KEYTRUDA aplicirati prvo. Bolnike je treba zdraviti do napredovanja bolezni ali nesprejemljivih toksičnih učinkov. Pri adjuvantnem zdravljenju melanoma je treba zdravilo uporabljati do ponovitve bolezni, pojava nesprejemljivih toksičnih učinkov oziroma mora zdravljenje trajati do enega leta. Pri bolnikih starih ≥ 65 let, bolnikih z blago do zmerno okvaro ledvic, bolnikih z blago okvaro jeter prilagoditev odmerka ni potrebna. Odložitev odmerka ali ukinitev zdravljenja: Za primere, kjer je treba zdravljenje zadržati, dokler se neželeni učinki ne zmanjšajo na stopnjo 0-1 in kadar je treba zdravilo KEYTRUDA trajno 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 4.948 bolnikih z napredovalim melanomom, kirurško odstranjenim melanomom v stadiju III (adjuvantno zdravljenje), NSCLC, cHL, urotelijskim karcinomom ali HNSCC s štirimi odmerki (2 mg/kg na 3 tedne, 200 mg na 3 tedne in 10 mg/kg na 2 ali 3 tedne). V tej populaciji bolnikov je mediani čas opazovanja znašal 7,3 mesece (v razponu od 1 dneva do 31 mesecev), najpogostejši neželeni učinki zdravljenja s pembrolizumabom so bili utrujenost (34,1 %), izpuščaj (22,7 %), navzea (21,7 %), diareja (21,5 %) in pruritus (20,2 %). 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. Varnost pembrolizumaba pri kombiniranem zdravljenju s kemoterapijo so ocenili pri 791 bolnikih NSCLC, ki so v kliničnih študijah prejemali pembrolizumab v odmerkih 200 mg, 2 mg/kg ali 10 mg/kg na vsake 3 tedne. V tej populaciji bolnikov so bili najpogostejši neželeni učinki naslednji: navzea (49 %), anemija (48 %), utrujenost (38 %), zaprtost (34%), diareja (31%), nevtropenija (29 %), in zmanjšanje apetita (28 %). Pri kombiniranem zdravljenju s pembrolizumabom je pojavnost neželenih učinkov 3. do 5. stopnje znašala 67 %, pri zdravljenju samo s kemoterapijo pa 66 %. Za celoten seznam neželenih učinkov, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. **Način in režim izdaje zdravila:** H – Predpisovanje in izdaja zdravila je samo 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. **Datum zadnje revizije besedila:** 27. junij 2019

MSD

**Merck Sharp & Dohme inovativna zdravila d.o.o.,**
Šmartinska cesta 140, 1000 Ljubljana, tel: +386 1/ 520 42 01, fax: +386 1/ 520 43 50
Pripravljeno v Sloveniji, junij 2019; SI-KEY-00008 EXP: 08/2021

**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.**

Več na
**foundationmedicine.si**

# OBŠIREN VPOGLED ZA NAČRTOVANJE BOLNIKU PRILAGOJENEGA ZDRAVLJENJA[1-6]

Odkrijte možnosti visoko kakovostnih storitev obširnega genomskega profiliranja FoundationOne®, ki olajšajo odločitev o najustreznejšem zdravljenju za posameznega bolnika z rakom, v različnih kliničnih stanjih.[4-6]

FOUNDATIONONE® CDx

FOUNDATIONONE® LIQUID

FOUNDATIONONE® HEME

Viri: 1. Frampton GM s sod. Nat Biotechnol 2013; 31:1023-1031. 2. Clark TA s sod. J Mol Diagn 2018; 20:686-702. 3. He J s sod. Blood 2016; 127:3004-3014. 4. FoundationOne® CDx Technical Information; dostopano april 2019 na: https://assets.ctfassets.net/vhribv12lmne/6Rt6csmCPuaguuqmgi2iY8/e3a9b0456ed71a55d2e4480374695d95/FoundationOne_CDx.pdf. 5. FoundationOne® Liquid Technical Specifications; dostopano april 2019 na: https://assets.ctfassets.net/vhribv12lmne/3SPYAcbGdqAeMsOqMyKUog/d0eb51659e08d733bf39971e85ed940d/F1L_TechnicalInformation_MKT-0061-04.pdf. 6. FoundationOne® Heme Technical Specifications; dostopano april 2019 na: https://assets.ctfassets.net/vhribv12lmne/zBxaQC12cScqgsEk8seMO/abf6133874f1e5929403f66d90c3b900/F1H_TechnicalInformation_06_digital.pdf.

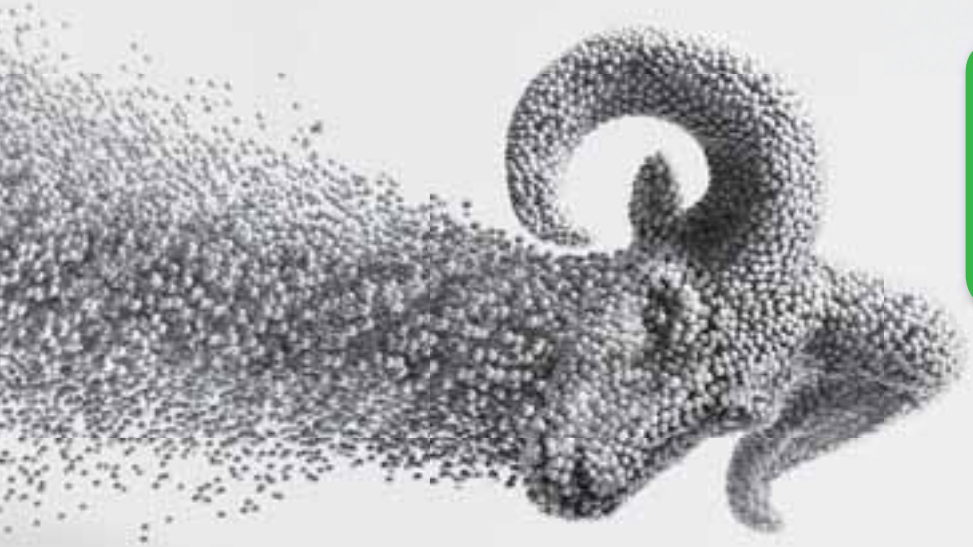
PM-0089-2019-FMI

Informacija pripravljena: maj 2019. Samo za strokovno javnost.
DODATNE INFORMACIJE SO NA VOLJO PRI:
Roche farmacevtska družba d.o.o., Vodovodna cesta 109, 1000 Ljubljana
rocheprotiraku.si / foundationmedicine.si

FOUNDATION MEDICINE®

Roche

# ODLOČNO PROTI RAKU TREBUŠNE SLINAVKE



**Prva odobrena terapija za zdravljenje metastatskega adenokarcinoma trebušne slinavke po zdravljenju na osnovi gemcitabina**

## ONIVYDE (liposomski irinotekan) je v kombinaciji s 5-fluorouracilom (5-FU) in levkovorinom (LV) pri odraslih bolnikih indiciran za zdravljenje metastatskega adenokarcinoma trebušne slinavke, pri katerih je bolezen po zdravljenju na osnovi gemcitabina napredovala.[1]

1. Povzetek glavnih značilnosti zdravila.

**Skrajšan povzetek glavnih značilnosti zdravila Onivyde 5 mg/ml**
**SESTAVA***: ONIVYDE 5 mg/ml koncentrat za raztopino za infundiranje: ena 10-mililitrska viala koncentrata vsebuje ekvivalent 50 mg irinotekanijevega klorida trihidrata (v obliki irinotekanijeve soli saharoznega oktalsulfata v pegilirani liposomski formulaciji), kar ustreza 43 mg irinotekana. **TERAPEVTSKE INDIKACIJE***: Zdravljenje metastatskega adenokarcinoma trebušne slinavke v kombinaciji s 5-fluorouracilom (5-FU) in levkovorinom (LV) pri odraslih bolnikih, pri katerih je bolezen po zdravljenju na osnovi gemcitabina napredovala. **ODMERJANJE IN NAČIN UPORABE***: ONIVYDE (liposomski irinotekan) smejo bolnikom predpisati in dajati samo zdravstveni delavci, ki imajo izkušnje pri uporabi zdravil za zdravljenje raka. Priporočeni odmerek in režim odmerjanja zdravila ONIVYDE je 80 mg/m$^2$ intravensko 90 minut, čemur sledi LV 400 mg/m$^2$ intravensko 30 minut in nato 5-FU 2400 mg/m$^2$ intravensko 46 ur, vsaka 2 tedna. Zdravilo ONIVYDE se ne daje kot samostojno zdravilo. Pri bolnikih z znano homozigotnostjo za alel UGT1A1*28 je treba razmisliti o manjšem začetnem odmerku zdravila ONIVYDE (liposomskega irinotekana) 60 mg/m$^2$. Če zdravilo ONIVYDE bolniki dobro prenašajo, lahko v naslednjih ciklih razmislimo o odmerku 80 mg/m$^2$. Prilagajanje odmerkov se priporoča za obvladovanje toksičnosti 3. ali 4. stopnje, povezane z zdravilom ONIVYDE. **KONTRAINDIKACIJE***: Anamneza hude preobčutljivosti na irinotekan ali katero koli pomožno snov. Dojenje. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI***: Zdravilo ONIVYDE (liposomski irinotekan) ni enakovredno drugim neliposomskim formulacijam irinotekana, zato jih ne smemo zamenjevati. *Mielosupresija/nevtropenija:* Med zdravljenjem z zdravilom ONIVYDE se priporoča nadziranje celotne krvne slike. Bolniki se morajo zavedati tveganja za nevtropenijo in pomena povišane telesne temperature. Febrilno nevtropenijo je treba nujno zdraviti v bolnišnici s širokospektralnimi intravenskimi antibiotiki. Pri bolnikih, ki doživijo hude hematološke neželene učinke, se priporoča zmanjšanje odmerka ali prekinitev zdravljenja. Bolnikov s hudo odpovedjo kostnega mozga ne smemo zdraviti z zdravilom ONIVYDE. *Imunosupresivni učinki in cepiva:* Dajanje živih ali atenuiranih cepiv bolnikom z oslabljenim imunskim sistemom lahko povzroči resne ali smrtne okužbe. Bolniki azijskega porekla imajo večje tveganje za hudo in febrilno nevtropenijo. Posamezniki s homozigotnostjo 7/7 za alel UGT1A1*28 imajo povečano tveganje za nevtropenijo. *Interakcije z močnimi induktorji encima CYP3A4, močnimi zaviralci encima CYP3A4 in močnimi zaviralci encima UGT1A1:* Zdravila ONIVYDE ne smemo dajati skupaj z močnimi induktorji encima CYP3A4, kot so antikonvulzivi, rifampicin, rifabutin in šentjanževka), močnimi zaviralci encima CYP3A4 (npr. grenivkinim sokom, klaritromicinom, indinavirjem, itrakonazolom, lopinavirjem, nefazodonom, nelfinavirjem, ritonavirjem, sakvinavirjem, telaprevirjem, vorikonazolom) ali z močnimi zaviralci encima UGT1A1, razen če ni drugih terapevtskih možnosti. Zdravljenje z močnimi zaviralci encima CYP3A4 moramo prekiniti vsaj 1 teden pred začetkom zdravljenja z zdravilom ONIVYDE. *Driska:* Pri bolnikih, ki doživijo zgodnji pojav driske (v ≤ 24 urah po začetku zdravljenja z zdravilom ONIVYDE), je treba razmisliti o terapevtskem in profilaktičnem zdravljenju z atropinom, razen če je kontraindicirano. Bolnike je treba opozoriti na tveganje za zapoznelo drisko (> 24 ur), ki je izčrpavajoča in v redkih primerih tudi življenjsko nevarna. Če driska traja tudi, ko bolnik prejema loperamid več kot 24 ur, je treba razmisliti o dodatni peroralni antibiotični podpori. Zdravljenje z zdravilom ONIVYDE je treba odložiti, dokler se driska ne umiri do ≤ 1. stopnje (2–3 odvajanja/dan več kot pred zdravljenjem). Zdravila ONIVYDE ne smemo dajati bolnikom z zaporo črevesja ali kronično vnetno črevesno boleznijo, dokler se ta ne pozdravi. *Holinergične reakcije:* Zgodnjo drisko lahko spremljajo rinitis, povečano slinjenje, zardevanje, diaforeza, bradikardija, mioza in hiperperistaltika. Pri bolnikih s holinergičnimi simptomi moramo uporabiti atropin. *Akutne infuzijske in povezane reakcije:* V primeru hudih preobčutljivostnih reakcij je treba zdravljenje z zdravilom ONIVYDE prekiniti. *Predhodna Whipplova operacija:* Večje tveganje za resne okužbe. Bolnike je treba spremljati glede znakov okužbe. *Žilne bolezni:* Zdravilo Onivyde je bilo povezano s trombemboličnimi dogodki, kot so pljučna embolija, venska tromboza in arterijska trombembolija. Bolnike je treba obvestiti o znakih in simptomih trombembolije in jim svetovati, da se v primeru katerega od teh znakov ali simptomov takoj obrnejo na svojega zdravnika ali medicinsko sestro. *Pljučna toksičnost:* Pri bolnikih, ki so prejemali neliposomski irinotekan, so se pojavili dogodki, podobni intersticijski pljučni bolezni (IPB), ki so vodili do smrtnih primerov. Pri bolnikih z dejavniki tveganja (obstoječo pljučno boleznijo, uporabo pnevmotoksičnih zdravil, kolonije stimulirajočimi dejavniki ali predhodnim zdravljenjem z obsevanjem) je treba pred zdravljenjem z zdravilom ONVYDE in po njem skrbno nadzirati respiratorne simptome. Dokler ni opravljena diagnostična ocena, je treba ob pojavu nove ali napredovale dispneje, kašlja in povišane telesne temperature zdravljenje z zdravilom ONIVYDE začasno prekiniti. Pri bolnikih s potrjeno diagnozo IPB moramo zdravljenje z zdravilom ONIVYDE dokončno prekiniti. *Jetrna okvara:* Bolniki s hiperbilirubinemijo so imeli povišane koncentracije skupnega SN-38, zato je tveganje za nevtropenijo povečano. Pri bolnikih z vrednostjo skupnega bilirubina 1,0–2,0 mg/dl je treba redno nadzirati celotno krvno sliko. Previdnost je potrebna pri bolnikih z jetrno okvaro (bilirubin > 2-kratna zgornja meja normalnih vrednosti [ULN]; aminotransferaze > 5-kratna ULN). *Ledvična okvara:* Uporaba zdravila ONIVYDE pri bolnikih s pomembno ledvično okvaro ni bila ocenjena. *Bolniki s premajhno telesno maso (indeks telesne mase < 18,5 kg/m$^2$):* Potrebna je previdnost. *Pomožne snovi:* En mililiter zdravila ONIVYDE vsebuje 0,144 mmol (3,31 mg) natrija. **INTERAKCIJE***: *Previdnostni ukrepi:* Sočasno dajanje zdravila ONIVYDE in induktorjev encima CYP3A4 lahko zmanjša sistemsko izpostavljenost zdravilu ONIVYDE. Sočasno dajanje zdravila ONIVYDE in zaviralcev encima CYP3A4 ali encima UGT1A1 (npr. atazanavirja, gemfibrozila, indinavirja) poveča sistemsko izpostavljenost zdravilu ONIVYDE. **PLODNOST* NOSEČNOST***: Uporaba ni priporočljiva. **DOJENJE***: Zdravilo je kontraindicirano. **KONTRACEPCIJA***: Ženske v rodni dobi morajo med zdravljenjem in še 1 mesec po zdravljenju z zdravilom ONIVYDE uporabljati učinkovito kontracepcijo. Moški morajo med zdravljenjem in 4 mesece po zdravljenju z zdravilom ONIVYDE uporabljati kondome. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA STROJEV***: Bolniki morajo biti med zdravljenjem pri vožnji in upravljanju strojev previdni. **NEŽELENI UČINKI***: *Zelo pogosti:* nevtropenija, levkopenija, anemija, trombocitopenija, hipokaliemija, hipomagneziemija, dehidracija, zmanjšan apetit, omotica, driska, bruhanje, navzea, bolečine v trebuhu, stomatitis, alopecija, pireksija, periferni edem, vnetje sluznic, utrujenost, astenija, zmanjšana telesna masa, *Pogosti:* septični šok, sepsa, pljučnica, febrilna nevtropenija, gastroenteritis, oralna kandidoza, limfopenija, hipoglikemija, hiponatriemija, hipofosfatemija, nespečnost, holinergični sindrom, dizgevzija, hipotenzija, pljučna embolija, embolija, globoka venska tromboza, dispneja, disfonija, kolitis, hemoroidi, hipoalbuminemija, akutna ledvična odpoved, z infuzijo povezana reakcija, edem, zvišana raven bilirubina, zvišana raven alanin-aminotransferaze, zvišana raven aspartat-aminotransferaze, zvišano mednarodno umerjeno razmerje. *Občasni:* biliarna sepsa, preobčutljivost, tromboza, hipoksija, ezofagitis, proktitis, makulopapulozni izpuščaj, obarvanje nohtov. **PREVELIKO ODMERJANJE***: Za preveliko odmerjanje zdravila ONIVYDE ni znanega antidota. Treba je uvesti maksimalno podporno nego, s katero preprečimo dehidracijo zaradi driske in zdravimo zaplete zaradi okužb. **FARMAKODINAMIČNE LASTNOSTI***: Učinkovina zdravila ONIVYDE je irinotekan (zaviralec topoizomeraze I), inkapsuliran v vezikel z lipidnim dvoslojem oziroma liposom. Irinotekan je derivat kamptotecina. Kamptotecini delujejo kot specifični zaviralci encima DNA-topoizomeraza I. Irinotekan in njegov aktivni presnovek SN-38 se reverzibilno vežeta na kompleks topoizomeraze I in DNA ter sprožita poškodbe v enoverižni DNA, kar zaustavi replikacijske vilice pri podvajanju DNA in povzroča citotoksičnost. Irinotekan se presnavlja s karboksilesterazo do SN-38, SN-38 je približno 1.000-krat močnejši kot irinotekan kot zaviralec topoizomeraze I, očiščene iz tumorskih celičnih linij človeka in glodavcev. **PAKIRANJE***: Pakiranje vsebuje eno vialo z 10 ml koncentrata. **NAČIN PREDPISOVANJA IN IZDAJE ZDRAVILA**: Rp/Spec. **Imetnik dovoljenja za promet**: Les Laboratoires Servier, 50, rue Carnot, 92284 Suresnes cedex, Francija. **Številka dovoljenja za promet z zdravilom**: EU/1/16/1130/001. **Datum zadnje revizije besedila: januar 2019. *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, tel: 01 563 48 11, www.servier.si.**

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onivyde®
liposomski irinotekan

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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.

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**BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA**

**XALKORI 200 mg, 250 mg trde kapsule**

**Sestava in oblika zdravila:** Ena kapsula vsebuje 200 mg ali 250 mg krizotiniba. **Indikacije:** Monoterapija za: - prvo linijo zdravljenja odraslih bolnikov z napredovalim nedrobnoceličnim pljučnim rakom (NSCLC – *Non-Small Cell Lung Cancer*), ki je ALK (anaplastična limfomska kinaza) pozitiven; - zdravljenje odraslih bolnikov s predhodno zdravljenim, napredovalim NSCLC, ki je ALK pozitiven; - zdravljenje odraslih bolnikov z napredovalim NSCLC, ki je ROS1 pozitiven. **Odmerjanje in način uporabe:** Zdravljenje mora uvesti in nadzorovati zdravnik z izkušnjami z uporabo zdravil za zdravljenje rakavih bolezni. Preverjanje prisotnosti ALK in ROS1: Pri izbiri bolnikov za zdravljenje je treba pred zdravljenjem opraviti točno in validirano preverjanje prisotnosti ALK ali ROS1. Odmerjanje: Priporočeni odmerek je 250 mg dvakrat na dan (500 mg na dan), bolniki pa morajo zdravilo jemati brez prekinitev. Če bolnik pozabi vzeti odmerek, ga mora vzeti takoj, ko se spomni, razen če do naslednjega odmerka manjka manj kot 6 ur. V tem primeru bolnik pozabljenega odmerka ne sme vzeti. *Prilagajanja odmerkov:* Glede na varnost uporabe zdravila pri posameznem bolniku in kako bolnik zdravljenje prenaša, utegne biti potrebna prekinitev in/ali zmanjšanje odmerka pri bolnikih, ki se zdravijo s krizotinibom 250 mg peroralno dvakrat na dan (za režim zmanjševanja odmerka glejte poglavje 4.2 v povzetku glavnih značilnosti zdravila). Za prilagajanje odmerkov pri *hematološki in nehematološki toksičnosti* (povečanje vrednosti AST, ALT, bilirubina; ILD/pnevmonitis; podaljšanje intervala QTc, bradikardija, bolezni oči) glejte preglednici 1 in 2 v poglavju 4.2 povzetka glavnih značilnosti zdravila. *Okvara jeter:* Pri zdravljenju pri bolnikih z okvaro jeter je potrebna previdnost. Pri blagi okvari jeter prilagajanje začetnega odmerka ni priporočeno, pri zmerni okvari jeter je priporočeni začetni odmerek 200 mg dvakrat na dan, pri hudi okvari jeter pa 250 mg enkrat na dan (za merila glede klasifikacije okvare jeter glejte poglavje 4.2 v povzetku glavnih značilnosti zdravila). *Okvara ledvic:* Pri blagi in zmerni okvari prilagajanje začetnega odmerka ni priporočeno. Pri hudi okvari ledvic (ki ne zahteva peritonealne dialize ali hemodialize) je začetni odmerek 250 mg peroralno enkrat na dan; po vsaj 4 tednih zdravljenja se lahko poveča na 200 mg dvakrat na dan. *Starejši bolniki (≥ 65 let):* Prilagajanje začetnega odmerka ni potrebno. *Pediatrična populacija:* Varnost in učinkovitost nista bili dokazani. Način uporabe: Kapsule je treba pogoltniti cele, z nekaj vode, s hrano ali brez nje. Ne sme se jih zdrobiti, raztopiti ali odpreti. Izogibati se je treba uživanju grenivk, grenivkinega soka ter uporabi šentjanževke. **Kontraindikacije:** Preobčutljivost na krizotinib ali katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Določanje statusa ALK in ROS1: Pomembno je izbrati dobro validirano in robustno metodologijo, da se izognemo lažno negativnim ali lažno pozitivnim rezultatom. Hepatotoksičnost: V kliničnih študijah so poročali o hepatotoksičnosti, ki jo je povzročilo zdravilo (vključno s primeri s smrtnim izidom). Delovanje jeter, vključno z ALT, AST in skupnim bilirubinom, je treba preveriti enkrat na teden v prvih 2 mesecih zdravljenja, nato pa enkrat na mesec in kot je klinično indicirano. Ponovitve preverjanj morajo biti pogostejše pri povečanjih vrednosti stopnje 2, 3 ali 4. Intersticijska bolezen pljuč (ILD)/pnevmonitis: Lahko se pojavi huda, življenjsko nevarna ali smrtna ILD/pnevmonitis. Bolnike s simptomi ILD/pnevmonitisa je treba spremljati, zdravljenje pa prekiniti ob sumu na ILD/pnevmonitis. Podaljšanje intervala QT: Opažali so podaljšanje intervala QTc. Pri bolnikih z obstoječo bradikardijo, podaljšanjem intervala QTc v anamnezi ali predispozicijo zanj, pri bolnikih, ki jemljejo antiaritmike ali druga zdravila, ki podaljšujejo interval QT, ter pri bolnikih s pomembno obstoječo srčno boleznijo in/ali motnjami elektrolitov je treba krizotinib uporabljati previdno; potrebno je redno spremljanje EKG, elektrolitov in delovanja ledvic; preiskavi EKG in elektrolitov je treba opraviti čimbližje uporabi prvega odmerka, potem se priporoča redno spremljanje. Če se interval QTc podaljša za 60 ms ali več, je treba zdravljenje s krizotinibom začasno prekiniti in se posvetovati s kardiologom. Bradikardija: Lahko se pojavi simptomatska bradikardija (lahko se razvije več tednov po začetku zdravljenja); izogibati se je treba uporabi krizotiniba v kombinaciji z drugimi zdravili, ki povzročajo bradikardijo; pri simptomatski bradikardiji je treba prilagoditi odmerek. Srčno popuščanje: Poročali so o hudih, življenjsko nevarnih ali smrtnih neželenih učinkih srčnega popuščanja. Bolnike je treba spremljati glede pojavov znakov in simptomov srčnega popuščanja in ob pojavu simptomov zmanjšati odmerjanje ali prekiniti zdravljenje. Nevtropenija in levkopenija: V kliničnih študijah so poročali o nevtropeniji, levkopeniji in febrilni nevtropeniji; spremljati je treba popolno krvno sliko (pogostejše preiskave, če se opazijo abnormalnosti stopnje 3 ali 4 ali če se pojavi povišana telesna temperatura ali okužba). Perforacija v prebavilih: V kliničnih študijah so poročali o perforacijah v prebavilih, v obdobju trženja pa o smrtnih primerih perforacij v prebavilih. Krizotinib je treba pri bolnikih s tveganjem za nastanek perforacije v prebavilih uporabljati previdno; bolniki, pri katerih se razvije perforacija v prebavilih, se morajo prenehati zdraviti s krizotinibom; bolnike je treba poučiti o prvih znakih perforacije in jim svetovati, naj se nemudoma posvetujejo z zdravnikom. Vplivi na ledvice: V kliničnih študijah so opazili zvišanje ravni kreatinina v krvi in zmanjšanje očistka kreatinina. V kliničnih študijah in v obdobju trženja so poročali tudi o odpovedi ledvic, akutni odpovedi ledvic, primerih s smrtnim izidom, primerih, ki so zahtevali hemodializo in hiperkaliemiji stopnje 4. Vplivi na vid: V kliničnih študijah so poročali o izpadu vidnega polja stopnje 4 z izgubo vida. Če se na novo pojavi huda izguba vida, je treba zdravljenje prekiniti in opraviti oftalmološki pregled. Če so motnje vida trdovratne ali se poslabšajo, je priporočljiv oftalmološki pregled. Histološka preiskava, ki ne nakazuje adenokarcinoma: Na voljo so le omejeni podatki pri NSCLC, ki je ALK in ROS1 pozitiven in ima histološke značilnosti, ki ne nakazujejo adenokarcinoma, vključno s ploščatoceličnim karcinomom (SCC). **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Izogibati se je treba sočasni uporabi z močnimi zaviralci CYP3A4, npr. atazanavir, ritonavir, kobicistat, itrakonazol, ketokonazol, posakonazol, vorikonazol, klaritromicin, telitromicin in eritromicin (razen če morebitna korist za bolnika odtehta tveganje, v tem primeru je treba bolnike skrbno spremljati glede neželenih učinkov krizotiniba), ter grenivko i n grenivkinim sokom, saj l ahko povečajo koncentracije krizotiniba v plazmi. Izogibati se je treba sočasni uporabi z močnimi induktorji CYP3A4, npr. karbamazepin, fenobarbital, fenitoin, rifampicin in šentjanževka, saj lahko zmanjšajo koncentracije krizotiniba v plazmi. Učinek zmernih induktorjev CYP3A4, npr. efavirenz in rifabutin, še ni jasen, zato se je treba sočasni uporabi s krizotinibom izogibati. Zdravila, katerih koncentracije v plazmi lahko krizotinib spremeni (midazolam, alfentanil, cisaprid, ciklosporin, derivati ergot alkaloidov, fentanil, pimozid, kinidin, sirolimus, takrolimus, digoksin, dabigatran, kolhicin, pravastatin: sočasni uporabi s temi zdravili se je treba izogibati oziroma izvajati skrben klinični nadzor; bupropion, efavirenz, peroralni kontraceptivi, raltegravir, i rinotekan, morfin, nalokson, metformin, prokainamid). Zdravila, ki podaljšujejo interval QT ali ki lahko povzročijo Torsades de pointes (antiaritmiki skupine IA (kinidin, disopiramid), antiaritmiki skupine III (amiodaron, sotalol, dofetilid, ibutilid), metadon, cisaprid, moksifloksacin, antipsihotiki) – v primeru sočasne uporabe je potreben skrben nadzor intervala QT. Zdravila, ki povzročajo bradikardijo (nedihidropiridinski zaviralci kalcijevih kanalčkov (verapamil, diltiazem), antagonisti adrenergičnih receptorjev beta, klonidin, gvanfacin, digoksin, meflokin, antiholinesteraze, pilokarpin) – krizotinib je treba uporabljati previdno. **Plodnost, nosečnost in dojenje:** Ženske v rodni dobi se morajo izogibati zanositvi. Med zdravljenjem in najmanj 90 dni po njem je treba uporabljati ustrezno kontracepcijo (velja tudi za moške). Zdravilo lahko škoduje plodu in se ga med nosečnostjo ne sme uporabljati, razen če klinično stanje matere ne zahteva takega zdravljenja. Matere naj se med jemanjem zdravila dojenju izogibajo. Zdravilo lahko zmanjša plodnost moških in žensk. **Vpliv na sposobnost vožnje in upravljanja strojev:** Lahko se pojavijo simptomatska bradikardija (npr. sinkopa, omotica, hipotenzija), motnje vida ali utrujenost; potrebna je previdnost. **Neželeni učinki:** Najresnejši neželeni učinki so bili hepatotoksičnost, ILD/pnevmonitis, nevtropenija in podaljšanje intervala QT. Najpogostejši neželeni učinki (≥ 25 %) so bili motnje vida, navzea, diareja, bruhanje, edem, zaprtje, povečane vrednosti transaminaz, utrujenost, pomanjkanje apetita, omotica in nevropatija. Ostali zelo pogosti (≥ 1/10 bolnikov) neželeni učinki so: nevtropenija, anemija, levkopenija, disgevzija, bradikardija, bolečina v trebuhu in izpuščaj. **Način in režim izdaje:** Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Izjemoma se lahko uporablja pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. **Imetnik dovoljenja za promet:** Pfizer Europe MA EEIG, Boulevard de la Plaine 17, 1050 Bruxelles, Belgija. **Datum zadnje revizije besedila:** 28.02.2019

**Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.**

**Vir: 1.** Povzetek glavnih značilnosti zdravila Xalkori, 28.02.2019

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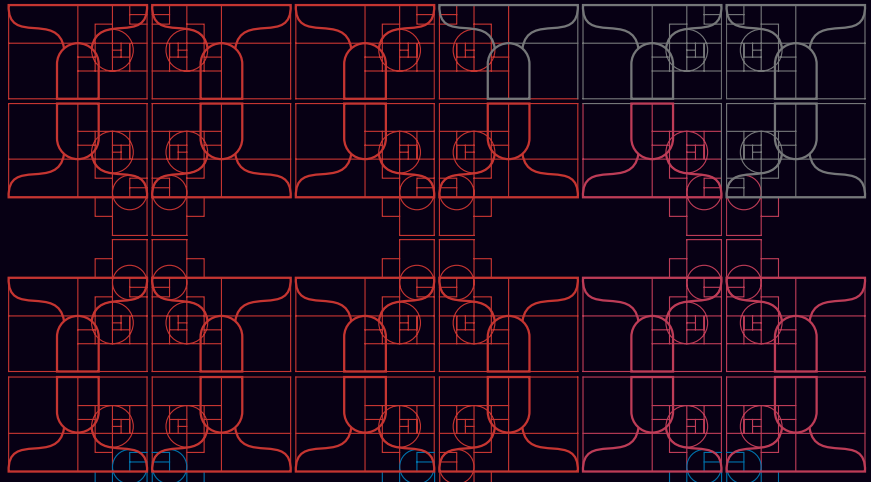

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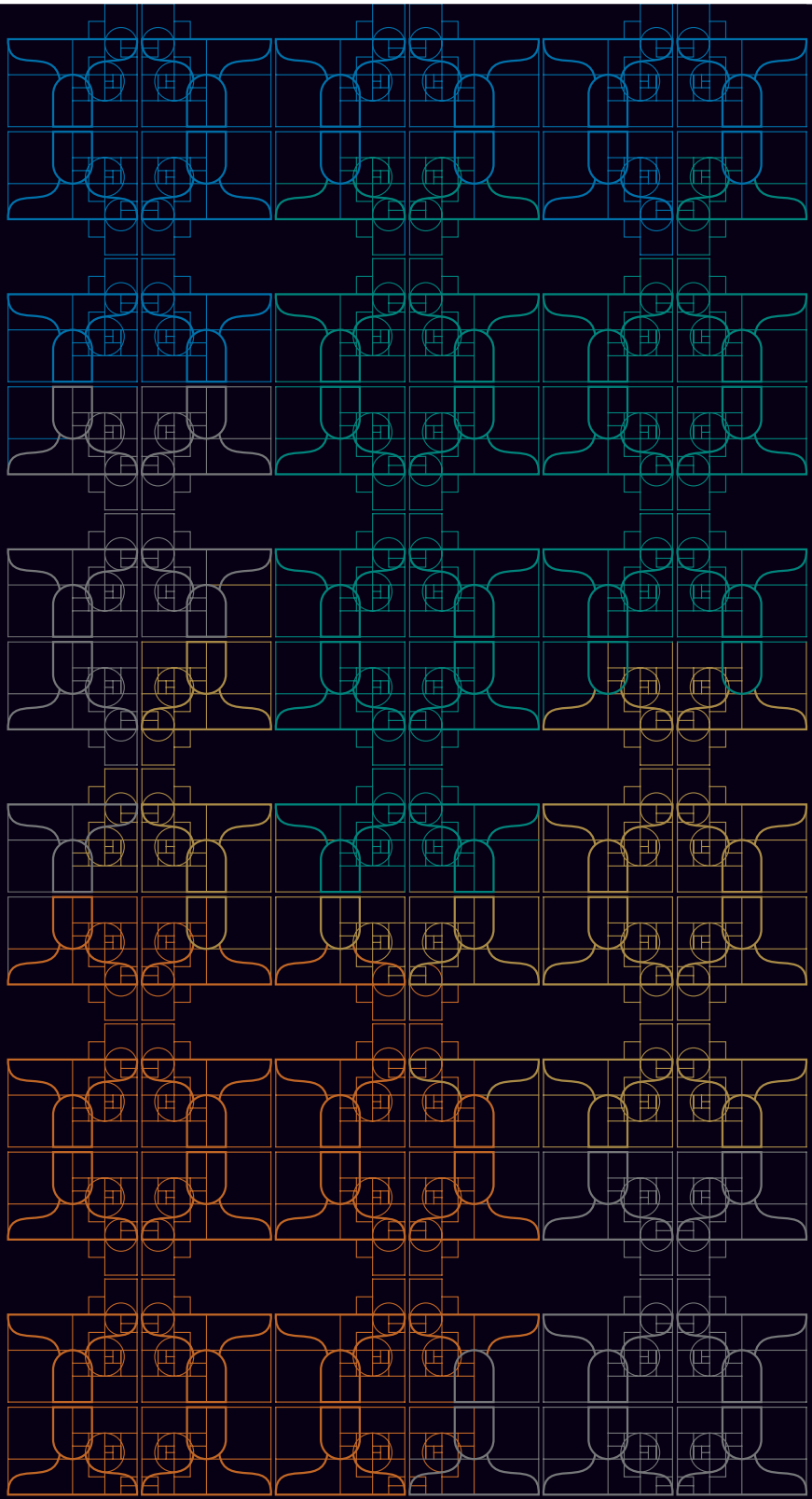

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