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Eisai

Prva in edina samostojna kemoterapija, ki v primerjavi z ostalimi možnostmi zdravljenja z enim zdravilom, pri bolnicah s predhodno že večkratno zdravljenim metastatskim rakom dojke, dokazano značilno podaljša celokupno preživetje.[1,2]

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Treba je razmisliti o antiemetični profilaksi, vključno s kortikosteroidi. **Preložitev odmerka med zdravljenjem:** Dajanje Halavena je treba preložiti, če se pojavi kaj od naslednjega: absolutno število nevtrofilcev (ANC) < 1 x 10^9/l, trombociti < 75 x 10^9/l ali nehematološki neželeni učinki 3. ali 4. stopnje. **Zmanjšanje odmerka med zdravljenjem:** Za priporočila za zmanjšanje odmerka ob pojavu hematoloških ali nehematoloških neželenih učinkov glejte celoten povzetek glavnih značilnosti zdravila. **Okvara jeter zaradi zasevkov:** Priporočeni odmerek pri blagi okvari jeter (stopnje A po Child-Pughu) je 0,97 mg/m^2 v obliki 2- do 5- minutne i. v. infuzije 1. in 8. dan 21-dnevnega cikla. Priporočeni odmerek pri zmerni okvari jeter (stopnje B po Child-Pughu) je 0,62 mg/m^2 v obliki 2- do 5- minutne i. v. infuzije 1. in 8. dan 21-dnevnega cikla. 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Dojenje. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Mielosupresija je odvisna od odmerka in se kaže kot nevtropenija. Pred vsakim odmerkom eribulina je treba opraviti pregled celotne krvne slike. Zdravljenje z eribulinom se lahko uvede le pri bolnikih z vrednostmi ANC ≥ 1,5 x 10^9/l in s trombociti > 100 x 10^9/l. Bolnike, pri katerih se pojavijo febrilna nevtropenija, huda nevtropenija ali trombocitopenija, je treba zdraviti v skladu s priporočili v celotnem povzetku glavnih značilnosti zdravila. Hudo nevtropenijo se lahko zdravi z uporabo G-CSF ali enakovrednim zdravilom v skladu s smernicami. Bolnike je treba skrbno nadzirati za znake periferne motorične in senzorične nevropatije. Pri razvoju hude periferne nevrotoksičnosti je treba odmerek prestaviti ali zmanjšati. Če začnemo zdravljenje pri bolnikih s kongestivnim srčnim popuščanjem, z bradiaritmijami, z zdravili, za katera je znano, da podaljšujejo interval QT, vključno z antiaritmiki razreda Ia in III, in z elektrolitskimi motnjami, je priporočljivo spremljanje EKG. Pred začetkom zdravljenja s Halavenom je treba popraviti hipokaliemijo in hipomagneziemijo in te elektrolite je treba občasno kontrolirati med zdravljenjem. Halavena ne smemo dajati bolnikom s prirojenim sindromom dolgega intervala QT. To zdravilo vsebuje majhne količine etanola (alkohola), manj kot 100 mg na odmerek. Eribulin je pri podganah embriotoksičen, fetotoksičen in teratogen. Halavena se ne sme uporabljati med nosečnostjo, razen kadar je to nujno potrebno. Ženske v rodni dobi naj ne zanosijo v času, ko same ali njihov moški partner dobivajo Halaven, in naj med zdravljenjem in še do 3 mesece po njem uporabljajo učinkovito kontracepcijo. Moški naj se pred zdravljenjem posvetujejo o shranjevanju sperme zaradi možnosti nepopravljive neplodnosti. **INTERAKCIJE:** Eribulin se izloča do 70 % prek žolča. Sočasna uporaba učinkovin, ki zavirajo jetrne transportne beljakovine, kot so beljakovine za prenos organskih anionov, P-glikoprotein, beljakovine, odporne na številna zdravila, z eribulinom se ne priporoča (npr. ciklosporin, ritonavir, sakvinavir, lopinavir in nekateri drugi zaviralci proteaze, efavirenz, emtricitabin, verapamil, klaritromicin, kinin, kinidin, dizopiramid itd). Sočasno zdravljenje z indukcijskimi učinkovinami, kot so rifampicin, karbamazepin, fenitoin, šentjanževka lahko povzroči znižanje koncentracij eribulina v plazmi, zato je ob sočasni uporabi induktorjev potrebna previdnost. Eribulin lahko zavira encim CYP3A4. Pri sočasni uporabi z učinkovinami, ki jih v glavnem presnavlja encim CYP3A4, se priporoča skrbno spremljanje zaradi povečanih koncentracij sočasno uporabljene učinkovine v plazmi. Če ima učinkovina ozek terapevtski razpon, je ne uporabljajte sočasno. **NEŽELENI UČINKI:** *Zelo pogosti (≥1/10):* nevtropenija (54,5 %), (3./4. stopnje: 48,3 %), levkopenija (22,1 %), (3./4. stopnje: 14 %), anemija (20,3 %), (3./4. stopnje: 1,4 %), zmanjšan apetit, periferna nevropatija (32,0 %), (3./4. stopnje: 6,9 %), glavobol, slabost (35,1 %), (3./4. stopnje: 1,1 %), zaprtost, driska, bruhanje, alopecija, artralgija in mialgija, utrujenost/astenija (52,8 %), (3./4. stopnje: 8,4 %), pireksija. *Pogosti (≥1/100 do <1/10):* okužba sečil, ustna kandidiaza, okužba zgornjih dihal, nazofaringitis, rinitis, febrilna nevtropenija (4,7 %), (3./4. stopnje: 4,6 %), trombocitopenija, limfopenija, hipokaliemija, hipomagneziemija, dehidracija, hiperglikemija, hipofosfatemija, nespečnost, depresija, disgevzija, omotičnost, hipoestezija, letargija, nevrotoksičnost, obilnejše solzenje, konjunktivitis, vrtoglavica, tahikardija, vročinski valovi, dispnea, kašelj, orofaringealna bolečina, epistaksa, rinoreja, bolečina v trebuhu, stomatitis, suha usta, dispepsija, gastroezofagealna refluksna bolezen, razjede v ustih, napihnjenost želodca, zvišanje alanin aminotransferaze (3,0 %), (3./4. stopnje: 1,1 %) in aspartat aminotransferaze, izpuščaj, pruritus, bolezni nohtov, nočno potenje, palmarno-plantarna eritrodisestezija, suha koža, eritem, hiperhidroza, bolečina v okončinah, mišični spazmi, mišično-skeletna bolečina in mišično-skeletna bolečina v prsih, mišična oslabelost, bolečina v kosteh, bolečina v hrbtu, vnetje sluznice (9,8 %), (3./4. stopnje: 1,3 %), periferni edem, bolečina, mrzlica, gripi podobna bolezen, bolečina v prsih, zmanjšanje telesne mase. *Občasni (≥1/1.000 do <1/100):* pljučnica, nevtropenična sepsa, ustni herpes, herpes zoster, tinitus, globoka venska tromboza, pljučna embolija, intersticijska pljučna bolezen, hiperbilirubinemija, angioedem, disurija, hematurija, proteinurija, odpoved ledvic. *Redki (≥1/10.000 do <1/1.000):* pankreatitis. 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Viri: (1) Povzetek glavnih značilnosti zdravila Halaven, november 2012; (2) Cortes J *et al. Lancet* 2011; 377: 914–23

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Dear authors, dear readers, dear reviewers of Radiology and Oncology. Our journal is steadily growing, and gaining international recognition. We are very pleased that Radiology and Oncology is now indexed in PubMed, starting the 2011 issues and onwards. Furthermore, the articles listed in PubMed Central are freely accessible. This achievement will greatly contribute to our visibility in international community, and citation index of the journal. However, the increase in quotation of our journal is expected with one or two years delay.

Radiology and Oncology has gained the Impact factor (IF) of 0.912 in the year 2012, which has unfortunately dropped substantially from the year before. However, we expect an increase of IF in 2013, due to the higher citation index of our journal on Thomson Reuters (Web of Knowledge). This variability in IF is quite common with the journals that have started indexing by Thomson Reuters. Our next challenge is to further grow our journal and to settle in the third quarter of the indexed journals in the field of Oncology. In order to achieve this goal, we have a team of dedicated and talented colleagues with a similar commitment. In addition to the Deputy Editors, the Editorial Board members contribute ideas and comments for improving the journal, and we expect from them to review at least 6 manuscripts per year. All of us continue to work on the quality of the published papers, and to gain trust of the international community.

By inclusion in world leading databases and indexes, the submission rate has increased considerably. Consequently also the rejection rate has increased, being now >60%. It is our strong belief that we need to maintain the quality of the published papers, and slowly grow the number of the published articles per issue. We encourage authors to submit through our editorial system at www.radioloncol.com high quality papers on almost all fields of oncology and radiology. On the other hand, we ask the reviewers to support our journal by their investment of their precious time for the reviewing process, and their objective but strict adherence to the scientific quality and novelty of the papers. Thank you for your effort.

The journal will adhere to the policy of the open access. The journal is freely accessible at the homepage of de Gruyter (http://www.degruyter.com/view/j/raon), and also through Versita homepage (http://www.versita.com/ro/). However, due to growing expenses of publishing, we are forced at the moment to urge the authors to subsidize the journal with voluntary fee of 500 EUR. Unfortunately we anticipate that 2013 is the last transitory year, in 2014 we will be charging the publication fee to all authors. Nevertheless, we will continue to publish hard copy of our journal.

The steady growth of the submissions puts great pressure on the Editors and Editorial Board. But we will continue on our endeavor to keep the journal independent from big publishers that have already expressed interest in us. We believe that in the independent way, we can in the best way promote Slovenian radiology and oncology in the world and also make our journal interesting for the international community. Thank you for your help and support, and please do so also in the future.

Best regards,

Prof. Gregor Serša, Ph.D.
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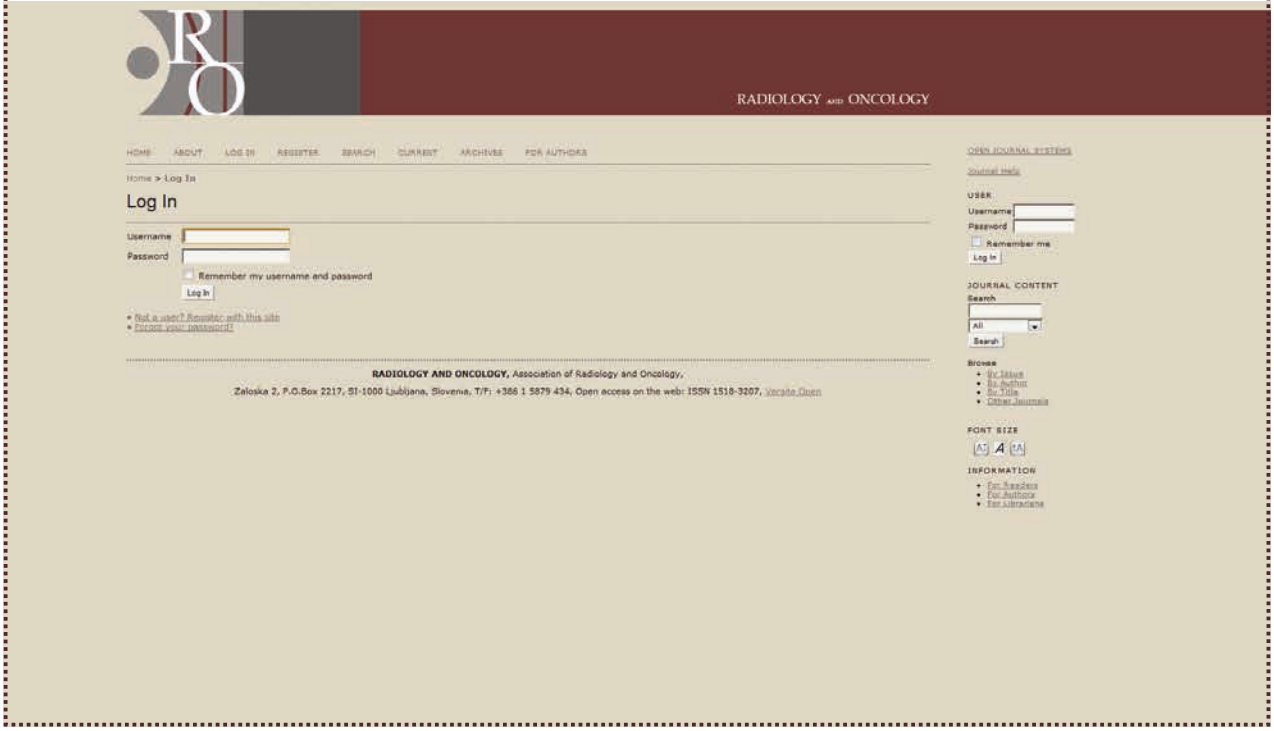

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review

Oral treatment with etoposide in small cell lung cancer – dilemmas and solutions

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Background. Etoposide is a chemotherapeutic agent, widely used for the treatment of various malignancies, including small cell lung cancer (SCLC), an aggressive disease with poor prognosis. Oral etoposide administration exhibits advantages for the quality of life of the patient as well as economic benefits. However, widespread use of oral etoposide is limited by incomplete and variable bioavailability. Variability in bioavailability was observed both within and between patients. This suggests that some patients may experience suboptimal tumor cytotoxicity, whereas other patients may be at risk for excess toxicity.

Conclusions. The article highlights dilemmas as well as solutions regarding oral treatment with etoposide by presenting and analyzing relevant literature data. Numerous studies have shown that bioavailability of etoposide is influenced by genetic, physiological and environmental factors. Several strategies were explored to improve bioavailability and to reduce pharmacokinetic variability of oral etoposide, including desired and undesired drug interactions (*e.g.* with ketoconazole), development of suitable drug delivery systems, use of more water-soluble prodrug of etoposide, and influence on gastric emptying. In addition to genotype-based dose administration, etoposide is suitable for pharmacokinetically guided dosing, which enables dose adjustments in individual patient.

Further, it is established that oral and intravenous schedules of etoposide in SCLC patients do not result in significant differences in treatment outcome, while results of toxicity are inconclusive. To conclude, the main message of the article is that better prediction of the pharmacokinetics of oral etoposide may encourage its wider use in routine clinical practice.

Key words: oral etoposide; bioavailability; pharmacokinetic variability; small cell lung cancer

Introduction

Etoposide is a topoisomerase II inhibiting anticancer drug, derived from podophyllotoxin. It has significant therapeutic activity in childhood leukemia, testicular tumors, Hodgkin's disease, large cell lymphomas and small cell lung cancer (SCLC).[1] In combined therapy with platinum compound (cisplatin or carboplatin), etoposide is used as a first-line therapy for SCLC, an aggressive disease with poor prognosis, which represents roughly 20% (15-25%) of all lung cancers.[2-7] With etoposide and cisplatin or carboplatin combination an overall response rate of approximately 75% can be anticipated. Radiation therapy to the thorax in addition to platinum/etoposide chemotherapy is associated with a small, but significant improvement in local control and overall survival in limited-stage disease.[8] Progress in the management of SCLC has been modest in recent years as initial results of cisplatinum plus irinotecan showed improved survival. However these results were not confirmed in subsequent trials and cisplatinum/etoposide chemotherapy remains cornerstone of

treatment for patients with SCLC.[9,10] In addition, some major contribution over the last 20 years has come also from radiotherapy.[11]

The mode of action of etoposide involves inhibition of topoisomerase II, a nuclear enzyme that is necessary for swivelling and relaxation of deoxyribonucleic acid (DNA) during replication and transcription. Etoposide inhibits the ability of topoisomerase II to relegate cleaved nucleic acid molecules by the formation and stabilisation of a topoisomerase II-etoposide-DNA ternary complex and thus increases topoisomerase II-mediated DNA breakage. The covalent topoisomerase II-cleaved DNA complex is normally a short-lived intermediate in the reaction and is tolerated by the cell. However, at high concentrations it has cytotoxic effects, probably due to impaired DNA repair, leading to apoptosis.[12,13]

The activity of etoposide is dose- and schedule-dependent, and etoposide efficacy might be improved markedly with repeated drug administration.[12-17] Etoposide directly interacts with the ATP-bound enzyme monomer in such a way that each molecule of etoposide stabilizes only a single-stranded break. Depending on the dose of etoposide, single-stranded or double-stranded DNA breaks are generated.[13,14] Furthermore, the inhibition of topoisomerase II by etoposide is reversible and discontinuation of ternary complex allows quick DNA repair and diminishes the cytotoxicity of the drug. Thus, prolonged exposure to etoposide could increase the anticancer activity of the drug.[12] Moreover, topoisomerase II is significantly expressed only in dividing cells during S and G2 phases of the cell cycle. Chronic scheduling maximizes the likelihood of exposing malignant cells to etoposide during sensitive periods of the cell cycle.[14] However, myelosupression as the dose-limiting toxicity should be taken into account when planning the chemotherapy regimen.[14] The chemotherapy treatment is therefore given in cycles, attacking cancer cells at their most sensitive periods, and allowing normal body cells time to recover.

A wide range of doses and schedules of etoposide are in use, depending on the treated disease. In patients with solid tumors, including SCLC, lower doses, such as 50–100 mg/m^2/day over 3-5 days are suggested by some authors, while other authors suggest prolonged schedule as superior.[18-20] In most regimens etoposide is administered in cycles, which are usually repeated every 3-4 weeks.[1,21]

Etoposide is commercially available in both intravenous and oral formulations. The oral formulation exhibits advantages for the patient as well as economic benefit compared with the intravenous one. The work from Liu and coworkers has indicated that 89% of incurable cancer patients preferred oral over intravenous chemotherapy, predominantly because of the convenience of administration, problems with intravenous access or needles, and a better chemotherapy-taking environment (outside of the clinic).[22] In general, the quality of life of patients receiving palliative chemotherapy for advanced cancer was significantly poorer in patients treated at hospital compared with those treated at home.[23] Most importantly, the oral formulation may provide an attractive alternative for patients who are unable to or have difficulty making the necessary and frequent visits to receive intravenous therapy.[24] In comparison with intravenous infusion, oral administration of etoposide represents a significant cost saving for hospital and health insurance. Results of an economic analysis, which was conducted within a randomised multicentre study comparing the use of intravenous etoposide versus oral etoposide treatment in SCLC patients, reported a 17% savings for patients receiving the oral regimen. The following costs were examined: antineoplastic drugs, intravenous fluids, supplies used for chemotherapy administration, and chemotherapy administration procedure fees.[25] Furthermore, the introduction of oral etoposide into combination chemotherapy regimens may shorten the hospitalization period and thus reduce non-drug related treatment costs as well.[26]

However, despite the numerous advantages of oral therapy, the intravenous formulation has been used more extensively.[24] The main drawback of oral etoposide is its incomplete and variable bioavailability.[27] Approximately 50% (30-97%) of the oral dose is bioavailable when compared with the intravenous route.[26-29] This means that the area under the curve (AUC) of a given oral dose is approximately 50% of what would be achieved after an intravenous dose. Additionally, variability in bioavailability was observed both within and between patients. Hande *et al.*[30] reported a mean etoposide bioavailability at a dose of 50 mg 64.6%, with intrapatient coefficient of variation (CV) 22.6% and interpatient CV 34.8%. A large CV suggests that some patients are receiving inadequate drug exposure, resulting in suboptimal tumor cytotoxicity, whereas others may be at risk for excess toxicity.[30] This is particularly important when using drugs with a narrow therapeutic window, like etoposide.[31] Additionally, a linear pharmacokinetic behaviour of oral etoposide was shown only for doses up to 200 mg.[32] In higher doses, the percentage of

absorbed etoposide may decrease while the CV in oral etoposide bioavailability may even increase.[29]

The absorption of etoposide is likely to depend on a number of interacting factors, the identification of which may be difficult. The improvement in the absorption of etoposide and the reduction in its variability, remain important goals to facilitate the clinical use of oral etoposide.[27]

This review focuses on the impact of various factors influencing bioavailability of etoposide, provides possibilities for its improvement and suggestions for treatment optimisation to ensure comparable pharmacokinetic parameters of oral and intravenous application. The review is restricted to treatment with etoposide in SCLC patients, for which etoposide-platinum doublet still represents the most effective standard therapy.

Bioavailability of oral etoposide

Bioavailability is the extent to which an administered drug enters the systemic circulation. It is defined by the AUC of the dose delivered by oral administration divided by the AUC of the intravenous application of the same dose. AUC of etoposide correlates with safety and efficacy as well as overall survival of patients with SCLC.[33,34] Oral administration may increase AUC variability because the drug must undergo additional processes such as being transported across the intestine, passing through the liver, and entering the systemic plasma circulation.[30] Those are pharmacokinetic processes called absorption, first-pass metabolism and elimination prior entering the systemic circulation.[35] Variation in the pharmacokinetics of a drug in a patient population is the net result of many complex interactions between genetic, physiological and environmental factors.[36] The impact of these factors on pharmacokinetic processes and consequently AUC and bioavailability is described in some details in the following sections.

Genetic factors

Genetic characteristics of metabolizing enzymes and transporters may influence drug blood level. Inherited differences in enzymes and transporters are known examples of pharmacogenetic variability. These factors may lead to inter-individual variation.[37]

Etoposide is a substrate of the efflux membrane transporters (ABC transporters) and metabolizing enzymes, which are located in the intestine and liver. Efflux membrane transporters limit the absorption of orally administered drug in the intestine and facilitate the pre-systemic elimination via bile, leading to poor bioavailability of drugs.[38] A study aiming to characterize the regional absorptive and secretory kinetics of etoposide in rabbit intestinal tissues revealed that the apical to basolateral (*i.e.* absorptive) transport of etoposide was not apparently mediated by specialized transporters, whereas basolateral to apical (*i.e.* secretory) transport by intestinal tissues was concentration dependent and saturable, mediated by transporters.[39]

Etoposide was shown to be a substrate of several ABC transporters, notably ABCB1 (MDR1, P-glycoprotein, P-gp) and ABCC1 (MRP1), ABCC2 (MRP2), ABCC3 (MRP3) and ABCG2 (BCRP).[40-45] The location of these transporters in enterocytes and hepatocytes is marked in Figure 1. Allen *et al.*[40] showed that ABCB1 can have a substantial effect on the oral availability of etoposide, while ABCG2 can have a little effect on oral etoposide pharmacokinetics. *In vitro* data showed that ABCC2 and ABCC3 can moderately transport etoposide.[42,43] Etoposide was shown to be a good ABCC1 substrate.[44] Lagas *et al.*[46] studied the impact of ABCB1, ABCC2 and ABCC3 on the pharmacokinetics of etoposide in wild-type, ABCC2$^{-/-}$, ABCB1a/1b$^{-/-}$, and ABCB1a/1b;ABCC2$^{-/-}$ mice. Results demonstrated that ABCB1, which is located in apical membrane of enterocytes, restricted the oral (re)uptake of unchanged etoposide, and mediated its excretion across the gut wall, while hepatobiliary excretion of both etoposide and etoposide glucuronide were almost entirely dependent on ABCC2, and not on ABCB1. Additionally, ABCC3 was responsible for the efflux of etoposide glucuronide from the liver to the systemic blood circulation, especially when ABCC2 was absent. Authors concluded that pharmacokinetics of etoposide and etoposide glucuronide is significantly affected by ABCB1, ABCC2, and ABCC3 and that high inter-individual variability of etoposide may be explained by variation in transporter expression or activity.[46]

Drug metabolism principally occurs in the liver, but also other tissues, like intestinal mucosa, must be considered.[12] Etoposide is O-demethylated primarily by cytochrome P450 (CYP) 3A4 and to a lesser extent by CYP3A5.[47,48] Furthermore, CYP1A2 and 2E1 are involved as the minor enzymatic components in this metabolic pathway.[49] O-demethylated metabolite of etoposide is catehol.[47] Catehol can undergo oxidation to form an ortho-quinone (and vice versa) via formation of a semi-quinone free radical. Studies suggest that radical species, in addition to the catehol and ortho-quinone, might also be involved in

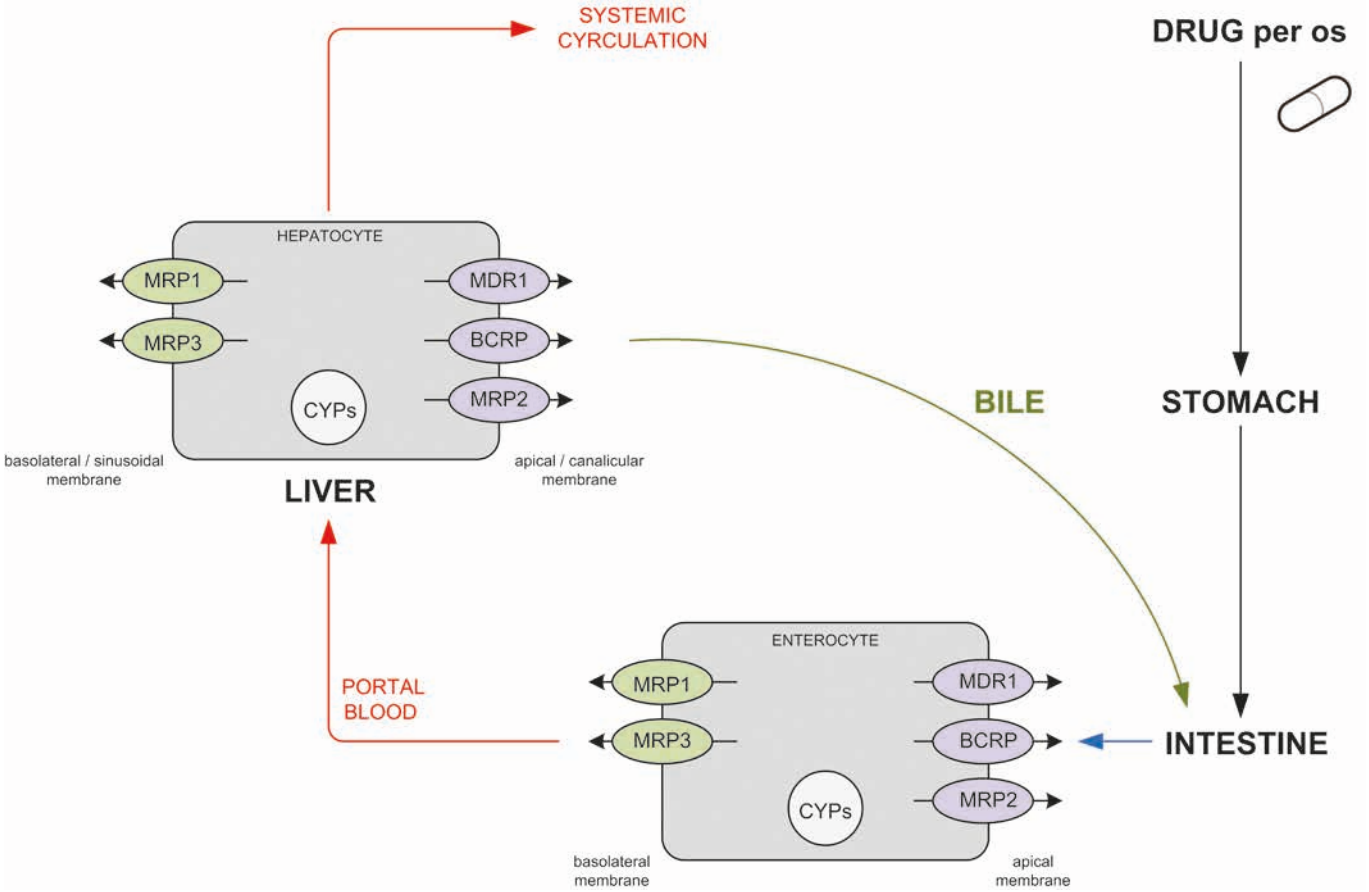

FIGURE 1. Schematic representation of the efflux transporters and metabolic enzymes (marked) possibly influencing etoposide bioavailability (modified by Ref. 58).

MDR1 = multi-drug resistance protein (ABCB1, P-glycoprotein); MRP1-3 = multidrug resistance-associated proteins (ABCC1-3); BCRP = breast-cancer resistance protein (ABCG2); CYPs = cytochrome P450

the cytotoxicity of etoposide.[50-52] Ortho-quinone is attenuated by glutathione conjugation.[53] The second way of etoposide metabolism is glucuronidation, mainly catalyzed by UGT1A1. Although etoposide glucuronidation is also catalyzed by UGT1A8 and 1A3, their activities are much lower than that of UGT1A1. The predominant form of etoposide glucuronide in liver and intestine is phenolic glucuronide, whereas two alcoholic glucuronides are the minor metabolites.[54,55] CYP isoform was reported to be directly involved in the oxidative metabolism of etoposide, therefore variation of the intestinal activity of this CYP isoform may directly affect the bioavailability of etoposide.[12]

As shown in Figure 1, once etoposide as a drug crosses the apical membrane of the enterocyte, a part is effluxed back to the intestinal lumen by ABC transporters ABCB1 (MDR1), ABCC2 (MRP2) and ABCG2 (BCRP) and part is possibly subjected to intestinal first-pass metabolism by metabolizing enzymes. The fraction of drug absorbed into the mesenteric blood circulation enters into the liver via the portal vein and may be transported from hepatocytes into the bile (metabolized or non-metabolized) or to the systemic circulation.[56,57]

Many enzymes and secretory transporters are subject to genetic polymorphisms with functional consequences. A complete description of these polymorphisms can be found in the article of Robert *et al*.[59] as well as on the dedicated websites (www.pharmgkb.org, www.imm.ki.se/CYPalleles, www.hapmap.org, www.ncbi.nlm.nih.gov/projects/SNP/). Genetic polymorphisms might be a one of the factors causing the interindividual differences in etoposide bioavailability. However, no study evaluated the association of these polymorphisms with etoposide bioavailability. Only one study explored the effect of polymorphisms in the *ABCB1* on etoposide pharmacokinetics. In this study *ABCB1* 3435TT genotype was associated with low-

er volume of distribution and contributed significantly to the inter-individual variability observed in etoposide pharmacokinetics.[60] However, effects of *ABCB1* polymorphisms, particularly 3435C>T, on digoxin plasma levels after oral administration were extensively studied.[61,62]

Summarized, genetic variability and functional polymorphisms in ABC transporters are relevant pharmacological factors that have to be considered together with drug-metabolizing enzymes, whose activity show a large degree of interindividual variability.[37]

Physiological factors

The metabolism of etoposide is partly hepatic, therefore hepatic insufficiency causes an increase in bioavailability of etoposide due to decreased first-pass effect.[12,63] However, Hande *et al*.[29,30] stated that variation in hepatic metabolism probably does not explain differences between oral and intravenous drug administration because etoposide's hepatic clearance rate is not high. Aita *et al*.[64] studied the pharmacokinetics of oral etoposide in patients with hepatocellular carcinoma and underlying cirrhosis. They found slightly high etoposide bioavailability and clearance resulting in a normal AUC.[64] Bioavailability of etoposide was not affected neither in patients with gastric carcinoma nor in patients with gastrectomy.[65]

Ando *et al*.[66] showed that gender does not affect the pharmacokinetics or pharmacodynamics of oral etoposide, while patient's age affect pharmacodynamics. Although there was no difference in pharmacokinetics between elderly (ages 75 years or older) and younger patients, equivalent exposure to etoposide resulted in greater pharmacodynamic sensitivity in elderly patients.[66,67] Contrary to the results of Ando *et al*.[66], Miyazaki *et al*.[68] showed that, although there were no significant differences in mean AUC values, plasma clearance and urinary excretion of oral etoposide, there were significant differences in elimination half life and bioavailability in the elderly group, compared with the younger adult group; both were significantly increased in the elderly patients. In comparison with intravenous administration, there was no statistically significant difference in these parameters between the elderly and younger adult group.[68]

Environmental factors

Cancer patients commonly receive multiple medications, including chemotherapy and supportive care drugs, the majority of them are elderly, and so require medications for co-morbid conditions, and have age-related decline in hepatic and renal function that reduce their ability to metabolize and eliminate drugs.[69] The possibility of drug-drug and drug-food interactions is therefore high. Interactions that affect bioavailability are usually pharmacokinetic interactions involving metabolising enzymes and drug transporters.

Many clinical and preclinical studies are documented wherein CYP450 and/or ABCB1 and/or UGT1A1 were prominently implicated to play an important role in etoposide bioavailability. Several CYP3A4 and ABCB1 inhibitors were described to enhance etoposide bioavailability, such as platinum compounds, cyclosporine A, hydroxyzine, quinidine, 20(S)-ginsenoside Rh2, GF120918, kaempferol, morin, quercetin, verapamil, PSC833 (valspodar), ketoconazole, piperine analogue and curcumin.

A study exploring the potential interaction between the two platinum drugs, cisplatin and carboplatin, and the oxidative metabolism of etoposide demonstrated that the interaction between etoposide and platinum drugs is small and the clinical impact is unlikely to be significant. The exact mechanism of interaction is unknown but may involve inhibition of etoposide metabolism.[70]

Cyclosporine A, hydroxyzine and quinidine were shown to increase systemic etoposide exposure through inhibition of the multidrug transporter ABCB1.[71-73] Increased AUC and additionally peak concentration (c_{max}) was observed by co-administration of an ABCB1 inhibitor 20(S)-ginsenoside Rh2, a trace constituent of ginseng.[74] Increased oral uptake of etoposide due to ABCB1 inhibition was shown also by GF120918.[40]

The oral bioavailability of etoposide increased significantly when the drug was combined with kaempferol, morin or quercetin, three ingredients of dietary supplements. Additionally, kaempferol also increased c_{max} of oral etoposide. A possible explanation to enhanced bioavailability of oral etoposide by these three aforementioned drugs could be due to an inhibition of CYP450-catalyzed metabolism and ABCB1-mediated efflux in the intestine and/or liver.[75-77] Similar results were obtained with verapamil and PSC833 (valspodar), a CYP3A and ABCB1 inhibitor.[40,78,79]

Ketoconazole was also shown to increase systemic exposure of oral etoposide. Ketoconazole is a commonly used antifugal drug known for its inhibitory effect on CYP3A4, UGT1A1, and ABCB1. However, Peng Yong *et al*.[80] reported that increased

TABLE 1. Quantitative effects of etoposide interactions with various drugs that can potentially affect etoposide bioavailability

Drug	Quantitative effect (method)	Reference
Cisplatin or carboplatin	Increased AUC of etoposide (8% with carboplatin, 28% with cisplatin) (patients, in vitro methods)	Thomas *et al.*[70]
Cyclosporine A	Mean increase of AUC of etoposide 89% (patients)	Bisogno *et al.*[71]
Hydroxyzine	Transport of etoposide increased from the luminal site to the serosal site in the jejunum by 2-fold (reduced efflux) (everted rat gut sacks)	Kan *et al.*[72]
Quinidine	Increased serum concentration of oral etoposide more than 2-fold (everted gut sacks prepared from rat jejunum and ileum)	Leu *et al.*[73]
20(S)-Ginsenoside Rh2	AUC of intragastric administration of etoposide in rats increased by 4.52-fold; c_{max} increased by 2.54-fold (rats)	Zhang *et al.*[74]
GF120918	Increased plasma levels of etoposide after oral administration 4-5-fold (wild-type mice)	Allen *et al.*[40]
Kaempferol	The absolute bioavailability of oral etoposide increased by 11.0-12.3%; the relative bioavailability of oral etoposide increased 1.15-1.64-fold; significantly increased c_{max} (rats)	Li *et al.*[75]
Morin	Increased absolute bioavailability of oral etoposide by 35,9% (rats)	Li *et al.*[76]
Quercetin	Increased absolute bioavailability of oral etoposide to 12.7 (quercetin 5 mg/kg) or 13.6% (quercetin 15 mg/kg) (rats)	Li *et al.*[77]
Verapamil	Increased absolute bioavailability of oral etoposide by 1.38 to 1.47-fold (rats)	Piao *et al.*[78]
PSC833 (valspodar)	Increased plasma concentration of orally administered etoposide at least 10-fold (rats)	Keller *et al.*[79]
Ketoconazole	Increased AUC of oral etoposide by a median of 20% (patients)	Peng Yong *et al.*[80]
Food (standard breakfast: milk, cornflakes, sugar, egg, sausage, bread, margarine, orange marmalade and coffee or tea, sweetened to taste)	Decreased AUC of oral etoposide from 40.8±10.7 $\mu gml^{-1}h1.7m^{-2}$ to 35.8±9.8 $\mu gml^{-1}h1.7m^{-2}$ (patients)	Harvey *et al.*[81]
Grapefruit juice	Decreased AUC of oral etoposide of 26.2%; median absolute bioavailability of 50 mg oral etoposide with and without pretreatment with grapefruit juice was 52.4% and 73.2%, respectively (patients)	Reif *et al.*[82]
Piperine analogue	Increased absolute bioavailability of oral etoposide 2.32-fold (in vitro and animal-derived models)	Najar *et al.*[83]
Curcumin	Increased AUC of oral etoposide by 35.1% (curcumin 2 mg/kg) and 50.8% (curcumin 8 mg/kg); increased F of oral etoposide by 36.0% (curcumin 2 mg/kg) and 52.0% (curcumin 8 mg/kg) (rats)	Lee *et al.*[84]

systemic exposure to etoposide by ketoconazole modulation is most likely mediated through the inhibition of etoposide metabolism in the liver rather than the inhibition of the transporters in the intestine.[80]

The results of a study conducted by Harvey *et al.*[81] showed that food does not significantly interfere with etoposide bioavailability, at least at doses of 100 mg. Grapefruit juice increases the bioavailability of some orally-administered drugs that are metabolized by CYP3A4. However, Reif *et al.*[82] reported that coadministration of grapefruit juice causes an unexpected decrease in systemic exposure of oral etoposide. A possible explanation for the observed effect may be an alteration of the intestinal ABCB1-mediated transport.[82] It was also shown that piperine analogue, a natural alkaloid of peppers significantly enhanced the plasma levels of etoposide. A mechanistic evaluation of this effect presented by Najar *et al.*[83] has shown that piperine analogue modifies ABCB1 and CYP3A4-mediated drug disposition mechanisms to enhance the intestinal absorption of etoposide, while preventing its efflux and metabolic inactivation during its transit from intestine to the systemic circulation. A similar effect was observed with curcumin which significantly increased the bioavailability of oral etoposide, while the pharmacokinetics of etoposide after intravenous application was not affected. Therefore, the enhanced oral bioavailability of etoposide in the presence of curcum might be due to inhibition of ABCB1 in the small intestine and possibly due to reduced first-pass metabolism via CYP3A also in the small intestine.[84]

Known interactions of oral etoposide with various drugs including their quantitative effects are summarized in Table 1. To our knowledge, interactions with other drugs are not well documented; however, this does not necessarily mean no interactions exist.

Some other drugs, like ifosfamide[34], phenytoin and phenobarbitone[85], also modify systemic expo-

sure (reduced AUC) of etoposide when administered concomitantly. However, in all these cases etoposide was administered intravenously.

On the other hand, low and variable etoposide bioavailability may be related also to its poor solubility in water and chemical instability in physiological fluids. Etoposide's aqueous solubility is considered as extremely low. The mean solubility of etoposide at 37°C over the pH range 1.30 to 10 is 116.44 to 167.25 μg/ml, respectively.[86] Assuming that stomach and intestine contain approximately 250 ml of fluid, the initial amount of solute in upper gastrointestinal tract is approximately 30 to 40 mg. Therefore, solubility may play an important role in higher doses.[39] Extensive degradation of etoposide is observed at pH 1.30 and 10. The intrinsic dissolution rate of etoposide increase with temperature, however, its magnitude is far less than 1.0 mg/min/cm^2 at 37°C, *i.e.* the absorption is limited by the dissolution rate. Additional proof for etoposide absorption to be dissolution rate limited rather than permeation rate limited is its partition coefficient between n-octanol and water which is 9.94 at 25°C, reflecting etoposide's high lipophilicity and consequently good permeability. The low aqueous solubility and slow intrinsic dissolution rate may account for the low and variable bioavailability of the drug. However, the problem of poor drug dissolution rate was resolved by the development of hydrophilic preparation: a soft gelatine capsule containing etoposide in the form of solution.[87] Additional factors that could contribute to the low and erratic bioavailability of etoposide is its chemical instability in physiological (gastric and intestinal) solutions. It is known that pH of the gastrointestinal tract ranges from 1 to 8. Considering etoposide's pH stability range, its maximal stability is at pH of 5-6.15, while it rapidly degrades at pH<2.03 and pH>8.[30,86] *In vitro* studies showed that the decrease in stability in intestinal fluid at pH 7.5 is concentration-dependent while there is no concentration effect on stability in gastric fluid at pH 3.0.[88]

Safety and efficacy of oral versus intravenous etoposide in SCLC

Safety and efficacy were shown to correlate with AUC of etoposide. Oral administration may increase the variability in AUC and may lead to a greater variability in safety and efficacy of oral etoposide.[30]

The first randomized phase II study compared 3-day oral *vs*. intravenous etoposide schedule in combination with cisplatin in SCLC patients, and assuming 50% bioavailability. Results of this study showed that overall response rates (complete and partial response), time to progression and survival were comparable for both treatment arms. Overall toxicity for both treatment arms was similar and included neutropenia, thrombocytopenia, anemia, alopecia, nausea, diarrhea, vomiting and weight loss. Septic episodes in neutropenic patients as well as moderate to severe anemia and more than 10% weight loss occurred more frequently with the intravenous when compared with the oral treatment. Based on this data it was concluded that the oral treatment regimen could be a suitable substitute for those patients to whom parenteral therapy cannot be given.[24]

Comparable results in terms of response were obtained in another SCLC study which compared safety and efficacy of intravenous and oral etoposide alone, in a 5-day schedule and not assuming 50% bioavailability. Intravenous dose was 80 mg/m^2/day while oral dose was 130 mg/m^2/day. Each study gave a similar response rate. The major dose-limiting factor, leukopenia, was observed more frequently in the intravenous administration. Other side effects were anemia, thrombocytopenia, anorexia, nausea, and alopecia[89,90]

Yet, two other randomized studies compared oral and intravenous etoposide administration in combination with cisplatin for the treatment of SCLC patients. In both studies etoposide was administered intravenously for 3 days and oral etoposide was administered for 21 days. Self-evidently, the daily dose of intravenously administered etoposide was higher than the dose of orally administered one, while the cumulative dose of etoposide per cycle was higher for orally administered etoposide. Results of both studies showed that the two schedules of etoposide in combination with cisplatin did not result in significant differences in treatment outcome with respect to tumor response and survival. However, a significantly greater rate of hematologic toxicity was noted in intravenous etoposide treatment schedule in the first study[91] and in oral etoposide treatment schedule in the second one.[92]

As expected, two randomized trials in patients with SCLC demonstrated that oral etoposide alone was inferior to intravenous combination treatment. Of note, although being less effective, oral etoposide alone was associated in the first trial with increased toxicity. However, in both studies intravenous etoposide was used in combined regimens with cisplatin or cyclophosphamide, doxorubicin,

and vincristine, while oral etoposide was administered as monochemotherapy. Treatment schedules of oral and intravenous etoposide were also very different.[93,94]

Aforementioned clinical trials are described in detail in Table 2.

Improvement of bioavailability of oral etoposide

Concomitant medications

Several strategies were explored to improve low and variable bioavailability of oral etoposide. A potential strategy for diminishing variability of oral etoposide is to minimize the sources of variability. Cancer patients are at especially high risk for drug interactions because they commonly receive multiple drugs. In addition, it is estimated that 50% of cancer patients use alternative and herbal medicines, often without their doctor's knowledge. To diminish these risks, it is important to take an accurate medication history which should be updated at each visit. However, all predictable drug interactions are not always avoidable.[69]

Some drugs were reported to be intentionally used to modulate oral availability of etoposide when administered concomitantly. The increase in bioavailability is a consequence of inhibition of metabolic enzymes and/or efflux transporters. One of these drugs is ketoconazole.[80] Combined use of etoposide with inhibitors of metabolizing enzymes and/or efflux transporters increases etoposide's bioavailability. Allen *et al*.[40] stated that raising the bioavailability closer to 100% might eliminate some variability and allow better control of etoposide exposure. On the contrary, Peng Yong *et al*.[80] showed that ketoconazole does not reduce the variability.

However, modulation of intestinal absorption of drugs that are substrates of metabolic enzymes and transporters is further complicated by the recognition that polymorphic enzymes and transporters can modulate drug uptake.[95]

Impact on drug and/or drug delivery system

To maximise bioavailability of oral etoposide, efforts should focus on ensuring rapid drug dissolution in the upper gastrointestinal tract, or delaying drug release to target the upper colon. These suggestions are based on results of directional study of etoposide from rabbit small intestine and colon which showed that secretory permeability was greatest in the ileum, whereas values in the upper small intestine and colon were approximately equal, and represented only 50% of the value in the ileum.[39]

Moreover, Zhang *et al*.[96] have successfully incorporated etoposide into various modified nanostructured lipid carriers. Pharmacokinetic studies revealed improved relative bioavailability (more than 3.5-fold) of etoposide nanostructured lipid carriers to etoposide suspension in rats after oral administration. They elucidated that the enhanced bioavailability by the modified nanostructured lipid carrier formulation might be attributed to uptake of nanoparticles through the GI tract, increased permeability by surfactants, and decreased degradation and clearance.[96] Furthermore, Wu *et al*.[97] developed a phospholipid complex self-emulsifying drug delivery system. Compared with etoposide suspension, the relative bioavailability of this formulation after oral administration in rats was enhanced by 60.21-fold.[97] Zhang *et al*.[98] used natural solubilizer rubusoside to form etoposide-rubusoside nanoparticles. This method showed a better solubilization effect and capability of improving physical and chemical stability profiles than a softgel capsule containing etoposide in a vehicle consisting of citric acid, glycerin, purified water, and polyethylene glycol 400. This may improve bioavailability and clinical efficacy as well as improve safety, benefiting from the GRAS (generally regarded as safe) status of rubusoside.[98] On another point, Mo *et al*.[99] suggested N-octyl-O-sulfate chitosan to be used as a formulation excipient for etoposide, since it has a potential by inhibiting ABCB1 to improve the absorption of etoposide.

Etoposide phosphate, a more water-soluble prodrug of etoposide has also been suggested for oral administration in an attempt to increase bioavailability and reduce inter-individual variability. Chabot *et al*.[100] reported a 19% higher extent of absorption for etoposide phosphate compared with literature data for oral etoposide while Sessa *et al*.[101] reported comparable or only slightly better bioavailability of etoposide phosphate compared with oral etoposide. de Jong *et al*. [102] found a small significant increase in bioavailability but inter-individual variability of bioavailability appeared to be unaltered.

Influence on the rate of gastric emptying

Joel *et al*.[27] investigated the use of agents that may influence etoposide stability in gastrointestinal tract and, thereby, bioavailability. Results showed that drugs that influence the rate of gastric empty-

TABLE 2. Clinical trials evaluating safety and efficacy of oral vs. intravenous (i.v.) etoposide regimen in SCLC

Trial(ref.)	Sample size	Treatment regimen	Results (oral etoposide regimen vs. i.v. etoposide regimen)			
			ORR (%)	mPFS (months)	mOS (months)	toxicity
Randomized phase II[24]	83 patients	*i.v. etoposide regimen (41 patients):* cisplatin 100 mg/m^2 i.v. day 1, etoposide 120 mg/m^2 i.v. day 1-3 *oral etoposide regimen (42 patients):* cisplatin 100 mg/m^2 i.v. day 1, etoposide 120 mg/m^2 i.v. day 1 and 240 mg/m^2 orally day 2 and 3 Every 4 weeks, maximum of 6 cycles.	50 vs. 59	5.9 vs. 6.6	8.6 for either treatment arm	hematologic toxicity comparable in both treatment arms, infectious episodes, moderate to severe anemia and weight loss more predominant with the i.v regimen
Randomized phase II[89,90]	47 patients	*i.v. etoposide regimen (22 patients):* etoposide 80 mg/m^2 i.v. 5 consecutive days *oral etoposide regimen (25 patients):* etoposide 130 mg/m^2 orally 5 consecutive days	similar for either treatment arm (PR: 28 vs. 36.4)	/	/	leukopenia observed in 32% patients of the oral administration and in 59% patients of the i.v. administration
Randomized trial[91]	21 patients	*i.v. etoposide regimen (14 patients):* cisplatin 80 mg/m^2 i.v. day 1, etoposide 100 mg/m^2 i.v. day 2, 3 and 4 *oral etoposide regimen (7 patients):* cisplatin 80 mg/m^2 i.v. day 1, etoposide 50 mg orally day 3-23 Both regimens were repeated every 4 weeks.	86 vs. 64	/	no significant difference	hematologic toxicity less severe for oral regimen than for i.v. regimen
Randomized phase III[92]	306	*i.v. etoposide regimen:* cisplatin 25 mg/m^2 i.v. 3 days, etoposide 130 mg/m^2 i.v. 3 days Regimen was repeated every 21 days for 8 cycles. *oral etoposide regimen:* cisplatin 33 mg/m^2 i.v. 3 days, etoposide 50 mg/m^2 orally 21 days Regimen was repeated every 28 days for 6 cycles.	14 vs. 15 (PR: 47 vs. 42)	7 months for either treatment arm	9.9 vs. 9.5	lethal toxicity due to neutropenia and infection: in 10% of patients on oral etoposide regimen and in 4% on i.v. etoposide regimen (difference not statistically significant)
Randomized trial[93]	339 patients	*i.v. etoposide regimen (168 patients):* standard intravenous regimen of etoposide and vincristine, or cyclophosphamide, doxorubicin, and vincristine, 4 cycles *oral etoposide regimen (171 patients):* etoposide 50 mg orally twice daily for 10 days, 4 cycles	45 vs. 51	/	4.3 vs. 6.1	grade 2 or worse haematological toxicity: in 29% of patients on oral etoposide regimen and in 21% on i.v. etoposide regimen
Randomized trial[94]	155 patients	*i.v. etoposide regimen (80 patients):* intravenous regimen consisting of alternating cycles of etoposide and cisplatin and cyclophosphamide, doxorubicin, and vincristine *oral etoposide regimen (75 patients):* etoposide 100 mg orally twice daily for 5 days Both regimens were repeated every 21 days for 6 cycles.	32.9 vs. 46.3	3.6 vs. 5.6	4.8 vs. 5.9	toxicity similar in the two treatment arms

ORR = overall response rate; mPFS = median progression free survival; mOS = median overall survival; PR = partial respons

ing (metoclopramide, propantheline), improve the stability of etoposide in artificial intestinal fluid (ethanol, bile salts), and that drugs that decrease stomach acidity (cimetidine) had no significant effect on improving the etoposide AUC.[27]

Individualization of etoposide dosage

Currently, the dose of etoposide is adjusted according to the body-surface area of the individual patient, but this does not yield the desired minimization in individual variation in the pharmacokinetics in adults.[103]

Etoposide is a suitable drug for pharmacokinetically guided dosing, because of its marked inter-individual pharmacokinetic variability, but relatively little intra-patient variation.[104] Various studies have been performed with dose adjustments based on pharmacokinetic sampling. These studies have involved the administration of etoposide orally or intravenously to treat patients with different kinds of cancer.

Optimisation of oral etoposide dosage was investigated by El-Yazigi *et al*.[105] in elderly patients with non-Hodgkin's lymphoma using individual fraction of dose absorbed and the therapeutic drug monitoring (TDM) approach. The extent of absorption (F) was calculated from the AUC generated from first oral and intravenous doses in the same patient. Etoposide was than given orally at a daily dose equivalent to D_{oral}/F. The data obtained indicated that adjustment of the oral dose of etoposide in specific group of patients using individual bioavailability data and TDM approach yielded good safety and efficacy results while keeping the toxicity at the level that is similar to that of the intravenous administration.[105]

AUC is the best pharmacokinetic parameter for predicting anticancer pharmacodynamic effects. Precise estimation of AUC based on plasma concentration requires the handling of many blood samples, usually 8-12, which is expensive, time-consuming and inconvenient.[12,106] Several limited sampling models (LSM) that are based only on a few sparse determinations of plasma concentrations and can obtain a good estimate of the AUC for oral etoposide, were developed and validated.[107-109] Several such models were developed also for intravenous etoposide.[106,110-112] The use of LSM in targeted dosing study was used in oral etoposide by Ando *et al*.[113] and in intravenous etoposide by Lowis *et al*.[114] Ando *et al*.[113] reported that during the first 4 days of chemotherapy, one 25-mg capsule was taken three times daily. On day 5, the number of etoposide capsules was adjusted to the individualized dose, depending on the mean etoposide concentration on days 3 and 4, to achieve target concentration range of 1.0 to 1.5 μg/ml.[113]

Another approach to optimizing etoposide dosing is to use population pharmacokinetics, which quantify pharmacokinetic variability among individuals who are the target population, and tries to explain the sources of variability. Individual pharmacokinetic parameters are estimated using the Bayesian approach by combining the population pharmacokinetic model with a limited number of plasma drug concentration measurments.[12,115]

A population pharmacokinetics of oral etoposide was studied in patients with various tumor types by Nguyen *et al*.[116] and Toffoli *et al*.[117] They indicated that the renal function is the most important variable to be taken into account in etoposide dosing.[116,117]

Ciccolini *et al*.[118] presented a Bayesian method for performing dose adjustment of etoposide when administered intravenously. A Bayesian method was proven to efficiently adjust the experimental values to the target values, thus suggesting that this approach could be routinely used for therapeutic drug monitoring of etoposide.[118]

Functional polymorphisms in metabolizing enzymes and ABC transporters are another relevant factors that have to be considered in personalized medicine. The determination of functional polymorphisms in individual patient enables the use of genotype-based dose administration, to ensure minimal adverse drug reactions and maximal therapeutic efficacy.[37,119]

On the other hand, pharmacodynamic model was developed and tested for TDM of 21-day oral etoposide in non-small cell lung cancer patients. The model was developed to predict the value of the neutrophil nadir as a function of the etoposide concentration. Depending of the target nadir (grade 3 neutropenia), the dose was adjusted. However, the pharmacodynamic model yields statistically significant results only when considering the population of patients. Conversely, when applied to individual patients for TDM, the model lacks accuracy and precision.[120,121]

Conclusions

Generally, oral etoposide administration compared to intraveneous administration may result in an improvement of patient's quality of life and reduced costs. Several studies confirmed comparable safety and efficacy of oral and intravenous etoposide. However, a greater use of oral etoposide is limited by its incomplete and variable bioavailability. Many researchers studied various factors that may influence etoposide bioavailability and, attempted to tailor etoposide dose to the individual patient. The strategy of limited sampling and estimation of individual pharmacokinetic parameters by the Bayesian method seems to efficiently adjust experimental values to the target value. Furthermore, dosage adjustment based on pharmacogenetic analysis may be of great importance for individualized treatment of cancer patients in future. Therefore, further studies are needed to show the accuracy and precision of Bayesian method and pharmacogenetic analysis in dosage adjustment of oral etoposide in SCLC patients.

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research article

Iodine based radiopacity of experimental blood clots for testing of mechanical thrombectomy devices

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Background. Barium sulfate powder used for radiopacity of experimental blood clots (EBCs) for testing mechanical thrombectomy devices (MTD) has negative effects on EBCs mechanical properties. *In vitro* and *in vivo* exploration was performed to determine if the iodine based contrast medium will have less negative effects on the EBCs than barium.

Materials and methods. Fresh blood from 2 swine was used to create fibrinogen enhanced and thrombin initiated EBC in tubes. Iodine radiopacity was achieved by mixing the blood with 65% Iohexol or by soaking the EBCs for 2 or 24 hours in Iohexol. The EBCs opacified with barium served as controls. *In vitro* study: The EBCs were subjected to four tests, manual elongation, catheter injection, radiopacity and contrast wash out tests. *In vivo* study: The common carotid arteries of 2 swine were embolized by either barium EBC or EBC soaked for 24 hours in Iohexol. The duration of radiopacity of the different EBCs was compared.

Results. The EBCs opacified with Iohexol initially had higher radiopacity than the barium opacified EBCs. However, their opacity rapidly decreased with saline soaking and, particularly, after they were embolized in live animals. The mechanical properties of Iohexol opacified EBCs were inferior to barium opacified EBCs. The Iohexol mixed EBCs were less firm and elastic and half of them fragmented during catheter injection. The Iohexol soaked EBCs exhibited decreased tensile strength and elasticity compared to the barium EBCs.

Conclusions. Compared to barium, iodine based contrast medium does not offer any advantage for opacifying EBCs.

Key words: experimental radiology; radioopaque experimental clot; stroke; preclinical interventional radiology; animal model

Introduction

Radiopacity of experimental blood clots (EBCs) is an important feature for evaluation of mechanical thrombectomy devices (MTDs) used for treatment of acute stroke. Radiopacity of EBCs allows fluoroscopic visualization during experimental embolization in an animal and direct observation of the MTD performance including fragmentation of the EBCs. Radiopacity of EBCs for the treatment of stroke with MTDs has been achieved by adding and carefully mixing barium sulfate powder with blood.[1-3] Chueh *et al.*, however, showed that the presence of barium sulfate significantly reduces the EBCs elasticity.[2] They also investigated using tantalum and tungsten silicic acid for EBC radiopacity, but found them unsuitable for testing MTDs.[2] We explored the iodine based contrast agent, Iohexol, to opacity EBCs for both *in vitro* and *in vivo* studies to determine if it provides sufficient radiopacity

and has less negative effects than barium sulfate on the mechanical properties of the EBCs.

Materials and methods

The research was approved by the Institutional Care and Use Committee. Two swine weighing 45 and 42 kg were used as donors and blood was obtained from their femoral arteries. The animals underwent standard general anesthesia with intubation and artificial ventilation as described previously.[4,5] Hemostasis was achieved as described previously.[6] After exposure of the right femoral artery, a 7 F sheath was introduced to obtain blood for EBC formation. The animals were then recovered and brought back the next day for embolization of the EBCs into their common carotid arteries. After embolization, x-ray of the EBCs and selective angiographic follow-up, the animals were euthanized. The procedures were done in an angiographic room equipped with GE-OEC 9800 cardiac mobile system with digital imaging (GE Medical System, OEC, Salt Lake City, UT).

Clot formation

The model used for testing was a fibrinogen enhanced, thrombin initiated EBC that had been formed in a tube.[3] Sixty five percent Iohexol (Omnipaque 300, GE Health Care Inc., Princeton, NJ) was used to opacify the EBC. Two techniques of Iohexol opacification were explored, mixing and soaking. With the *mixing technique*, 2 ml of Iohexol were added to 200 mg of bovine fibrinogen powder (Calbiochem, La Jolla, CA) and mixed well with 10 ml of blood in between two 12 ml syringes connected with a 3-way stopcock. Twenty five IU of bovine thrombin (Gen Trac, Inc., Middleton, WI) were then added to the mixture and carefully flushed about 10 times between the syringes. The final mixture was then injected into an 80 cm long polyvinyl chloride (PVC) tube with inner diameter of 4 mm. With the *soaking technique*, EBCs were created by mixing 200 mg of fibrinogen with 10 ml of blood and then with 25 IU of thrombin and injected into a PVC tube. EBCs initiated by 200 mg fibrinogen and 25 IU thrombin and made opaque by addition of 1 gr of barium sulfate powder (Spectrum Quality Products Inc., Gardena, CA) served as a control for comparison.[3] The filled tubes were kept at room temperature for 1 hour. The EBCs produced with these techniques were then flushed into a basin filled with saline and cut into 2 cm long pieces.

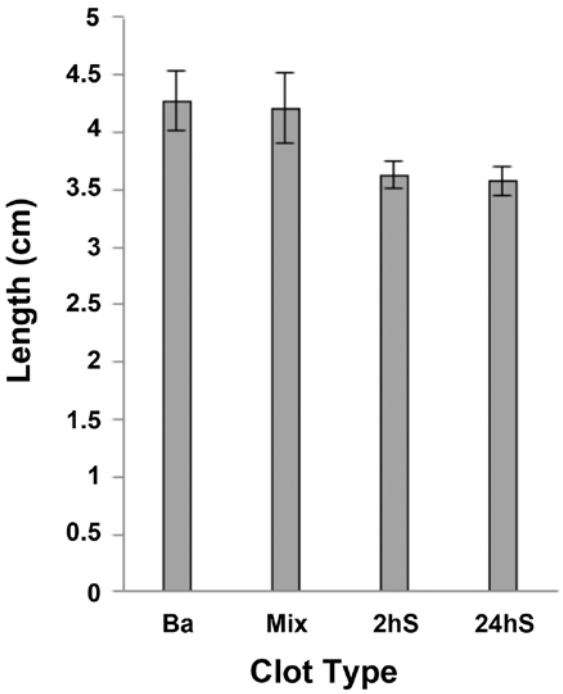

FIGURE 1. Results of manual elongation tests for four types of EBCs with starting length of 2 cm. The bar length indicates the average maximum elongation of 20 tested EBCs. Ba – control EBC with barium, Mix – EBC obtained by mixing with Iohexol, 2hS and 24hS – EBCs obtained by soaking in Iohexol for 2 hours and 24 hours respectively.

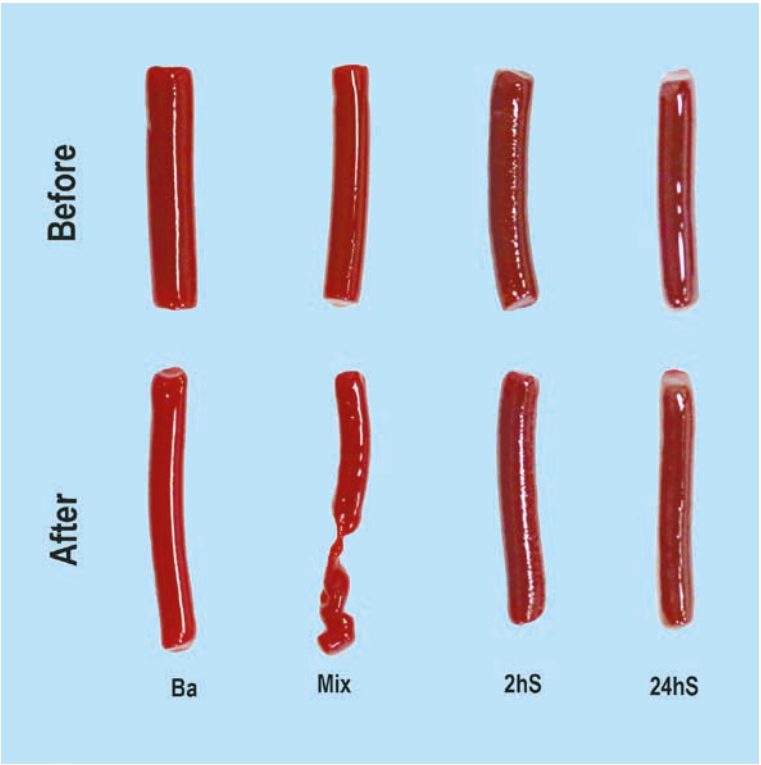

FIGURE 2. Examples of catheter injection tests of four types of EBCs. Ba – control EBCs with barium, Mix – EBCs obtained by mixing with Iohexol, 2hS and 24hS – EBCs obtained by soaking in Iohexol for 2 hours and 24 hours respectively.

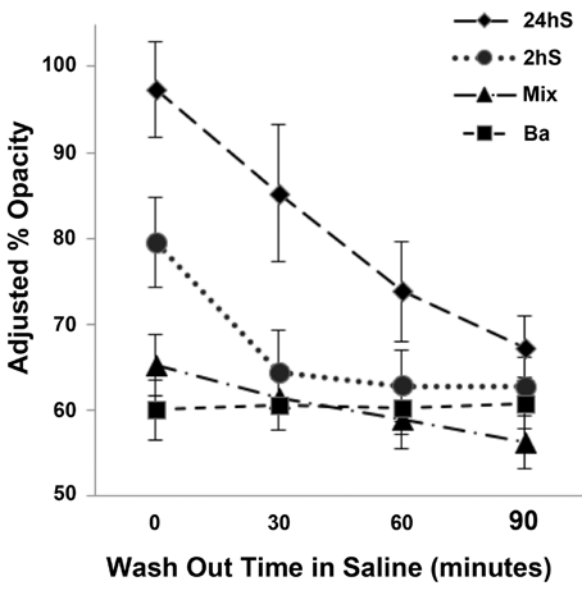

FIGURE 3. Results of radiopacity and its duration tests in four types of EBCs. Ba – control EBCs with barium, Mix – EBCs obtained by mixing with Iohexol, 2hS and 24hS. EBCs obtained by soaking in Iohexol for 2 hours and 24 hours respectively. The radiopacity of Iohexol EBCs is initially high, but rapidly decreases. The radiopacity of barium EBC is low, but stable.

The EBCs formed by mixing technique (Mixs) and the control barium opaque EBCs (Bas) were immediately tested. The EBCs formed by soaking technique were first placed into a basin containing 65% Iohexol. These EBCs were tested after soaking in Iohexol for either 2 hours (2hSs) or 24 hours (24hSs). The EBCs soaked for 24 hours were kept soaking in a basin that was refrigerated at 4^{0}C.

In vitro study

Clot testing. The EBCs were subjected following four tests, manual elongation, catheter injection, opacity and contrast wash out tests. Twenty EBCs were tested in each group, ten from each animal.

Manual elongation test. The manual elongation test measuring the EBC tensile strength was performed in a room temperature saline solution.[3] Both ends of the 2 cm long EBC were grasped with forceps and slowly stretched until the EBC fragmented. The length of stretching was measured with a ruler and recorded. A low tensile strength EBC usually fragmented well before its length was doubled. A firm EBC withstood stretching to almost double its original length.

Catheter injection test. The catheter injection test was done to evaluate the EBCs' elasticity and to determine if the EBCs were able to withstand injection through a long, small diameter sheath.[3] Tested EBCs were 4 mm in diameter. They were aspirated into a short plastic tube and injected with a saline filled 20 mL syringe through a 90 cm long 8F (2.2 mm inner lumen) Flexor-Shuttle Select introducer set (Cook Medical, Bloomington, IN) onto a smooth white surface. The size, shape and the degree of fragmentation of the EBC after injection were recorded by photography. The EBC's length and diameter were measured before and after injection.

Radiopacity evaluation. For evaluation of radiopacity, ten pieces of 2 cm long EBCs were placed in a Petri dish. A small tube containing 6% barium suspension was added for reference. Digital images of EBCs before and after injection through the catheter were obtained on the cardiac mobile system with a standard brightness of 50 and contrast of 54. The relative mean gray values of EBCs in the digital images were then measured using the Image J software (NIH, Bethesda, MD). These values were converted into adjusted opacity percentages where 100% indicated the opacity of the reference barium tube.

Radiopacity duration test – "a washout test". For evaluation of the radiopacity duration, the EBCs were immersed into room temperature saline. After soaking for 30 minutes, 60 minutes and 90 minutes their digital images were obtained to evaluate their loss of radiopacity. The manual elongation test was also performed on the washout EBCs.

In vivo studies

Embolization of EBCs into the common carotid artery (CCA) was done to evaluate their mechanical properties, *i.e.* fragmentation and the degree and duration of their radiopacity. After EBC creation, a 90 cm long 8 F Flexor-Shuttle Select Introducer Set with inner lumen of 2.2 mm was introduced via the femoral artery and baseline arteriograms of both CCAs were obtained. In both animals, a 3 cm long piece of barium EBC was injected into the right CCA and a 3 cm long EBC formed with 24 hour soaking was injected into the left CCA. Radiographs of the neck and head were obtained immediately and 30, 60 and 90 minutes after injection. They were evaluated for homogenicity and radiopacity of the injected EBCs.

Results

All EBCs formed well within 60 minutes and had dark red color. With the Bas, the color was slightly lighter, particularly after catheter injection. During

the manual elongation test, the Mixs broke at a mean of 4.19 cm (SD± 0.25 cm) (Figure 1). Similarly, the Bas broke at median of 4.27 cm (SD± 0.22 cm). The 2hSs broke at a mean of 3.59 cm (SD± 0.13 cm) and 24hSs at a mean of 3.63 cm (SD± 0.16 cm). All soaked EBCs retained their tensile strength during 30, 60 and 90 minutes saline soaking and broke with a mean difference of less than 0.1 cm.

With the catheter injection test, the Mixs exhibited deformity and fragmentation in 10 of 20 tested clots (Figure 2). This was similar for all three times of these EBCs soaked in saline. The unbroken Mixs became elongated by a mean of 0.2 cm (SD± 0.05 cm) and narrowed by a mean of 0.7 mm (SD ±0.04 mm) after injection. The Bas, the 2hSs and 24hSs did not exhibit any deformity or breakage after catheter injection (Figure 2). The elongation and narrowing for Bas was a mean of 0.53 cm (SD ± 0.21 cm) and a mean of 0.25 mm (SD ±0.12 mm), respectively. The elongation and narrowing of 2hSs was a mean of 0.12 cm (SD ± 0.12 cm) and a mean of 0.41 mm (SD ± 0.01 mm), respectively. The 24hSs elongated at a mean of 0.21 cm (SD ± 0.02 mm) and narrowed at a mean of 0.42 mm (SD ± 0.01 mm) after catheter injection (Figure 2).

Initial radiopacity of the Mixs was a mean of 64.2% (SD ±5.2%) and decreased after saline soaking gradually to a mean of 55% (SD ±4.3%) at 90 minutes (Figure 3). The Bas exhibited initial opacity of a mean of 58% (SD ± 4.2%) without changes with saline soaking. The 2hSs had initial opacity of a mean 79.5% (SD ± 5.2%) that gradually decreased to a mean of 62.6% (SD ± 3.3%) after 90 minutes of saline soaking. After catheter injection, they exhibited a decrease in opacity of a mean of 5.5% (SD ± 0.5%). The 24hSs had initial mean opacity of 97.3% (SD ± 5.6%) that decreased to a mean opacity of 67.1% (SD ± 3.4%) after 90 minutes of saline soaking. The opacity decrease after injection for 24hSs was a mean of 3.75% (SD ± 3.7%).

With embolization of EBCs in the CCAs of live animals, the Mixs had moderate opacity. However, their opacity rapidly decreased after embolization and by 90 minutes was only minimal. The barium EBCs retained good opacity for 3 hours (Figure 4). Selective arteriographies of CCAs done at the end of studies revealed CCA occlusions.

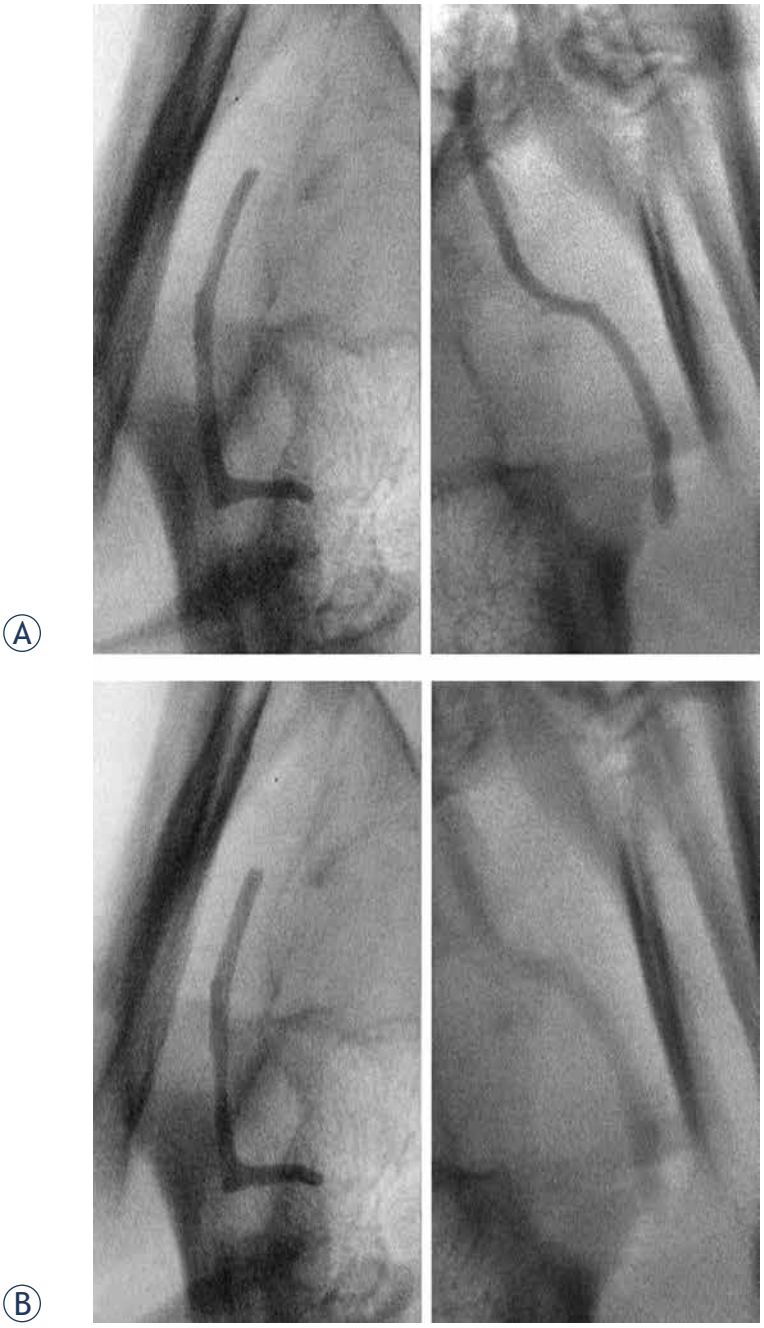

FIGURE 4. Close up radiographs of EBCs embolized into the common carotid arteries (CCA) of a live swine. Barium EBC is in the right CCA, 24 hour Iohexol soaked EBC is in the left CCA. (A) Initial radiographs show similar opacity of both EBCs. (B) Radiographs 60 minutes after embolization show no radiopacity change of the barium EBC and significant decrease of radiopacity of the Iohexol EBC.

Discussion

We used the EBC model formed with 200 mg fibrinogen and 25 IU thrombin plus 1 g barium sulfate powder as the control because we found it sufficiently firm and elastic in our previous work.[3] We are using it for testing new MTDs and for hands-on courses instructing physicians on MTDs use. Two types of EBC opacification (mixing and soaking) with the iodine based contrast material, Iohexol were explored. With the mixing technique, 2 ml of

Iohexol was used because 1 ml did not give sufficient opacity. Both the Mixs clots and the Bas clots were available in 1 hour as acute EBCs. The 2hSs clots can also be used in the same session. These differ from the 24 hSs, made using the Ponomar et al technique that are less practical because they require two days to be available for testing.[7]

Results of this study did not show an advantage to opacifying EBCs by iodinated contrast material compared to barium. On the contrary, the mechanical properties and duration of radiopacity of Iohexol EBCs were inferior to barium opacified EBCs making them less suitable for MTD testing. The tensile strength of Iohexol mixed EBCs is similar to that of the barium EBCs. However, they are less firm and elastic and a high percentage fragments during catheter injection. The Iohexol soaked EBCs don't break during catheter injection, but have lesser tensile strength and elasticity than the barium EBC model. The initial opacity of Iohexol EBCs is greater than the barium EBCs, particularly, those prepared with 24 hour soaking. However, this is only short lasting and rapidly decreases with saline soaking. The decrease in radiopacity of Iohexol EBCs in live animals is faster than during saline soaking, undoubtedly due to fast absorption of Iohexol. On the other hand, barium EBCs don't have loss of opacity during saline soaking or as thromboemboli in living animals. Post mortem examination of these EBCs revealed the barium powder is well mixed with red cell clumps and fibrin.[3]

In conclusion, iodine based contrast medium has greater negative effects on the mechanical properties and radiopacity of EBCs than barium sulfate powder. Barium tube EBCs enhanced by fibrinogen and initiated by thrombin are preferable for MTD testing.

Acknowledgement:

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research article

The value of the sagittal-oblique MRI technique for injuries of the anterior cruciate ligament in the knee

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Background. Complete rupture of the anterior cruciate ligament (ACL) does not represent a diagnostic problem for the standard magnetic resonance (MR) protocol of the knee. Lower accuracy of the standard MR protocol for partial rupture of the ACL can be improved by using additional, dedicated MR techniques. The study goal was to draw a comparison between sagittal-oblique MR technique of ACL imaging *versus* flexion MR technique of ACL imaging and, *versus* ACL imaging obtained with standard MR protocol of the knee.

Patients and methods. In this prospective study we included 149 patients who were referred to magnetic resonance imaging (MRI) examination due to knee soft tissues trauma during 12 months period. MRI signs of ACL trauma, especially detection of partial tears, number of slices per technique showing the whole ACL, duration of applied additional protocols, and reproducibility of examination were analysed.

Results. Accuracy of standard MRI protocol of the knee comparing to both additional techniques is identical in detection of a complete ACL rupture. Presentations of the partial ruptures of ACL using flexion technique and sagittal-oblique technique were more sensitive ($p<0.001$) than presentation using standard MR protocol. There was no statistically significant difference between MRI detection of the ruptured ACL between additional techniques ($p> 0.65$). Sagittal-oblique technique provides a higher number of MRI slices showing the whole course of the ACL and requires a shorter scan time compared to flexion technique ($p<0.001$).

Conclusions. Both additional techniques (flexion and sagittal-oblique) are just as precise as the standard MR protocol for the evaluation of a complete rupture of the ACL, so they should be used in cases of suspicion of partial rupture of the ACL. Our study showed sagittal-oblique technique was superior, because it did not depend on patient's ability to exactly repeat the same external rotation if standard MR protocol was used or to repeat exactly the same flexion in flexion MR technique in further MR examinations. Sagittal-oblique technique does not require the patient's knee to be repositioned, which makes this technique faster. We propose this technique in addition to the standard MR protocol for detection of partial ACL tears.

Key words: knee MRI; anterior cruciate ligament; partial rupture; partial tears; sagittal-oblique technique

Introduction

Anterior cruciate ligament (ACL) injuries occur due to a strong contact or indirect knee trauma, and results in stretching or tearing of non-contractile, elastic soft-tissue articular structures. ACL is the most frequently injured large ligament in the knee.[1] As injured ACL recovery is very limited long-term consequences are frequent including cartilage loss, secondary meniscal injuries and degenerative changes.

Magnetic resonance imaging (MRI) examination gives precise information about lesions in different organs as well as in the knee.[2-5] It determines the

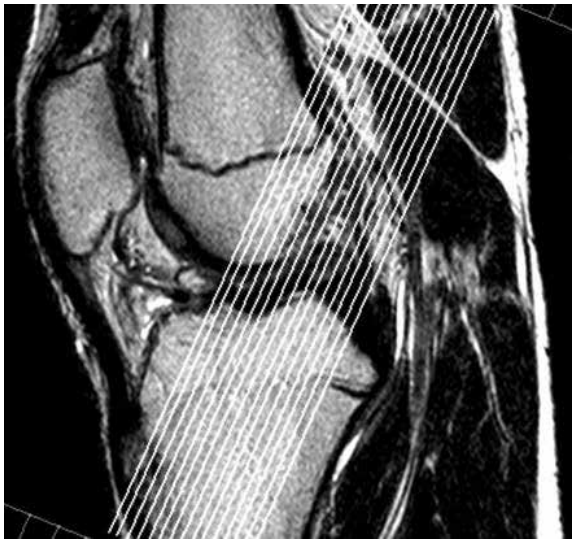

FIGURE 1. Sagittal image of standard MRI examination as a topogram for planning the paracoronal oblique T2 FSE 2 mm image.

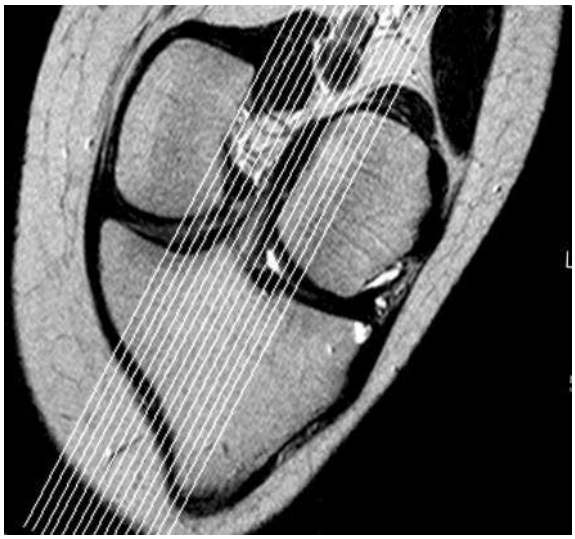

FIGURE 2. Anterior cruciate ligament (ACL) paracoronal oblique image.

course and length of patient's treatment. MRI diagnostic protocols and technical abilities have almost completely excluded the use of diagnostic arthroscopy nowadays.

Partial ACL tears occur in 10-43% of all ACL injuries.[5-8] In cases of partial ACL tears conservative treatment is effective in less than 25% in diameter. Larger tears progress into a complete rupture in 50-86% of cases.[8]

Partial ACL tears are difficult to diagnose during physical examination. On the other hand, many studies emphasise insufficiency of standard MRI protocol in diagnosing partial ACL tear.[9-11] There are, predominantly, two approaches in published studies, considering additional MRI examination of the ACL:

- dedicated MRI examination with one's knee in a slightly flexed position (approximately 17 degrees) and sagittal slices along the ACL, trying to avoid partial averaging with the intercondylar roof in the ACL central and proximal part – flexion technique;[12-15]
- dedicated MRI examination consisting of images trough longitudinal course of the ACL obtained with angled coronal and angled sagittal tomograms, the "double-oblique" or sagittal-oblique technique.[16,17]

The study goal was to compare sagittal-oblique MR technique *versus* flexion MR technique for ACL imaging with standard knee MR protocol. We analysed diagnostic value of MRI data related to ACL trauma, especially detection of partial tears, number of slices per technique showing the whole ACL, duration of additional protocols, ergonomics and reproducibility of examination.

Patients and methods

The study included 149 patients (age distribution Table 1) referred to MRI examination in Centre for Diagnostic Radiology in Clinical Centre of Montenegro during 12 months period, due to trauma of knee soft tissue structures. Inclusion criteria were positive history for ACL lesion and/or positive one or two ACL lesion physical tests (the Lachman and anterior drawer test).[6,8,18]

Radiological criteria for diagnosing ACL lesions obtained by standard MRI protocol and by additional techniques were identical: complete rupture (total discontinuity or an area of increased signal intensity extending completely across the ACL, partial rupture (inhomogeneity and increased signal intensity within the ACL, but with some fibres intact) and normal.[19,20] Partial ACL rupture parameters were: direct presentation of partial rupture of ACL, course of the ligament, presented by "increased curvature of posterior convexity of ACL"[9,10] and intraligamentous hyperintense MRI signal.[21,22]

Additionally, the number of slices showing the whole ACL, duration of particular sequences and total time of additional techniques were analyzed for two dedicated ACL imaging techniques.

All patients were examined with standard MRI protocol for the knee followed by sagittal-oblique technique and flexion technique using MR 1.0T scanner (Philips, Intera, Eindenhoven, The

Netherlands) with omni gradient 23 mT/m and, software version 11. Standard MRI protocol for the knee consisted of triplanar images using T2 fast-spin echo sequences (repetition time [TR] 3382, echo-time [TE] 100, flip angle 90 degrees), 3 mm slices with 0.3 mm gap and field of view [FOV] of 170 mm, 512x256 matrix. Patient was in supine position with extended knee in external rotation.[23] After standard MRI protocol, additional sagittal-oblique technique and flexion technique were performed in all patients using same parameters as standard MRI protocol with the exception for slice thickness of 2 mm and 0.2 mm gap.

Sagittal tomogram of the knee with the best image of the ACL, obtained with the previously applied standard MRI protocol was used as a topogram for paracoronal oblique sequence that followed the actual course of ACL (Figure 1). Obtained paracoronal oblique image was angled depending on actual anatomy of the patient's knee (Figure 2). This image, showing the full course of ACL, was used as a topogram for the sagittal-oblique protocol (Figure 3).

After the sagittal-oblique MRI examination, the flexion MRI technique was performed. This technique requires repositioning of patients knee in 17 degree flexion, followed by a sagittal topogram with orientation of slices without angling (Figure 4).

Two independent radiologists analysed the MRI figures of the patients, their history and physical data.

Data collected from the patients that showed a variety of ACL injuries diagnosed by the compared techniques (standard MR protocol, sagittal-oblique MR technique and flexion MR technique) were assessed by a chi-square test. Statistical differences between variants expressed as arithmetic averages and percentages were assessed by the Student's t-test.

All patients signed the consent of participation in the study. The investigators strictly followed recommendations of the Helsinki Declaration (1964, with later amendments) and of the European Council Convention on Protection of Human Rights in Bio-Medicine (Oviedo 1997).

Results

All 149 patients (108 males, 41 females) were sent to MRI examination with a clinical diagnosis: internal knee lesion-meniscal or ACL lesion. In 59 patients (39.6%) referring diagnosis was decisive – ACL lesion.

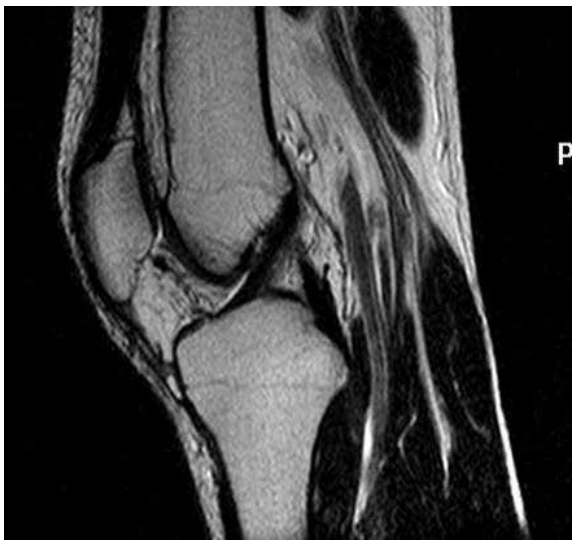

FIGURE 3. Anterior cruciate ligament (ACL) sagittal-oblique T2 FSE image.

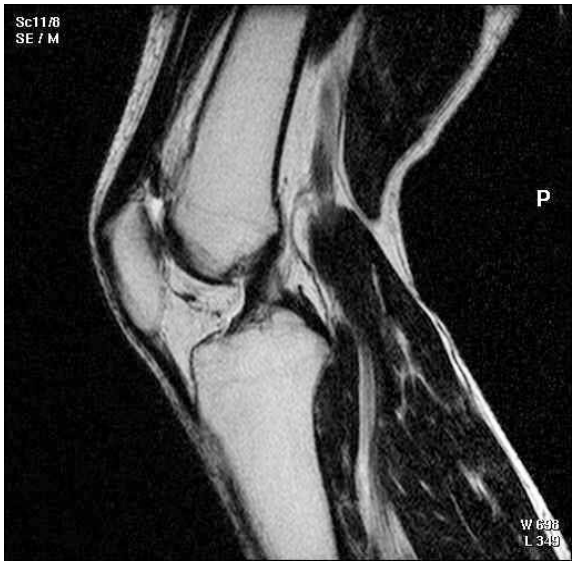

FIGURE 4. Anterior cruciate ligament (ACL) flexion MRI technique.

All patients suffered from a knee injury which mechanism pointed to the lesion of internal soft tissue structures and thus potentially to the ACL injury. The anterior drawer test was positive in 94 patients (63.1%), out of them 86 (57.7% of total patients) had positive Lachman test. In the remaining 55 (36.9%) patients, MR examination was performed on the basis of the mechanism of injury (from patient's history) and clinical suspicion of a violation of the ACL.

Standard knee MR protocol was positive (complete or partial ACL lesion) in 134 patients (89.9%) out of 149 patients. The percentage of complete

TABLE 1. Patient's distribution by age groups

Age groups	No of patients	%
<10 years	4	2.7%
10-20 years	17	11.4%
20-30 years	49	32.9%
30-40 years	71	47.6%
40-50 years	8	5.4%
Total	149	100%

lesion of ACL using standard MR protocol was 42.3%. The percentage of partial lesion of ACL using standard MR protocol was 47.6% (Table 2).

Both, flexion MRI technique and sagittal-oblique technique showed the same number of complete ACL ruptures, 63 (42.3%), as in standard MR protocol of the knee. The number of diagnosed partial ACL ruptures using flexion technique and sagittal-oblique technique was 86 in both techniques respectively, comparing to 71 ACL ruptures diagnosed by standard MR protocol.

The percentage (47.9%) of direct presentation of the partial rupture of ACL using standard MR protocol was statistically significantly lower ($p<0.001$) than in flexion technique (77.9%).

Also, higher percentage of direct presentation of partially ruptured ACL of 82.5% using sagittal-oblique technique compared to 47.9% using standard knee MR examination was statistically significant ($p<0.001$) (Table 3). The percentage of direct presentation of partially ruptured ACL using sagittal-oblique technique in comparison to flexion technique was without statistical significance ($p> 0.65$).

The site of partial ACL rupture detected by MRI (upper attachment, middle part or lower attachment) using flexion and sagittal-oblique technique showed no statistically significance between these techniques with *p* value less than 0.05 (Table 4).

Number of slices showing the whole course of the ACL and the time of examination comparing flexion and sagittal-oblique technique are presented in Table 5. Sagittal-oblique technique provides a higher number of slices presenting the whole course of ACL. The necessity of repositioning of the patient in order to perform flexion technique reduces reproducibility in possible subsequent examinations. Shorter scan time of 1.9 minutes required for sagittal-oblique technique (1 minute 54 seconds) compared to the duration of flexion technique is statistically significant ($p<0.001$).

Discussion

Complete rupture of the ACL does not represent a diagnostic problem for the standard MR protocol of the knee as overall accuracy of the procedure ranges from 86% to 100%.[10,11,20,21,24-31] Lower accuracy of standard MR protocol for partial rupture of ACL can be improved by using additional, dedicated MR techniques, described in recent literature as "standard orthogonal sequences plus oblique axial intermediate weighted imaging".[32] We tested two additional MR techniques for diagnosing partial ACL tear and confirmed them to be more sensitive ($p<0.001$) than standard MR protocol, with no statistical difference between them.

Sagittal-oblique technique clearly shows partial rupture because its' double angulation follows the specific course of the patient's ligament, due to approximate orientation of the external rotation of the foot. The advantages of this technique were described in MR studies of the knee after ligamentoplasty.[28,33-35]

In flexion technique, the flexed knee position avoids partial volume effect of intercondylar fossa of the ACL, which is one of the major diagnostic problems in diagnosing partial ACL rupture using standard MR protocol.[7,30,36,37] In addition, unstreched ligament in the knee in flexion is wider and thus can be precisely analysed.[14]

We shoved that both additional MR techniques reveal partial rupture of ACL from the "grey zone" of indirect signs such as course angulations of ACL, hyperintense MR signal, unclear contours of the ligament with the characteristics of fluid, bleeding and/or fibrosis (depending on the time between trauma and MRI examination).[7,11,21,26-29,34]

Intraligamentous hyperintense signal on T2 sequences, as a sign of fluid accumulation between the ACL-bundles due to fibre microruptures, found on standard MR procedure and additional techniques, showed the same level of sensitivity. Flexion technique seemed to be slightly more precise in detecting an intraligamentous hyperintense signal than standard MR protocol and sagittal-oblique technique because the flexion of the knee during examination results in shortening and widening of ACL, making the fluid in the ligament thicker and thus more available for detection.

In assessment of the location of ACL partial rupture (upper attachment, middle part, lower attachment) both flexion technique and sagittal-oblique technique showed the same results, confirming lower attachment as the most common site of ACL partial rupture.[11,35]

TABLE 2. Standard knee MR protocol, flexion MR technique and sagittal-oblique technique findings of anterior cruciate ligament (ACL)

Anterior cruciate ligament (ACL)	Standard MR protocol	Flexion technique	Sagittal-oblique technique
Complete rupture	63 (42.3%)	63 (42.3%)	63 (42.3%)
Partial rupture	71 (47.6%)	86 (57.7%)	86 (57.7%)
Normal	15 (10.1%)	0	0
Total	149 (100%)	149 (100%)	149 (100%)

TABLE 3. Standard knee MR protocol, flexion MR technique and sagittal-oblique MR technique findings of partial rupture of anterior cruciate ligament (ACL)

ACL partial rupture	Standard MR protocol	Flexion technique	Sagittal-oblique technique
Direct presentation	34 (47.9%)	67 (77.9%)	71 (82.5%)
Course of ACL	22 (31%)	0	0
Hyperintens signal	15 (21.1%)	19 (22.1%)	15 (17.5%)
Total	71 (100%)	86 (100%)	86 (100%)

TABLE 4. Flexion MR technique and sagittal-oblique MR technique findings of the position of partial rupture of anterior cruciate ligament (ACL)

Position of the partial rupture of ACL	Flexion technique	Sagittal-oblique technique
Upper attachment	30 (34.9%)	29 (33.7%)
Middle part	20 (23.2%)	16 (18.6%)
Lower attachment	36 (41.9%)	41 (47.7%)
Total	86 (100%)	86 (100%)

TABLE 5. Differences between flexion MR technique and sagittal-oblique MR technique in terms of number of tomograms that show the whole course of the anterior cruciate ligament (ACL) and the total duration of examination

	Flexion technique	Sagittal-oblique technique
Number of tomograms showing all coarse of ACL	2.7	3.8
Reposition of the patient	5.1 min	0
Scout / T2, 2 mm	0.5 min	3.9 min
T2, 2 mm / T2, 2 mm	4.2 min	4 min
Total time	9.8 min	7.9 min

In our study we found the statistically significant difference between standard procedure and both, flexion technique and sagittal-oblique technique regarding the number of MR slices that showed the whole course of ACL. Sagittal-oblique technique offers a larger number of MR tomograms showing the whole ACL, due to angulations in two planes with two phases of sagittal and paracoronal tomograms which present the true ligament axis of the patient's knee, making the ACL clearly visible. This technique does not require repositioning of the patient as flexion technique, thus the reproducibility of this method is easier and we have recommended it in patients with expected re-examinations. On the other hand, this technique is more ergonomic and faster because there is no need for patient knee repositioning and for a new scout.

Clinical parameters (patient's history, clinical examination) are not the subject of this study, but in our study we found low percentage of precise

referring diagnoses (only 39.6%), many unclear referring diagnosis (internal knee lesion) and various time between knee trauma and MR examination.

Lachman test was positive in fewer patients than the anterior drawer test, mostly in patients with strong quadriceps muscles as a result of greater physical effort that had to be applied by physician while performing anterior drawer test. This statement differs from the literature[25], probably due to a larger number of partial ruptures of the ACL in our study that are difficult to diagnose with physical examination.[8] Our study emphasises the role of MRI examination, especially for partial ACL lesions and confirms literature statements.[5,7,11,21,26-28]

The limitation of our study is the lack of arthroscopic procedures (as a "gold standard") after MR examinations. Arthroscopies were not performed in patients with clinical suspicion of isolated ACL injury, or minor lesions of the knee (mostly referred as "internal knee trauma"). If isolated partial tear of ACL was diagnosed on MR, patients were treated conservatively with physiotherapy and rehabilitation therapy. These approaches reduce the risk of invasive diagnostics and also the coast of patient's treatment.[19]

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research article

The effect of breast shielding during lumbar spine radiography

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Background. The aim of the study was to determine the influence of lead shielding on the dose to female breasts in conventional x-ray lumbar spine imaging. The correlation between the body mass index and the dose received by the breast was also investigated.

Materials and methods. Breast surface dose was measured by thermoluminescent dosimeters (TLD). In the first phase measurements of breast dose with and without shielding from lumbar spine imaging in two projections were conducted on an anthropomorphic phantom. In the second stage measurements were performed on 100 female patients, randomly divided into two groups of 50, with breast shielding only used in one group.

Results. On average, breast exposure dose in lumbar spine imaging in both projections (anteroposterior (AP) and lateral) was found reduced by approximately 80% ($p < 0,001$) when shielding with 0.5 mm lead equivalent was used (from 0.45±0.25 mGy to 0.09±0.07 mGy on the right and from 0.26±0.14 mGy to 0.06±0.04 mGy on the left breast). No correlation between the body mass index (BMI) and the breast surface radiation dose was observed.

Conclusions. Although during the lumbar spine imaging breasts receive low-dose exposure even when shielding is not used, the dose can be reduced up to 80% by breast shielding with no influence on the image quality.

Key words: breast dose; lead shielding; scattered radiation; lumbar spine radiography

Introduction

Protection of the most radiosensitive organs is recommended during radiography procedures as even low exposure to ionizing radiation can damage cellular material and consequently lead to cancer.[1] The most radiosensitive organs with the highest tissue weighting factor (0.12) include breast, lungs, stomach, colon, and bone marrow. According to ICRP the quoted weighting factor for breast represents an average over both sexes and is thus larger for females, further increasing the importance of breast shielding. Additionally the weighing factor is higher for females of younger age.[1]

In lumbar spine imaging which is one of the examinations with the highest radiation dose in conventional radiography breasts are located in close proximity of the primary x-ray beam.[2-6] As lead shielding results in reduction of dose to different superficial organs in many radiological procedures[7-12], the influence of breast shielding on breast exposure was considered an interesting subject of investigation.

Fordham *et al.* investigated whether breast dose is reduced in abdominal fluoroscopic examinations when lead shielding is used. They determined that, on average, the use of shielding with 0.5 mm lead equivalent reduces breast dose by 50%.[7] Beaconsfield *et al*. examined whether lead shielding of breast during CT imaging of the head reduces exposure of the thyroid and breast. The measurements were performed both on a phantom and on patients. Results of the measurements on the phantom showed that the average of 0.27 mGy for unshielded breast was reduced by 90% when

the shield was used. Measurements on the patients showed a reduction from on average 0.32±0.038 mGy to 0.075±0.042 mGy (approximately 70% reduction).[8]

Brnić *et al*. and Beaconsfield *et al*. also investigated the dose reduction to the breast in computed tomography (CT) of the head.[8,9] They determined that using lead shielding of 0.35 mm lead equivalent reduced the dose to the breast by 57%, from on average 0.28±0.07 mGy to 0.13±0.05 mGy.[9]

Clancy *et al*. examined how different placing of the lead shield influences the dose to the gonads from lumbar spine imaging in AP and lateral positions. The measurements on a phantom were performed with and without the lead shield in the following positions: lead shield between the primary beam and phantom, lead shield between the phantom and the panel detector, and lead shield wrapped around the part of the phantom where gonads are located. Shielding with 0.4 mm lead equivalent was used. In the AP projection the dose to the testicles with a lead shield placed between the testicles and the x-ray tube was reduced by 42% ($p \leq 0.01$) compared to the dose received without the shield; with the lead shield wrapped around the pelvis, the dose was only reduced by 36% ($p \leq 0.01$). In the lateral projection, the dose received by the testicles when using the lead shield, wrapped around the pelvis, was reduced by 12% ($p \leq 0.06$), compared to the dose received when the shield was not used.[12]

Review of the literature indicates that a significant dose reduction (over 50%) to the breast can be expected in the lumbar spine imaging by shielding the breasts. The aim of this research was to confirm this assumption and to determine the actual value of the dose reduction. Correlation of the breast dose with the body mass index (BMI) was also investigated using the linear regression and Pearson correlation coefficients.

Materials and methods

The study was conducted in two phases. In the first phase measurements were conducted on an anthropomorphic phantom and in the second phase on 100 female patients, randomly divided into two groups of equal size, with breast shielding only used in one group. In both phases, breast dose was estimated from measurements of the surface dose.

In both phases measurements were carried at the Radiology department at Department of Orthopedic Surgery, University medical centre Ljubljana on the AXIOM Iconos R200 system with digital fluoroscopy, manufactured by Siemens. The grid ratio used was 17:1, with 70 line pairs/cm. The focus-detector distance (FDD) was 115 cm. The image detectors used were computed radiography (CR) imaging plates, AGFA CR 35-X (manufactured by Agfa-Gevaert N.V., Belgium) and MD 4.0 image plate's size 35 × 43 cm.

FIGURE 1. Image of the phantom with 340 ml implant size on the right and 500 ml implant size on the left.

Beam positioning was done as referred in the literature.[4-6] In the AP projection, the transverse line of the central ray was positioned at the height of the lowest points of the rib cage, and the longitudinal on the central body line. In the lateral projection, the transverse line was at the same height whereas the longitudinal was in the frontal plane, 6 to 8 cm anterior from the posterior border of the

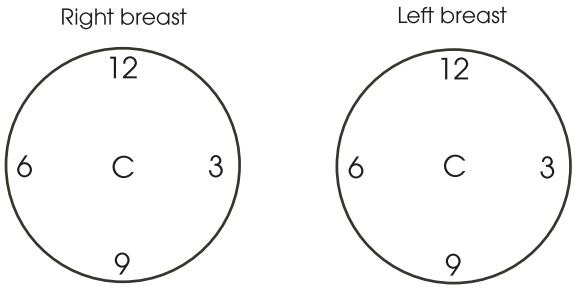

FIGURE 2. The position of the TLDs on the implants.

skin of the back. In the lateral position the phantom and the patients were lying on the left side. The breasts were outside of the primary imaging field.

Dosimetry

The entrance surface doses (ESD) were measured by $LiBO_4$ thermolumnescent dosimeters (TLD). The TLDs were provided by and readings conducted at the Institute of Occupational Safety (Dosimetry Laboratory), one of the three approved dosimetry services in Slovenia. For each set of dosimeters five control dosimeters were used to record the background radiation, which was subtracted from the measurements. In addition the dose area product (DAP) measured with a built-in DAP meter (Kermax plus DDP, IBA Dosimetry) was also recorded.

Measurements on phantom

In the first part of the study the influence of breast shielding on their exposure was determined by measurements on an anthropomorphic phantom. A whole body phantom PBU 60 (Kyotokagaku Co., Ltd, Japan), simulating a man of 165 cm and 50 kg was used (Figure 1). The breasts were simulated by breast implants of two different sizes, 340 ml and 500 ml.[13] The 340 ml size implants were positioned between 2nd and 6th rib and the medial edge was aligned with the edge of the sternum; the 500 ml size implants were positioned between 2nd and 7th rib with the medial edge aligned with the edge of the sternum, following the article from Marolt Mušič *et al*.[14] TLDs were placed at the centre of the implant and on its edge as shown on Figure 2.

The phantom study was performed using the same protocol as used for lumbar spine radiography at this radiology department. After each positioning of the phantom, fluoroscopy was used to verify positioning of the lumbar spine. In the AP projection the tube voltage was 55 kV and in lateral projection 66 kV. The middle automatic exposure control (AEC) was used for both positions. Dose measurements were conducted for AP and lateral projection together so the results determine the breast dose for complete lumbar spine radiography.

Measurements on patients

The second part of the study was conducted on 100 adult women (age range 35 – 89 years with average of 68.5 years) referred to lumbar spine radiography. The study was approved by the National Medical Ethics Committee. The patients were informed about the study and a written informed consent was obtained from all patients. None of the patients declined participation in the study.

The weight of the participating patients varied between 47 kg and 122 kg and their height between 150 cm and 183 cm. The patients were divided into two groups of 50 patients each. Kolmogorov-Smirnov test confirmed a natural distribution of variables BMI, DAP and breast dose in both groups (p was greater than 0.05 in all cases).

For each patient a single TLD was attached to the central part of each of the patients' breasts. In the first group breasts were left unshielded as referred in the literature [3-6] and in the second group a shield of 0.5 mm lead equivalent was used to cover both breasts. The breast area was covered as tightly as possible with the shield remaining outside the primary beam.

As in the phantom study the locally used clinical protocol for the lumbar spine radiography was used. Thus, after positioning of the patient, fluoroscopy was used to examine the position of the lumbar spine. In the AP projection the tube voltage ranged from 55 kV to 75 kV and AEC was used. In the lateral projection the tube voltage ranged from 66 kV to 83 kV also with AEC. Again, the measurements show sum of exposure from both projections.

Results

Phantom study

In the phantom study 40 measurements with 1 TLD per measurement were performed. Tables 1 and 2 show absolute and percentile dose reduction due to the shielding for the 340 ml implant size and 500 ml implant size respectively. Positions of the numbers on the tables correspond to TLD positions on the implants as shown on Figure 1. In average

TABLE 1. Dose reduction on the phantom study with 340 ml implant size

Right breast (mGy)			Left breast (mGy)		
	0.07 (- 73%)			0.02 (- 61%)	
0.16 (- 65%)	0.14 (- 84%)	0.16 (- 73%)	0.14 (- 71%)	0.10 (- 78%)	0.04 (- 59%)
	0.56 (- 67%)			0.81 (- 95%)	

TABLE 2. Dose reduction on the phantom study with 500 ml implant size

Right breast (mGy)			Left breast (mGy)		
	0.11 (- 96%)			0.03 (- 86%)	
0.28 (- 86%)	0.16 (- 88%)	0.11 (- 64%)	0.32 (- 80%)	0.06 (- 80%)	0.06 (- 60%)
	0.65 (- 70%)			0.024 (- 65%)	

the results of the phantom study show a dose reduction of approximately 80%.

Patient study

In the second phase of the study, 200 measurements with TLD were carried out on 100 patients and for 94 patients DAP measurements were also recorded.

Results of the breast dose measurements are summarised in Tables 3 and 4. The results for both groups were analysed using two-sided t-test and statistically significant differences between the doses to the shielded and unshielded breast were confirmed with a significance of $p < 0.05$ (Figure 3, 4).

As a consequence of the lead shielding, the average dose for the right breast decreased from 0.45±0.25 mGy to 0.09±0.07 mGy, representing a statistically significant decrease confirmed by the two-sided t-test of independent samples ($p < 0.001$). The average dose value for the left breast decreased from 0.26±0.14 mGy to 0.06±0.04 mGy by using lead shield. The statistically significant decrease was confirmed by two-sided t-test of independent samples ($p < 0.001$). The difference between the dose to right and left breast was due to the patient's positioning in the lateral projection. Patients were lying on their left side so the left breast was further away from the primary beam then the right.

In average, the breast surface radiation dose decreased by ~80% when the lead shielding was used. Breast dose measurements (Table 3, 4) in both groups were analysed using two-sided t-test. Statistically significant differences between the doses to the shielded and unshielded breast were confirmed with a significance of $p < 0.05$ (Figures 3,4).

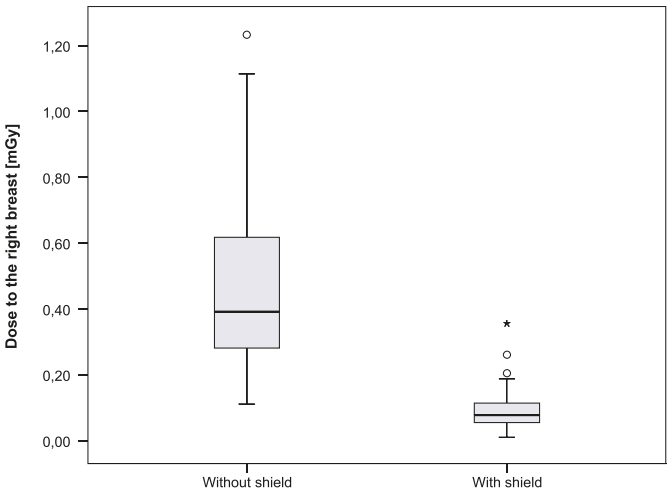

FIGURE 3. The comparison of the dose between shielded and unshielded right breast.

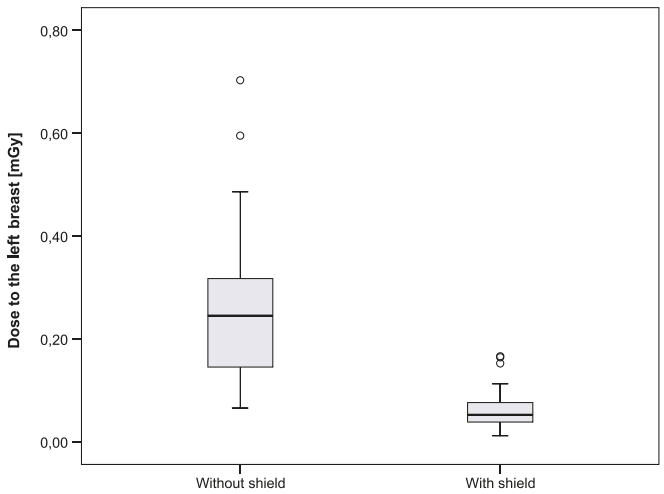

FIGURE 4. The comparison of the dose between shielded and unshielded left breast.

TABLE 3. Basic statistical characteristics of right breast dose measurement with and without the lead shielding

Personal protective equipment	Average (mGy)	Median	Standard deviation	Minimum	Maximum
Right breast without the lead shield	0.45	0.39	0.25	0.11	1.23
Right breast with the lead shield	0.09	0.08	0.07	0.01	0.36

TABLE 4. Basic statistical characteristics of left breast dose measurement with and without the lead shielding

Personal protective equipment	Average (mGy)	Median	Standard deviation	Minimum	Maximum
Left breast without the lead shield	0.26	0.24	0.14	0.07	0.70
Left breast with the lead shield	0.06	0.05	0.04	0.01	0.17

Discussion

The results of the patient study were found to be consistent with the results of the phantom study as well as with observations of previous studies.[7-9] They are showing a major (approximately 80%) reduction of the breast dose as a result of breast shielding.

In order to exclude the influence of other parameters in the patient study, eventual significant differences between the two groups of patients were checked for. The body mass index (BMI) and dose-area product (DAP) were identified as the main parameters that could influence the results. Distributions of those two parameters for both groups of patients were compared and checked for statistically significant differences. The two-sided t-test of independent samples was used to compare the body mass index (BMI) distributions and no statistically significant differences were found ($p = 0.399$). Despite differences in the size of the imaging field due to variations in physique among the patients, the two-sided t-test also showed no statistically significant differences ($p = 0,195$) in the DAP values between the two groups. It can thus be concluded that the observed differences in the breast doses can be attributed to the effect of the shielding.

In addition, the correlation between the breast dose and BMI was checked for. Using Pearson's correlation coefficient, no linear correlation between BMI and dose to the breast was observed in either group ($r = 0.211$, $p = 0.141$; $r = - 0.248$, $p = 0.082$). These results are mostly consistent with findings of Brnić *et al*.[9] who found no correlation between BMI and dose to the breast under the lead shield ($c=0.28$; $p>0.05$) and weak correlation between BMI and the breast without the lead shield ($c=0.08$; $p>0.05$).

The authors expect that further reduction of the breast doses could be achieved by using breast displacement device to remove the breasts further from the primary imaging field, as in the research conducted by Foley *et al*.[15] Another option is the use of the PA projection instead of AP, as recommended by Brennan and Madigan[16], and yet another alternative would be lead shielding, completely wrapped around the patient, as determined by Jackson and Brennan[11], and Clancy *et al*.[12] However, those approaches are much less practicable in regular clinical work and were not investigated in this study.

Various approaches such as optimisation of the imaging protocols and avoidance of fluoroscopy for verification of positioning could be implemented to achieve the breast dose reduction (and generally reduced patient exposure) during lumbar spine radiography. Although those might influence the relative effectiveness of shielding for breast protection (*e.g.* reduced shielding factor of lead at higher tube voltages), overall optimisation of the protocols was not the aim of this study.

Conclusions

A breast dose reduction of approximately 80% ($p < 0.001$) during lumbar spine imaging was found to be achievable by using shield of 0.5 mm lead equivalent with no influence on the image quality. Despite a relatively low exposure of breast during this procedure, the use of breast shielding might thus be beneficial with little disturbance to established clinical practice. Although a higher dose reduction might be achieved by using displacement devices or full wrapping of lead shielding around

the thorax, such measures are probably limited to academic interest due to their impracticality in clinical settings. No correlation between the BMI and the dose to the breast was observed.

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research article

Tumor size and effectiveness of electrochemotherapy

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Background. Electrochemotherapy (ECT) is an effective and safe method for local treatment of tumors. However, relatively large variability in effectiveness of ECT has been observed, which likely results from different treatment conditions and tumor characteristics. The aim of this study was to investigate the relationship between tumor size and effectiveness of a single-session ECT.

Materials and methods. A systematic search of various bibliographic databases was performed and nine studies eligible for this study were extracted. Different statistical methods including meta-analysis were applied to analyze the data.

Results. The results of analysis based on data from 1466 tumors of any histotype show significantly lower effectiveness of ECT on tumors with maximal diameter equal to or larger than 3 cm (complete response (CR) of 33.3%, objective response (OR) of 68.2%) in comparison to smaller tumors (CR% of 59.5%, OR% of 85.7%). The results of meta-analysis indicated that ECT performed on tumors smaller than 3 cm statistically significantly increases the probability of CR by 31.0% and OR by 24.9% on average in comparison to larger tumors. The analysis of raw data about the size and response of tumors showed statistically significant decrease in effectiveness of ECT progressively with increasing tumor diameter. The biggest drop in CR% was detected at tumor diameters as small as 2 cm.

Conclusions. The standard operating procedures for ECT should be reexamined and refined for the treatment of large tumors. We propose that future clinical trials should include accurate ECT treatment planning and/or multiple ECT cycles, besides a prolonged observation for tumor response evaluation.

Key words: electrochemotherapy; cutaneous tumors; effectiveness; tumor size; meta-analysis

Introduction

Treatment of cutaneous and subcutaneous tumors using electrochemotherapy (ECT) has gained its role in routine clinical practice. The reason for an increasing use of ECT in clinics arises from favorable treatment characteristics, which are high effectiveness, safety, simplicity, low toxicity, possible application in an out-patient setup and cost-effectiveness.[1–7] The standard operating procedures (SOP) for ECT using the Cliniporator device were prepared during the European Standard Operating Procedures of Electrochemotherapy (ESOPE) project.[1,8] The aim of the SOP document was to define guidelines for safe and effective ECT of cutaneous and subcutaneous tumors. Different treatment procedures were proposed within the SOP with respect to the number, size (maximal diameter) and depth of tumors. The SOP document was developed based on the experience from the leading European cancer centers using ECT, and tested during the ESOPE project in which also tumors larger than 3 cm in

diameter were treated but they were excluded from ECT treatment evaluation reported in ESOPE study.[1] More recently, some researchers apply ECT also for the treatment of tumors larger than 3 cm.[9-11] Although general recommendations for ECT procedures on large tumors are given in the SOP, it is unclear whether this recommendations are appropriate for tumors with diameters larger than 3 cm.

The purpose of this study was therefore to examine the relationship between tumor size and tumor response to treatment based on local tumor control of single-session ECT (using merged evidence from different studies) and to address the issue of the SOP for large tumors.

Materials and methods

Study selection and data extraction

All steps for a systematic review from PRISMA guidelines were applied in this study.[12–14]

The publicly available literature was systematically searched to obtain relevant published articles about clinical evaluation of effectiveness of ECT on tumors of various sizes. The following 16 databases were searched: Web of Science, Science Direct, PubMed, Wiley Online Library, OvidSP, HighWire Press, IEEE Xplore, SpringerLink, nature.com, Compendex, BioMed Central, Ingenta, Inspec, Journal Storage, The Cochrane Library, and Medscape. The search terms "electrochemotherapy" and "clinical" were used and the time span between 1st January 1991 and 22nd November 2011 was considered. Author BM first examined the titles and abstracts of the studies identified with the search strategy to narrow the initial selection of studies and then made the final selection based on full text reading. Authors TJ and GS independently checked the preliminary selection of studies. Bibliographies of original articles, review articles and relevant books were also screened to identify other potentially eligible studies. Articles published electronically were included but abstracts, posters, reviews, editorials, lectures and commentaries were not included in systematic review. In addition, the data collected at the Institute of Oncology Ljubljana (denoted as IO data from this point onwards) was also recognized as appropriate and was hence included in the analysis.

A study was considered eligible for meta-analysis if the following criteria were met:

1) inclusion of data for single-session ECT of cutaneous or subcutaneous tumors of any histotype performed on human patients;
2) inclusion of data about number of patients and tumors, size and response of tumors, histotype of tumor; electrode type, drug type and route of administration;
3) response of tumors evaluated at least 4 weeks after ECT treatment according to WHO or RECIST criteria, or with diagnostic imaging or biopsy;[15,16]
4) data about size and response of tumors was reported in such a way that separation of tumors into two groups was possible: tumors with maximal diameter smaller than 3 cm and tumors with maximal diameter equal to or larger than 3 cm.

The cutoff dimension of tumor size of 3 cm in the last (fourth) criterion was selected because the majority of studies included in data analysis reported data of tumor responses only for group of tumors smaller and equal to or larger than 3 cm without details that would allow using a different cutoff value. The custom cut off value can be set only for two studies with full access to raw data (IO data and data from Campana *et al.*[9]).

The following data was extracted from eligible studies by two of the authors (BM and TJ) independently: author and year of publication, number of patients, number, size and response of tumors, tumor histotype, electrode type, chemotherapeutic drug and route of its administration, criteria for tumor response evaluation, duration of follow-up and assessment of risk of bias of the study. Differences in extracted data between both authors were discussed to find the source of disagreement and to reach a common final decision. If the same data was used in two or more studies, either the first published or the more comprehensive study was included in the analysis. Authors of three studies included in the analysis were contacted for additional data, which were not included in published articles, but were needed for this study.[9–11]

The risk of bias of the studies was assessed following the Cochrane Collaboration recommendations.[14] Ratings for each bias issue (low, high or unclear bias) were extracted independently by two authors (BM and TJ) who were not blinded to names of the authors or locations of the studies. Ratings of both authors were compared and dissimilarities were discussed until consensus was reached. Studies were further rated as having an overall low (all bias issues rated with low), high (any bias issue rated with high) or unclear risk of bias (no bias issue rated with high and any bias issue rated with unclear).

TABLE 1. Assessment of risk of bias for studies included in the analysis (except for IO data)

First author, year of publication, reference	Adequate sequence generation	Allocation concealment	Blinding of participants and operators	Incomplete outcome data	Selective outcome reporting	Other bias	Overall risk of bias
Byrne, 2005[18]	unclear	unclear	unclear	low	unclear	unclear	unclear
Campana, 2009[9]	unclear	unclear	unclear	unclear	high	high	high
Curatolo, 2011[10]	unclear	unclear	unclear	low	unclear	low	unclear
Landstrom, 2010[19]	unclear	unclear	unclear	low	low	low	unclear
Larkin, 2007[20]	unclear	unclear	unclear	low	unclear	unclear	unclear
Matthiessen, 2011[21]	unclear	unclear	unclear	low	unclear	low	unclear
Quaglino, 2008[11]	unclear	unclear	unclear	unclear	unclear	low	unclear
Rols, 2000[22]	high	unclear	unclear	low	high	unclear	high

In this study, the tumor response to a single-ECT application was evaluated. The tumor response was classified as either complete response (CR), partial response (PR), no change (NC) or progressive disease (PD), according to the response criteria adopted in the studies (WHO or RECIST), or the pathologic response, assessed by biopsy.[15,16] Although WHO and RECIST criteria are different in some respects, these criteria are essentially equivalent for the evaluation of tumor response on individual lesions (the per-tumor effectiveness), which is the level of response considered in this study. CR is defined as a disappearance of tumor, PR as a decrease of at least 50% in the products of the two largest perpendicular diameters of the tumor (corresponding to tumor area), PD as an increase of more than 25% of lesion area. In all other cases, a response is determined as NC. Tumor response was determined not earlier than 4 weeks post treatment by two observations not less than four weeks apart. Tumors with CR and PR responses were further combined in the so called objective response group (OR) and tumors with NC and PD responses were grouped in the no response group (NR).

Statistical analyses

The overall effectiveness of ECT was determined across all eligible studies by pooling the response data of individual tumors of all studies together. For this purpose, complete and objective response rate (denoted as CR% and OR% respectively) were calculated across all eligible studies. The same calculations were also performed separately for the group of tumors with maximal diameter smaller and larger than (or equal to) 3 cm. CR% and OR% results of these two groups were compared using two-sided Chi-square test and the difference was considered statistically significant for $p < 0.05$.

The CR% and OR% values result in a summary in which all individual tumors from all studies contribute equally. Consequently, the relative contribution of each study to these values is proportional to its relative size. When applying statistical analysis on data accumulated from a series of studies that had been performed by researchers operating independently, it would be unlikely that all the studies were functionally equivalent. In such cases, a meta-analysis based on the random-effects model is generally the preferred method for pooling the results of independent studies.[14,17] By applying meta-analysis we obtained the most reliable estimate of the difference in effectiveness of ECT correlated to tumor size. The software for meta-analysis calculations was written in Matlab following the procedures published in the literature.[14,17] The so-called risk difference (RD) was used as the measure of the effect because of dichotomous nature of tumors' response data. RD is defined as the probability of response (either CR or OR) in one group minus the probability of the same response in the other group. The between-study heterogeneity was assessed with the I^2 statistic. The summary effect of meta-analysis was combined using a so-called random-effects model. This model considers the within-study variance and the between-studies variance and as a consequence the confidence interval (CI) of the summary effect is wider than in case of the fixed-effects model (thus requiring a larger difference between the two groups in order to find this difference significant). But by using the random-effects model, the larger studies (with many tumors) are also less likely to dominate the overall effect and smaller studies (with few tumors) are less likely to be trivialized than with the

fixed-effects model.[14,17] The difference between the two groups of tumors was considered statistically significant for $p < 0.05$.

A sensitivity analysis was applied to investigate the influence of studies of high risk of bias on the overall results of data analysis.

The raw data about the size (maximal diameter of tumor) and response for tumors from article by Campana *et al.* were used for examination of relationship between tumor size and response.[9] Spearman's rank correlation coefficient and its significance were used for determination of statistical dependence between these two parameters. The tumors were also grouped by their size into four groups with 1 cm step size with the last group including all tumors equal to and larger than 3 cm, *i.e.* <1 cm, 1–2 cm, 2–3 cm and >3 cm. The differences in proportion of CR, PR and NR were tested between neighbor groups using Chi-square test in order to find the range of tumor size with statistically significant decrease of CR and OR and increase of NR with respect to its neighbor group. The same statistical tests were also performed on IO data. Due to a full access to IO data, the statistical comparisons of additional parameters (tumor area, volume, histotype and location; drug type and route of administration; current, voltage and energy per area delivered on tumor; electrode type; median follow-up) were performed between the groups. Rank Sum test on ordinal data and Chi-square test on nominal data were applied using statistical toolbox in Matlab and the difference was considered statistically significant for $p < 0.05$.

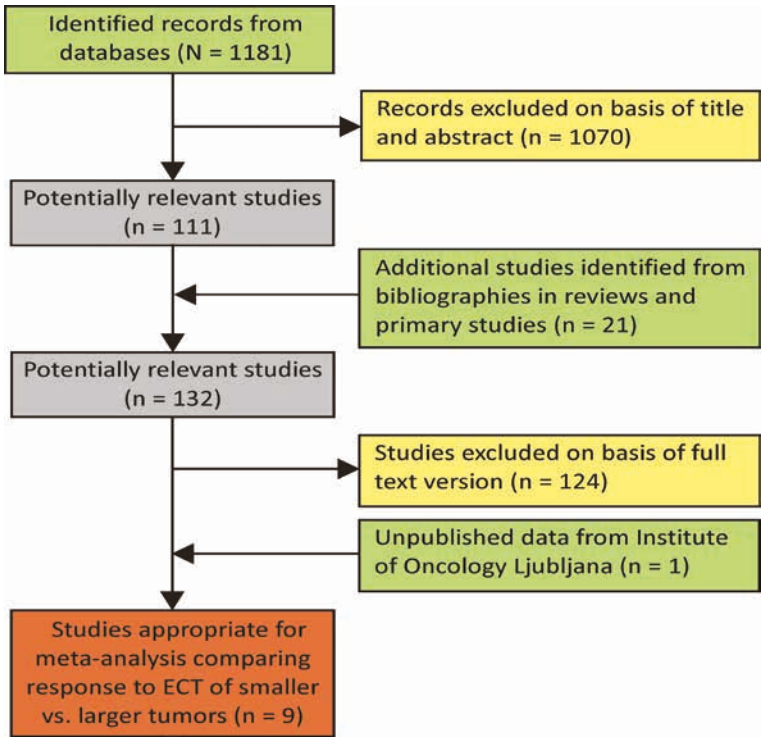


FIGURE 1. Selection process for the studies included in the data analysis.

Results

Study selection and data extraction

The flow chart of the selection process for the studies included in data analysis is given in Figure 1. The initial search of 16 databases resulted in 1181 records after removal of duplicates but finally only eight articles satisfied all criteria.[9-11,18–22] The IO data from a clinical database of ECT performed on cutaneous and subcutaneous tumors at Institute of Oncology Ljubljana also met all the selection criteria and was therefore included as the ninth study. All these studies were non-randomized phase I or II studies.

The risk of bias (rated as low, high or unclear) was assessed for individual studies included in the analysis (Table 1). No assessment of overall risk of bias was possible for IO data because most of this data has not been previously published. Note that some of these data (about 40% of patients and 25% of tumors) has been published previously in the ESOPE study.[1]

The characteristics of the studies used for systematic review are shown in Table 2. In total, 1466 tumors and 197 patients were included. There were 252 (17.2%) tumors with maximal diameter larger than or equal to 3 cm, and 1214 (82.8%) tumors smaller than 3 cm.

Statistical analyses

Overall CR% and OR% of 55.0% and 82.7% were determined respectively, across all included studies irrespective of tumor size (Table 2). The results show higher effectiveness of ECT on tumors with the largest diameter smaller than 3 cm (CR% and OR% of 59.5% and 85.7%, respectively) in comparison to tumors with the largest diameter equal to 3 cm or larger (CR% and OR% of 33.3% and 68.2%, respectively). The differences of CR% and OR% between these two groups of tumors were statistically significant, both with $p < 0.001$. Consequently, the proportion of tumors with NR (combining the cases of NC and PD after a single application of ECT) was statistically significantly higher for the

larger tumors' group in comparison to the smaller tumors' group (NR% of 31.8% and 17.3%, respectively, p <0.001).

Similarly, the results of meta-analysis demonstrated that ECT performed on tumors smaller than 3 cm increases the probability of CR and OR by 31.0% and 24.9% on average, respectively, in comparison to tumors equal to or larger than 3 cm (summary RD values, see Figure 2). The results of summary risk difference (RD) for CR and OR were statistically significant with significances of <0.001 and 0.002, respectively.

For the sensitivity analysis, two studies (Rols *et al.*, 2000 and Campana *et al.*, 2009) with an overall rating of high risk of bias were removed from the statistical analysis to see if overall results are affected by the inclusion of studies with high risk of bias.[9,22] These two studies accounted for 22.4% of all tumors included in this study. The differences in CR% and OR% between groups of tumors of different size both remained statistically significant with p <0.001 (CR% and OR% of 62.5% and 87.9% respectively for smaller tumors, CR% and OR% of 35.4% and 70.8% respectively for larger tumors). When compared to CR% and OR% values in Table 1, the change of these values was relatively small in comparison to the variability of results between different studies. Similarly small changes in results were also found for meta-analysis when studies with a high risk of bias were excluded. Namely, RD for CR of 0.34 (CI between 0.13 and 0.55) and RD for OR of 0.26 (CI between 0.05 and 0.46) were obtained (compare these values to data in both summary lines of Figure 2). Both results for CR and OR however remained statistically significant with p = 0.002 and p = 0.015, respectively.

The analysis of raw data for the size and response for tumors from the study by Campana *et al.* showed that the effectiveness of ECT, defined as CR%, was decreasing progressively with increasing maximal tumor diameter (Spearman's rho = 0.418, p <0.001) (Figure 3A). The statistically significant drop in CR% (but not significant drop in OR% and increase in NR%) was detected between group of tumors of size <1 cm and 1–2 cm (p = 0.017), as well as between group of tumors of size 1–2 cm and 2–3 cm (p = 0.001), where the most evident drop in CR% was detected.

TABLE 2. Summary of studies eligible for meta-analysis comparing the response to ECT of tumors smaller than 3 cm with tumors larger than 3 cm

First author, year of publication, reference	No. of patients/tumors		No. of responses of tumors < 3 cm				No. of responses of tumors ≥ 3 cm			
	All	Included	OR	CR	PR	NR	OR	CR	PR	NR
Byrne, 2005[18]	19/63	15/18	11	11	0	5	1	1	0	1
Campana, 2009[9]	52/608	52/267	184	124	60	26	35	16	19	22
Curatolo, 2011[10]	23/532	23/489	330	225	105	38	102	57	45	19
Landstrom, 2010[19]	6/6	6/6	4	4	0	1	1	1	0	0
Larkin, 2007[20]	30/148	26/111	82	64	18	8	8	2	6	13
Matthiessen, 2011[21]	52/196	24/94	72	57	15	10	4	1	3	8
Quaglino, 2008[11]	14/233	14/233	202	133	69	8	14	3	11	9
Rols, 2000[22]	5/61	5/61	24	6	18	34	1	0	1	2
IO data	52/379	32/187	131	98	33	44	6	3	3	6
Summary (%)	**253/2226**	**197/1466**	**1040 (85.7)**	**722 (59.5)**	**318 (26.2)**	**174 (14.3)**	**172 (68.2)**	**84 (33.3)**	**88 (34.9)**	**80 (31.8)**
Summary of all tumors (%)			**1212 (82.7)**	**806 (55.0)**	**406 (27.7)**	**254 (17.3)**				

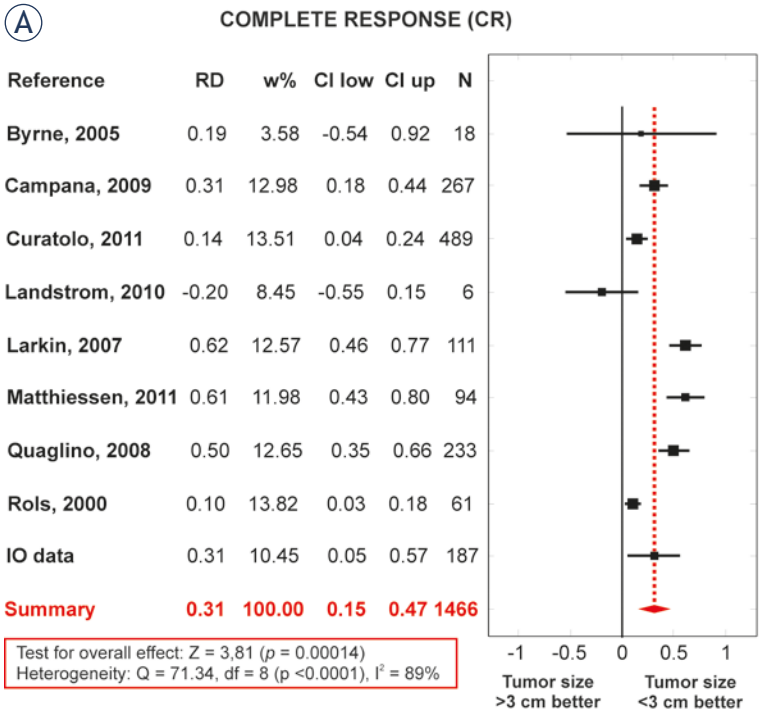
OR = objective response (including CR and PR); CR = complete response; PR = partial response; NR = no response (including tumors with no change and progressive disease status); bleo = bleomycin; CDDP = cisplatin; i.t. = intratumoral route of administration; i.v. = intravenous route of administration; BCC = basal cell carcinoma; SCC = squamous cell carcinoma; AC = adenocarcinoma; mo. = month. nd = no data

Similar results were obtained for the IO data, in which also similar tendency of decrease in effectiveness of ECT (expressed as CR%) with increasing size of treated tumors was detected (Spearman's rho = 0.129, p = 0.078) (Figure 3B). The maximal drop in CR% was detected between group of tumors of size 1–2 cm and 2–3 cm, and was statistically significant with p = 0.041. Due to full access to IO data, we were able to investigate if there was some other parameter beside the tumor size (such as tumor histotype and location; drug type, dose and route of administration; current, voltage and energy per area delivered on tumor; electrode type; median follow-up) that could be correlated with the observed difference in tumor response between these two size groups of tumors. Based on statistical comparison, these two groups of tumors of size 1–2 cm and 2–3 cm proved to be imbalanced with respect to upper listed parameters; therefore, no other parameter that would correlate with the difference in tumor response between these two size groups of tumors could be found. Among them, significant imbalance in proportion of melanoma and non-melanoma tumors, and drug type and route of administration used was identified.

Discussion

The main prerequisites for an effective ECT treatment are an adequate extracellular concentration of the chemotherapeutic drug in the entire tumor at the time of pulse delivery and the coverage of tumor volume with an electric field able to permeabilize the cell membrane and therefore to enable drug uptake.[23–26] Sufficiently high electric field in the tumor tissue can be assured by delivery of pulses of adequately high voltage and appropriate positioning of the electrodes. In addition, some other conditions or parameters could be relevant, such as patient and tumor (histotype, size and location) characteristics and treatment parameters (drug, dose and route of administration, electrode type, protocol and timing of pulse delivery). In this study, we investigated the correlation between tumor size and effectiveness of ECT. Individual tumor data were gathered from heterogeneous non-randomized studies with

Drug, route	Electrode type, electroporator	Histotype of tumor(s)	Response evaluation	Median follow-up in mo. (range)
bleo, i.t.	needle, Medpulser	melanoma	WHO, biopsy	6 (3-6)
bleo, i.t. or i.v. or both	needle, Cliniporator	melanoma, breast cancer, sarcoma, SCC, head and neck cancer	RECIST	nd (2-21)
bleo, i.v.	plate or needle, Cliniporator	Kaposi sarcoma	RECIST	18 (2-50.4)
bleo, i.t.	needle, Medpulser	BCC and SCC	biopsy	18.5 (3-24)
bleo, i.t or i.v.	plate or needle, Cliniporator	melanoma, SCC, AC, chondrosarcoma	WHO	nd (2-12)
bleo, i.t or i.v.	plate or needle, Cliniporator	melanoma, SCC, AC, BCC, breast cancer	RECIST	nd (2-6)
bleo. i.v.	plate or needle, Cliniporator	melanoma	WHO	21 (5-28)
bleo, i.v.	plate, PS 15, Jouan	melanoma, SCC	WHO	1.6 (1-2)
bleo, i.t. or i.v., CDDP, i.t.	plate or needle, Cliniporator	melanoma, carcinoma, sarcoma	WHO	3.2 (1-16)



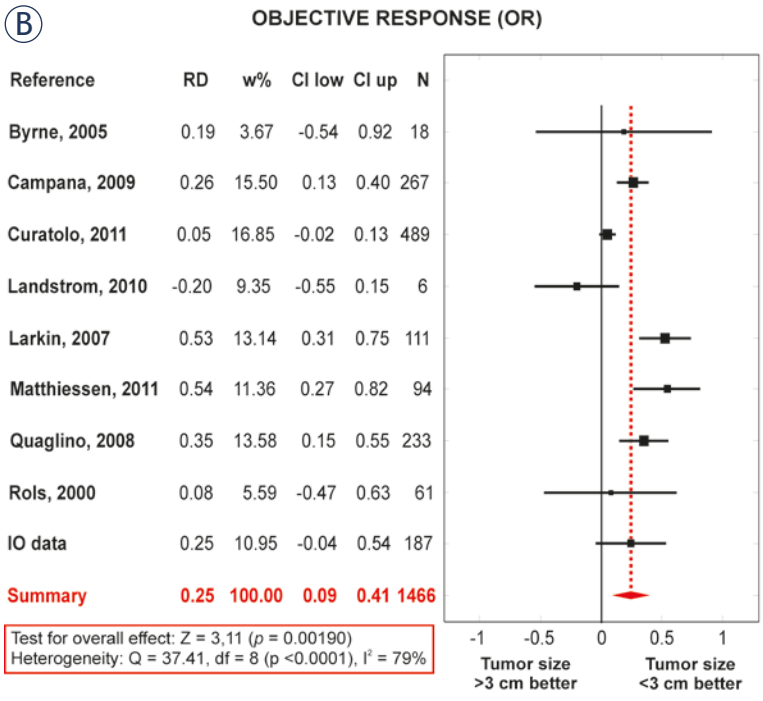


FIGURE 2. Results of meta-analysis. Data for individual studies and pooled results (Summary) demonstrating: (A) a statistically significant 31% increase in probability of CR for tumors smaller than 3 cm in comparison to tumors equal to or larger than 3 cm with ECT, and (B) a statistically significant 25% increase in probability of OR for tumors smaller than 3 cm in comparison to tumors equal to or larger than 3 cm with ECT.

RD = individual and summary risk difference for studies included in meta-analysis; w% = weight of study in comparison to all studies; CI low and CI up = the lower and upper confidence interval of RD, respectively; N = the number of tumors per each study and total number of tumors included in meta-analysis

various levels of additional information available; therefore we were not able to assess the possible cause-effect relationship between other parameters and the treatment response.

Our results showed that ECT was less effective on tumors larger than 3 cm in comparison to tumors smaller than 3 cm (CR% and OR% of 59.5% and 85.7%, respectively, versus CR% and OR% of 33.3% and 68.2%, respectively, Table 2). On the other hand, the no response rate (NR%) had more than doubled on larger tumors when compared to NR% on smaller tumors (from 14.3% to 31.8%, Table 2). The results of meta-analysis confirmed these findings, by showing that the effectiveness of ECT on the smaller tumors was significantly higher than on the larger ones, when the size limit between smaller and larger tumors was set to 3 cm (Figure 2) regardless of large heterogeneity of the included studies (Table 2). All results remained statistically significant when studies with an overall high risk of bias were excluded. The sensitivity analysis thus showed that the overall results and conclusions are not affected by the inclusion of studies with high risk of bias. Therefore, our results can be considered with a higher degree of certainty.

The trend of decreasing ECT effectiveness with the increasing tumor size was clearly demonstrated with the analysis of raw data derived from the paper by Campana *et al.* and confirmed by the analysis of unpublished data from Institute of Oncology Ljubljana (IO data) (see Figure 3).[9] The results of the analysis based on the data from these two independent sources (the only two available for more detailed analysis) revealed that proportion of CR% was statistically significantly decreased already for tumors with maximal diameter around 2 cm (see Figure 3).

When treating large tumors with ECT, the SOP document suggests the administration of bleomycin by the intravenous route and use of needle electrodes in order to cover the whole tumor with sufficiently high electric field.[8] Almost all studies included in our survey were conducted according to the SOP recommendations, except for the study by Byrne *et al.* in which only intratumorally administered bleomycin was used and the study by Rols *et al.* in which strictly plate electrodes were used (Table 2).[18,22] Both these studies predate the publication of the SOP. Even though the SOP recommendations were generally followed in the studies included in the analysis, a relatively low response rates (CR% or OR%) were obtained in ECT treatment of tumors larger than 3 cm.

The first possible explanation for decreased effectiveness of ECT in tumors larger than 3 cm that

should be considered is inadequate concentration of chemotherapeutic drug reached in the target tumor due to improper timing of pulse delivery. In the analyzed studies, pulses were applied either around 2 minutes after intratumoral bleomycin or cisplatin administration (which is within 10 min after drug administration as recommended in SOP), or within the therapeutic window of 8-28 minutes after intravenous bleomycin administration.[8,23] The interval between intratumoral drug administration and pulse delivery is adequate according to study by Cemazar *et al*.[27] The "optimal" therapeutic window for intravenous bleomycin administration (originally proposed by Domenge *et al*.) was actually determined based on data from a single patient on whom the ECT was performed in two sessions.[23] But according to the study by Front *et al*., the concentration of intravenously administered bleomycin in interstitial fluid around tumor is high enough for efficient ECT treatment for considerably longer period after the injection than the "optimal" therapeutic window recommended within the SOP.[28] Plasma concentration of bleomycin declines biexponentially with a mean distribution half-life of approximately 24-30 min and mean elimination half-life of 2-4 hours,[29–31] which means that bleomycin concentration within tumors declines relatively slowly in the first two hours after intravenous administration. Therefore, if the insufficient extracellular drug concentration in tumors was indeed responsible for the demonstrated lower effectiveness of ECT on tumors larger than 3 cm, it is very unlikely that it happened due to missed optimal therapeutic window for application of pulses. Nevertheless, further studies are needed to re-examine the current SOP recommendations for "optimal" treatment window. A more appropriate definition of the "optimal" therapeutic window should probably take into account other factors such as histotype, size and anatomical location of tumors to be treated, in addition to the drug type and time and route of its administration.

The second very likely reason for reduced effectiveness of ECT in large tumors is the insufficient exposure of the tumor to the drug, due to heterogeneous distribution of blood flow. It was reported that the periphery of the tumor is considerably better perfused than the inner portion, thus suggesting that the concentration of the drug in the center of the tumor can be lower than in the periphery of the tumor.[32–34] In addition, large temporal and spatial heterogeneity in blood flow is typical for tumors.[35] Higher drug concentrations in the inner portion of large tumors could be achieved by an appropriate combination of both intratumoral and systemic (intravenous) administrations.

The third possible explanation for the lower effectiveness of ECT in large tumors might be the insufficient coverage of the entire tumor volume with sufficiently high electric field. To overcome this problem, an individualized treatment planning based on radiological imaging could be adopted to determine the appropriate voltages based on the size, geometry and electrical properties of the

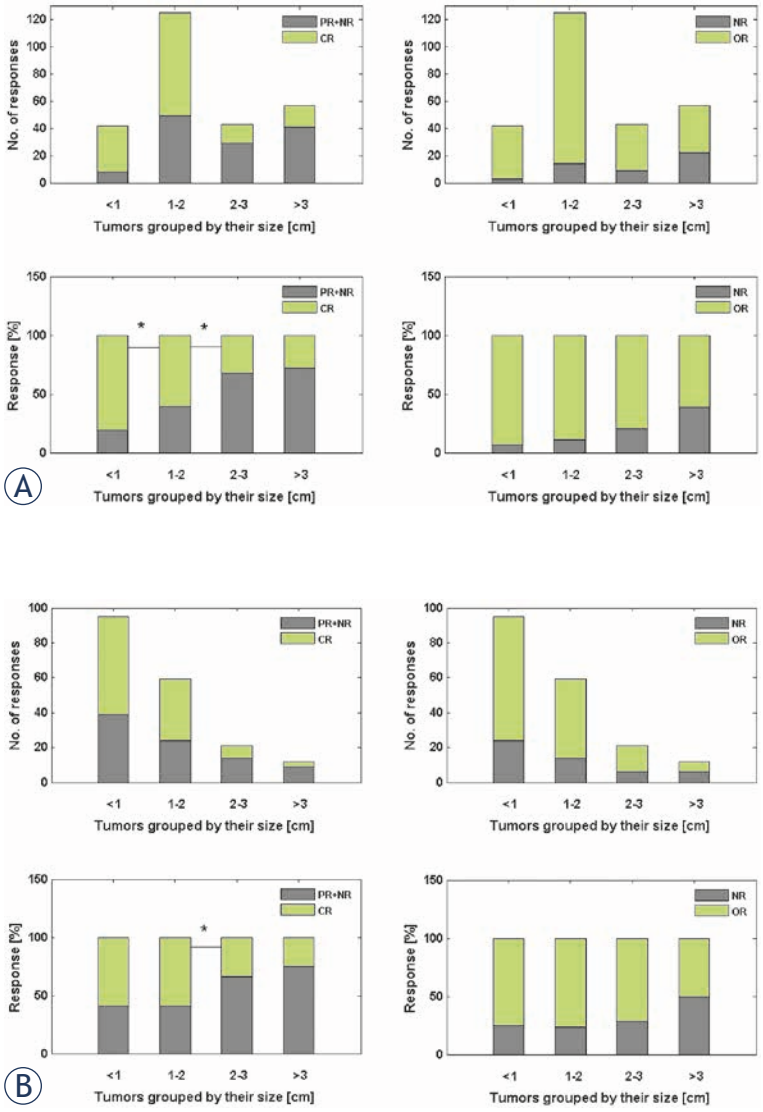


FIGURE 3. Number and proportion of tumor CR and OR to ECT with respect to tumor size for data: (A) from Campana *et al* and (B) from unpublished IO data. Tumors were grouped by their size using a 1 cm step. Each pair of neighbor groups, for which a statistically significant difference in proportion of CR and OR was found, is indicated with *.

OR = objective response; CR = complete response; PR = partial response; NR = no response

target region.[36–39] Another option to maximize the tumor response could be to perform ECT treatment with fixed-geometry electrodes and their multiple and overlapped insertions.

The timing of response evaluation after ECT treatment for tumors should also be taken into consideration when interpreting the results of clinical studies. In this study, we considered tumors whose response assessment was performed at least 4 weeks after ECT, according to the SOP document.[8] However, longer healing time can be expected for larger tumors and 4 weeks after ECT may be too soon for evaluation of the response to ECT in many if not all large tumors. A healing time for smaller tumors is expected to be between 4 and 8 weeks, whereas for larger tumors (larger than 1.5 cm) can be prolonged to up to 10 weeks.[8] In this study, it turned out that 7 out of 9 studies reported response of tumors at least 8 weeks after ECT treatment. The remaining two studies (Rols *et al*.[22] and IO data) reported response evaluated less than 8 weeks after treatment only for small portion of tumors. If tumors with the response evaluated earlier than 8 weeks after ECT are not included into analysis, the results remain practically identical and the conclusions of this study remain unchanged. Nevertheless, for more accurate assessment of correlation between tumor size and response, longer follow-up observations should probably be more appropriate, especially because in general the kinetics of response of various tumors after ECT is unknown.

In this study, we considered exclusively the effect of a single-session of ECT. However, several clinical studies reported that the result of ECT on large tumors can be improved with repetitive treatments.[9,11] Moreover, ECT retreatment is not only recommended to achieve a better response in case of larger tumors, but also for smaller tumors unresponsive to the first ECT treatment.

In addition to investigation of correlation between tumor size and response, we intended to evaluate the influence of other tumor and treatment parameters on effectiveness of ECT (tumor area, volume, histotype and location; drug, dose and route of administration; current, voltage and energy per area delivered on tumor; electrode type; median follow-up). Such multivariate data analysis was unfortunately not possible due to unavailability of the details concerning these parameters for individual tumors in the analyzed studies. With such data reported in future clinical reports or with initiated ad hoc study, the reliable estimation of the most important influential parameters on response of large tumors will become possible.

In conclusion, the response of large tumors to ECT treatment seems not to be as good as that reported in smaller tumors. Tumor size starts to play a significant role in the final treatment outcome for tumors as small as about 2 cm in diameter. Therefore, we suggest that the SOP should be refined to improve the effectiveness of ECT for larger tumors. The optimal way to treat larger tumors should include individualized treatment planning to determine the appropriate electrode geometry and voltages, or, alternatively, the application of fixed-geometry electrodes with their accurate repositioning in order to overlap the treated volumes. Moreover, bleomycin could be administered combining both the intravenous and the intratumoral routes to achieve sufficient extracellular concentration in the portion of the tumor. The possibility of repetitive treatments on large tumors (already introduced in the clinical practice in some centers[9,11]) should be explicitly suggested within the SOP document, including the recommended interval between ECT cycles. Finally, for an accurate assessment of the correlation between tumor size and response to ECT in larger tumors, a longer follow-up (at least 3 months) could be required.

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research article

p38 MAPK regulates the expression of ether à go-go potassium channel in human osteosarcoma cells

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Background. The ether à go-go (Eag) channel has been shown to be overexpressed in a variety of cancers. However, the expression and function of Eag in osteosarcoma are poorly understood. In addition, the molecular mechanisms responsible for Eag overexpression in cancer cells remain unclear.

Methods. The expression of Eag in human osteosarcoma cell line MG-63 was detected by reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis. The effect of Eag inhibition on MG-63 cell proliferation was assessed *in vitro*. The effect of short hairpin RNA (shRNA) mediated knockdown of Eag on osteosarcoma growth was evaluated in xenograft model *in vivo*. The activation of mitogen-activated protein kinase (MAPK) pathway and p53 in MG-63 cells was detected by Western blot analysis.

Results. Eag was overexpressed in MG-63 cells. Imipramine or Eag shRNA significantly suppressed the proliferation of MG-63 cells *in vitro* and *in vivo*. MG-63 cell proliferation was specifically inhibited by p38 MAPK inhibitor SB203580 or small interference RNA (siRNA). The inhibition of p38 MAPK activation by SB203580 or siRNA reduced Eag protein level but increased p53 protein level. Moreover, the activation of p53 by nutlin-3 induced cell growth arrest in MG-63 cells and reduced Eag protein level, while the inactivation of p53 by pifithrin-alpha (PFT-a) promoted MG-63 cell growth and increased Eag protein expression.

Conclusions. Eag channel functions as an oncogene to promote the proliferation of human osteosarocma cells. Furthermore, the high expression of Eag in osteosarcoma cells is regulated by p38 MAPK/p53 pathway.

Key words: Ether à go-go; cell proliferation; MAPK pathway; p53; osteosarcoma

Introduction

Voltage-gated potassium channels (Kv) play a vital role in the function of diverse cell types. Ether à go-go (Eag) channels are a unique group of Kv channels due to their close relation to tumor growth, progression and metastasis. Eag channels were originally cloned from *Drosophila melanogaster*[1] and consist of three subfamilies: EAG, ERG (the eag-related gene) and ELK (the eag-like gene).[2] The physiological expression of Eag is restricted to the central nervous system and placenta[3], but Eag is aberrantly expressed in several tumor cell lines and more than 75% of the primary solid tumors.[4,5] In addition, Eag appears to induce tumor angiogenesis by the induction of functional increase of hypoxia inducible factor-1 (HIF-1) and the secretion of vascular endothelial growth factor (VGEF) upon hypoxia[6] and participates in the acquisition of malignant phenotypes

in lung tumor cell.[7] Moreover, siRNA targeting of Eag[4,8] or non-specific blockers[9] have been shown to suppress the proliferation of tumor cells. Later studies confirmed that aberrant expression of Eag in tumors is associated with a poor prognosis.[10,11] Taken together, these studies reveal the oncogenic potential of Eag.[12,13]

Osteosarcoma is the most common primary bone tumor,[14] which is characterized by a high incidence among youngsters and the potential of lung metastasis.[15,16] Despite the recent development of neoadjuvant chemotherapy and advances in surgical techniques, the overall relapse-free survival rate of osteosarcoma over 5 years remains approximately 65%.[17] Moreover, 30% of osteosarcoma patients die of lung metastases when presenting with metastases at the time of diagnosis.[18] Therefore, there is an urgent need to develop new treatment methods for osteosarcoma in the clinic.[19]

Although a clear relationship between Eag and tumour progression has been established, it is unclear whether Eag plays a similar oncogenic role in osteosarcoma. This study aimed to investigate the expression and function of Eag in human osteosarcoma. In addition, we explored the signaling mechanisms that contribute to Eag overexpression in osteosarcoma cells. In this study we employed loss of function of approach to inhibit Eag expression or activity and found the consequent inhibition of the proliferation of human osteosarcoma cells *in vitro* and *in vivo*. Furthermore, our data suggest that Eag expression is regulated by p38 MAPK/p53 pathway.

Materials and methods

Cell culture

Human osteosarcoma cell line MG-63, human osteoblastic cell line hFOB 1.19, human breast cancer cell line MDA-MB435S and human embryonic kidney cell line 293 (HEK293) were purchased from the American Type Culture Collection. MG-63, MDA-MB435S and HEK293 cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 in the air at 37°C. hFOB 1.19 cells were cultured in ham's F12/ Dulbecco's modified Eagle medium (DMEM) (Gibco) supplemented with 10% (v/v) FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 33.5°C. All cells were subcultured every 3-4 days.

Drugs and siRNAs

Imipramine was purchased from Sigma (St. Louis, MO, USA), dissolved in distilled water as 1 mM stock solution and stored at -20°C. The p38 MAPK inhibitor SB203580 was from Calbiochem (Darmstadt, Germany), dissolved in dimethyl sulfoxide (DMSO) as 10 mM stock solution and stored at -20°C. The c-jun N-terminal kinase (JNK) MAPK inhibitor CEP11004 was from Sigma, dissolved in DMSO as 4 mM stock solution, and stored at -20°C. The extracellular-regulated kinase 1/2 (ERK1/2) MAPK inhibitor PD98059 was obtained from Santa Cruz Biotechnology (CA, USA), dissolved in DMSO as 10 mM stock solution, and stored at -20 °C. p38 MAPK siRNA (#6564), JNK MAPK siRNA (#6232), ERK1/2 MAPK siRNA (#6560) and control siRNA (#6568) were obtained from Cell Signaling Technology® (Danvers, MA) and stored at -20°C. Nutlin-3 (p53 activator) and PFT-α (p53 inhibitor) were purchased from Sigma. Stock solution of Nutlin-3 (20 mg/ml) was prepared in DMSO and stored at -20°C. PFT-α was protected from light and stored under inert gas.

Preparation of adenoviral shRNA vectors

The oligonucleotides targeting human Eag was designed and selected as the template: AGC CAT CTT GGT CCC TTA TAA, which shared no homology with other coding sequences in human by BLAST analysis. A ring sequence of 9 base pairs (TTC AAG ACG) existed between the sense and antisense strands. The shRNA was synthesis by Sangon Biotech (Shanghai, China). Plasmid pGeneSil-1, which was purchased from GeneSil Biotechnology (Wuhan, China), contained the human U6 promoter inserted between the BamHI and the HindIII sites. The shRNA-expressing cassette was subcloned into pAdTrack vector between the HindIII and the XbaI sites.[20] The recombinant plasmid was linearized by digestion with restriction endonuclease, and subsequently cotransformed into *E. coli* BJ5183 cells with an adenoviral backbone plasmid, pAdEasy-1. Recombinant plasmids were selected for kanamycin resistance, and transduced into the HEK293 cells. A recombinant adenovirus expressing shRNA against Eag (Ad5-Eag-shRNA) was generated. The recombinant adenovirus control (Ad5-Control-shRNA), which carries a CTA GGT GTT CTA GTC TGG ACT and did not target any known human genes, was generated as control. All viruses were propagated and purified on a CsCl gradient using standard methods. The vi-

ruses were titered for viral particles using standard methods based on spectrophotometry at 260 nm. Functional titer (plaque forming units) was determined with a plaque assay on HEK293 cells according to the method developed by Quantum Biotechnology.

Adenovirus infection

MG-63 cells (1×10^5) in serum-free RPMI-1640 were infected with Ad5-Eag-shRNA or Ad5-Control-shRNA at 5 MOI (multiplicity of infection, calculated as PFU/cell numbers) in a humidified atmosphere of 5% CO_2 at 37°C. Virus-containing medium was removed 8 h later and replaced with fresh RPMI-1640 medium containing 10% (v/v) FBS. Cells were incubated for another 48 h.

RT-PCR

The total RNA was isolated from the cultured cells by Trizol reagent (Invitrogen, Rockville, MD, USA). RNA purity and integrity was checked by running an aliquot on a denaturing 1% (w/v) agarose gel. cDNA was then synthesized from 1 μg of total RNA using 200 U reverse transcriptase (Takara, Tokyo, Japan), plus 200 μM dNTPs and 2.5 μM oligo-dT primer, in a 20 μL reaction volume, for 10 min at 30°C then 60 min at 42°C and finally at 80°C for 5 min. 1 μL of cDNA were then amplified by PCR in 25 μL reaction containing 2.5 U DNA polymerase and 200 μM dNTPs. Sequences of forward and backward primers, amplified fragment sizes, annealing temperatures were as follows: Eag, 5′-GCT TTT GAG AAC GTG GAT GAG-3′, 5′-CGA AGA TGG TGG CAT AGA GAA-3′, 475 bp, 56°C. β-actin, 5′-TCC ACC TTC CAG CAG ATG TG-3′, 5′-GCA TTT GCG GTG GAC GAT-3′, 75 bp, 54°C. Samples of PCR products were run on a 2% (w/v) agarose gel and the bands were visualized by ethidium bromide staining on a UV trans illuminator. Each experiment was repeated three times. Some of PCR products were sequenced to check the PCR specificity.

Western blot analysis

5-6 × 10^7 cells were collected and lysed in ice-cold lysis buffer containing 50 mmol/L Tris-Cl (pH 7.5), 150 mmol/L NaCl, 0.2 mmol/L EDTA, 1 mmol/L PMSF and 1% (v/v) Nonidet-P40 for 30 min. The lysates were centrifuged at 13,200 rpm for 10 min at 4°C and the supernatants were collected. 25 μg protein were resolved by a 12% SDS-PAGE and blotted on nitrocellulose membranes (Bio-Rad, Richmond, CA). Membranes were blocked with 10% (w/v) nonfat milk powder at room temperature for 1 h, and then incubated with antibodies to Eag (Alomone laboratories, Jerusalem, Israel), actin, phospho-ERK1/2, ERK1/2, phospho-JNK, JNK, phospho-p38 MAPK, p38 MAPK (Cell Signaling) and p53 (Abcam, Cambridge, MA) overnight, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibody (Santa Cruz Biotechnology). Then the membranes were developed with chemiluminescent detection kit (Zhongshan Biotechnology, Beijing, China) and exposed to X-ray films. Experiments were performed at least three times with representative data presented.

Cell proliferation assay

The cell proliferation was analyzed by using Cell Counting Assay Kit-8 (CCK-8) (Dojindo Molecular Technologies, Gaithersburg, MD) according to the manufacturer's protocol. In brief, 1×10^5 cells were starved in serum-free medium for 12 h and then the cells were transduced. After 48 h, cells were harvested. Ten microliters of Cell Counting Assay Kit-8 solution was added to each well, the cells were incubated for another 1 h, and the absorbance (A) at 450 nm was measured by using spectrophotometer (Bio-Rad). Experiments were performed at least three times with representative data presented.

Tumour model

Thymus-null BALB/c nude mice (female, age 6-8 weeks) were obtained from the Animal Center of Chinese Academy of Medical Sciences. All animal procedures were performed according to approved protocols and in accordance with recommendations for the proper use and care of laboratory animals. Osteosarcoma xenografts were established in nude mice according to a previous report.[21] A total of 1.5×10^6 MG-63 cells in 150 μL Phosphate-buffered Saline (PBS) were subcutaneously injected in the hind right leg. One week later, the tumors grew to visible size. The osteosarcoma-bearing mice were randomly divided into 3 groups (6 in each group). Group 1 received intra-tumor injections with Ad5-Eag-shRNA (10 MOI) every 2 days (6 injections totally). Group 2 received intra-tumor injections of Ad5-Control-shRNA (10 MOI) every 2 days (6 injections totally). Group 3 received normal saline injection as controls. Tumor volume

(cm^3) was determined based on the following formula: $ab^2/2$ where a was the length and b was the width of the tumor.[21]

Statistical analysis

All the data were presented as mean ± standard error of mean (SEM). Statistical significance was determined using t-test or analysis of variance (ANOVA) using the SPSS18.0 program. $p < 0.05$ was considered as statistically significant difference.

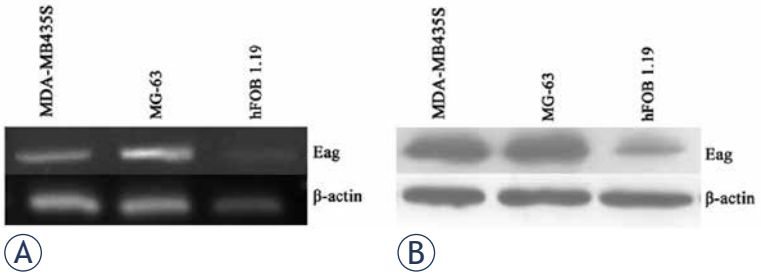

FIGURE 1. Expression of Eag is high in MG-63 cells. RT-PCR analysis of Eag mRNA level in three cell lines MDA-MB435S, MG-63 and hFOB 1.19 (A). Western blot analysis of Eag protein level in three cell lines MDA-MB435S, MG-63 and hFOB 1.19 (B). Shown were representative images from three independent experiments with similar results. MDA-MB435S and hFOB 1.19 cell lines served as positive and negative control, respectively. β-actin was used as a loading control.

Results

Expression of Eag in osterosarcoma cells

RT-PCR analysis was performed to examine the expression of Eag mRNA in osteosarcoma cell line MG-63 and osteoblastic cell line hFOB 1.19. As a positive control, we used breast cancer cell line MDA-MB435S.[6] Compared with hFOB 1.19 cells, the transcript of Eag was significantly increased in MG-63 cells (Figure 1A). Western blot analysis further confirmed the aberrant expression of Eag protein in MG-63 cells (Figure 1B).

Eag silencing reduces the proliferation of osteosarcoma cells

To characterize the oncogenic role of Eag in osteosarcoma cells, we inhibited Eag expression by virus mediated silencing. Western blot analysis showed that Eag expression was suppressed in Ad5-Eag-shRNA infected MG-63 cells compared with Ad5-Control-shRNA infected cells (Figure 2A). Consequently, cell proliferation was inhibited by 56% (n = 8) in Ad5-Eag-shRNA infected MG-63 cells (Figure 2B). To further confirm the oncogenic role of Eag, we treated MG-63 cells with imipramine, a nonspecific blocker of Eag activity. The results showed that MG-63 cell proliferation was inhibited by 48% (n = 8) after the treatment with 20 μM imipramine (Figure 2C). Collectively, these results suggest that Eag promotes the proliferation of MG-63 cells *in vitro*.

Eag silencing inhibits osteosarcoma growth *in vivo*

To investigate the in vivo role of Eag in osteosarcoma, we made a xenograft model of osteosarcoma using nude mice, and treated the xenografts by intra-tumor injection of Ad5-Eag-shRNA, Ad5-Control-shRNA or saline. The results showed that the tumor volume was significantly smaller in Ad5-Eag-shRNA injected animals compared to saline or Ad5-Control-shRNA injected animals at each evaluating time point (Figure 3). These *in vivo*

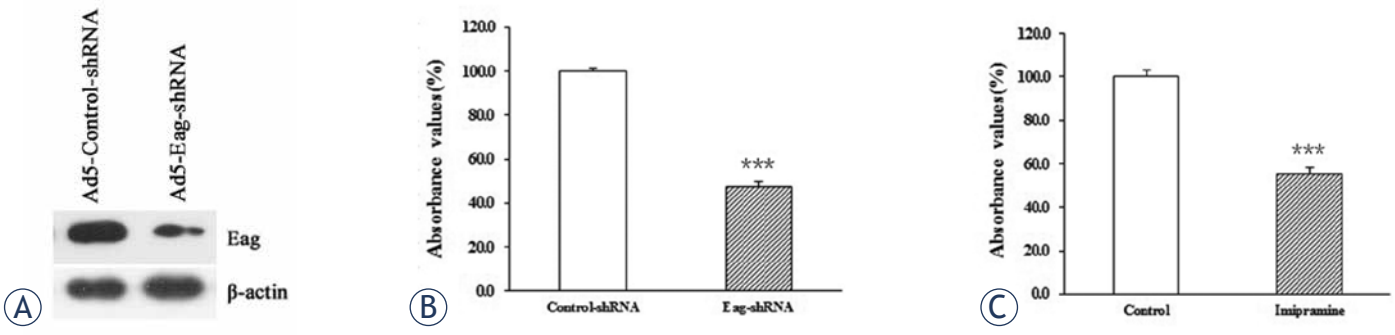

FIGURE 2. Inhibition of Eag expression or activity leads to reduced proliferation of MG-63 cells *in vitro*. The knockdown efficiency of Eag-shRNA in MG-63 cells was examined by Western blot analysis. β-actin was used as a loading control (A). CCK-8 assay showing that the proliferation of MG-63 cells was significantly reduced after transduction of Eag-shRNA (B) and treatment by 20 μM imipramine (C). Data were normalized as the value obtained for control or control-shRNA and presented as mean ± SEM (n = 8). ***p<0.001 vs. control.

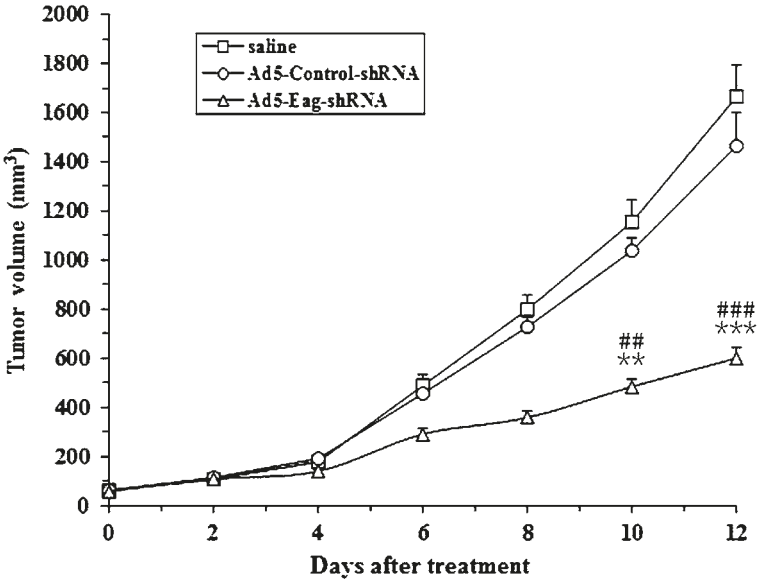

FIGURE 3. Eag silencing inhibits the growth of MG-63 cells in nude mice. Intra-tumor injection of Ad5-Eag-shRNA significantly reduced the size of MG-63 derived tumor implanted subcutaneously in nude mice during the 12-day follow-up period as compared with the saline control and the Ad5-Control-shRNA. **p<0.01; ***p<0.001, vs. the saline control; ##p<0. 01; ###p<0.001, vs. Ad5-Control-shRNA; (n = 6)

data confirm our *in vitro* results and suggest the oncogenic role of Eag in osteosarcoma.

p38 MAPK pathway is activated and promotes the proliferation of osteosarcoma cells

To explore the molecular mechanism underlying the oncogenic role of Eag in osteosarcoma, we focused on MAPK pathway because Eag was shown to have a role in the activation of MAPK pathway which is frequently activated in a variety of tumors.[22-24] The activation of p38 MAPK, JNK MAPK and ERK1/2 MAPK were detected in MG-63 cells and hFOB 1.19 cells by Western blot analysis. As shown in Figure 4A, both total p38 MAPK and phospho-p38 MAPK levels were higher in MG-63 cells than hFOB 1.19 cells. Next we employed MAPK inhibitors and siRNA against MAPK to treat MG-63 cells. CCK-8 assay showed that the proliferation of MG-63 cells was significantly decreased by p38 MAPK inhibitor SB203580 and p38 MAPK siRNA, but not by JNK inhibitor CEP11004, JNK siRNA, ERK1/2 MAPK inhibitor PD98059, or ERK1/2 siRNA (Figure 4B,C). Taken together, these data indicate that high expression of Eag may induce the activation of p38 MAPK which then promotes the proliferation of MG-63 cells.

p38 MAPK pathway modulates the expression of Eag in osteosarcoma cells

It has been proposed that p53 acts downstream of p38 MAPK pathway to modulate cell growth and apoptosis.[25,26] Thus, we examined the expression level of p53 and Eag by Western blot analysis. A significant increase of p53 protein level and reduction of Eag protein level were observed in MG-63 cells treated by SB203580 or p38 MAPK siRNA (Figure 5A).

Next we treated MG-63 cells with p53 activator nutlin-3 and found that p53 activation induced cell growth arrest and reduced Eag protein level in

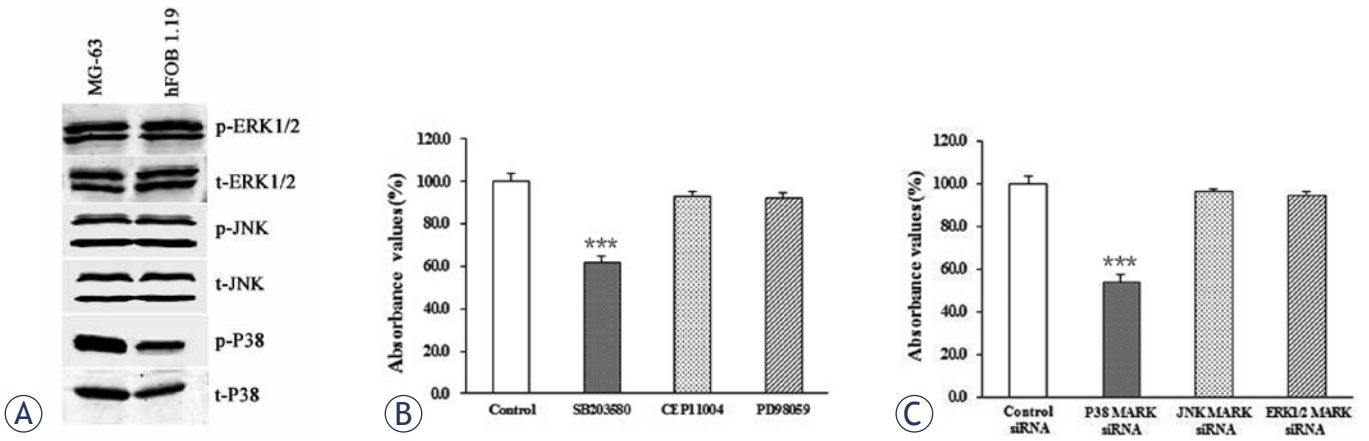

FIGURE 4. p38 MAPK pathway is activated and promotes the proliferation of MG-63 cells. Western blot analysis of the activation of MAPK pathways in MG-63 and hFOB 1.19 cells. Shown were representative blots from three independent experiments with similar results. Specifically, the phospho-p38 MAPK level was higher in MG-63 cells than in hFOB 1.19 cells. β-actin was used as a loading control (A). MG-63 cells were treated with different MAPK pathway inhibitors and cell proliferation was evaluated by CCK-8 assay (B). MG-63 cells were treated with different siRNAs against MAPK pathway components and cell proliferation was evaluated by CCK-8 assay (C). Data were normalized as the value obtained for control or control-siRNA and presented as mean ± SEM (n = 8); ***p<0.001 vs. control

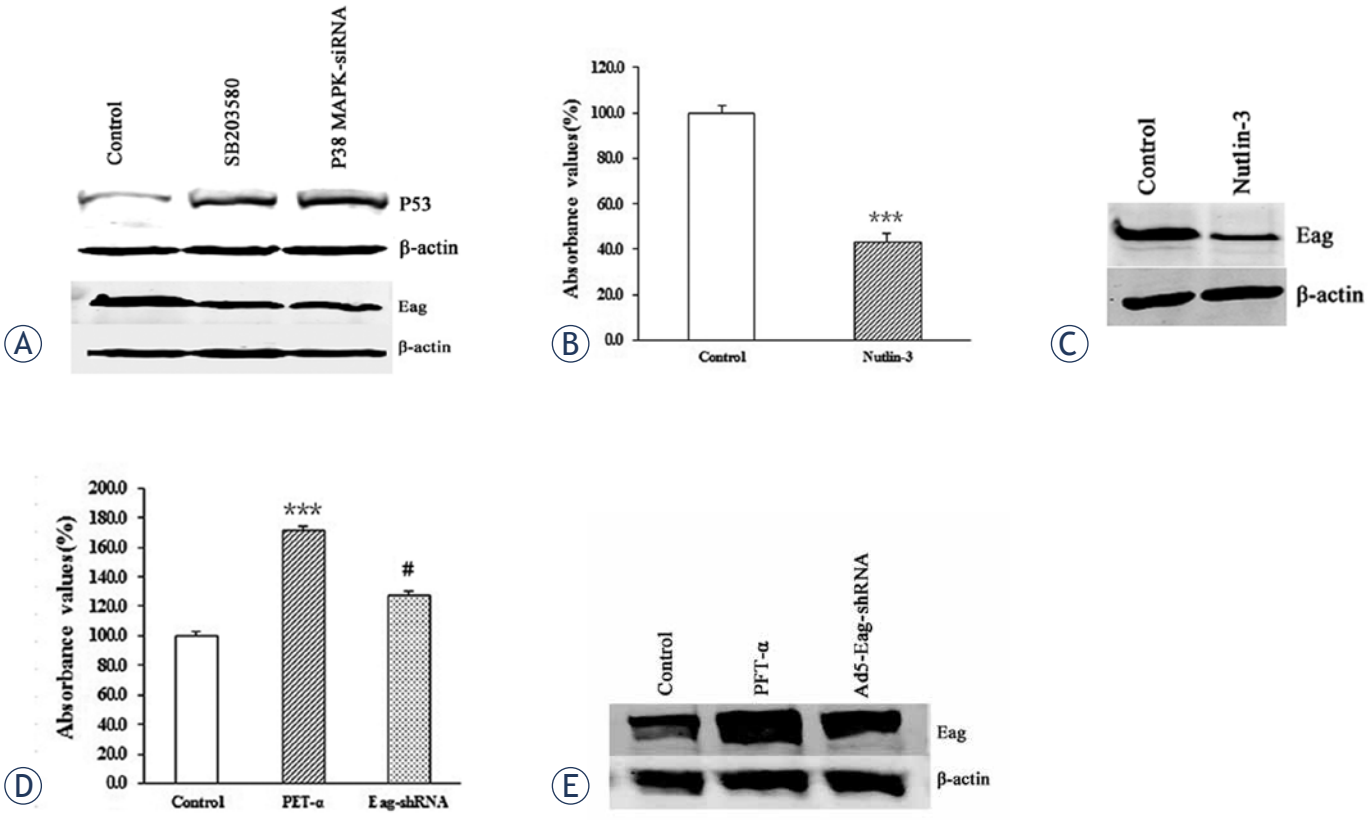

FIGURE 5. p38 MAPK pathway modulates the expression of Eag in MG-63 cells. Western blot analysis of p53 and Eag protein levels in MG-63 cells treated by SB203580 or p38 MAPK-siRNA (A). MG-63 cells were treated with nutlin-3 (1 μM) and cell proliferation was evaluated by CCK-8 assay (B). ***p < 0.001 vs. control. MG-63 cells were treated with nutlin-3 (1 μM) and Eag protein level was detected by Western blot analysis (C). MG-63 cells were treated with PFT-α (30 μM) alone or together with Ad5-Eag-shRNA and cell proliferation was evaluated by CCK-8 assay (D). MG-63 cells were treated with PFT-α (30 μM) alone or together with Ad5-Eag-shRNA and Eag protein level was detected by Western blot analysis (E). Data were normalized as the value obtained for control or control-siRNA and presented as mean ± SEM (n = 8). *** p<0.001 vs. control; # p<0.05 vs. PFT-α alone. Shown were representative blots from three independent experiments with similar results. β-actin was used as a loading control.

MG-63 cells (Figure 5B,C). On the other hand, inactivation of p53 by PFT-α promoted cell growth and increased Eag protein expression, which were abrogated by Eag-shRNA (Figure 5D,E).

Discussion

Imipramine, a tricyclic antidepressant, has been widely used in the psychiatric treatment for more than 50 years. The recent study showed that imipramine had a relative high affinity to Eag channels and permeate the membrane to inhibit the hEag1 current by selectively binding to open channels.[9] The non-specific cytotoxic doses of imipramine is 50 μM.[27] Thus, we used 20 μM imipramine in this study to avoid its cytotoxicity. We observed a significant reduction of proliferation in MG-63 cells treated by 20 μM imipramine for 2 days, which is consistent with previous reports.[4,28,29] However, the side effects of imipramine limit its applicability in the cancer treatment.[6] Compared with imipramine, siRNA is a more specific tool to investigate the role of Eag in cancer progression. siRNA mediated knockdown of Eag resulted in reduced proliferation of tumor cell lines without observable nonspecific responses.[8] In our study, Eag-shRNA could effectively downregulate Eag expression in osteosarcoma cells with high specificity. In addition, Eag-shRNA inhibited the proliferation of osterosarcoma cells *in vitro* and *in vivo.*

Eag overexpression is known to be implicated in tumor progression. However, the intracellular signaling pathways downstream of Eag have not been fully characterized. MAPK pathway plays critical roles in the pathogenesis of various cancers[30-33], including osteosarcoma.[34,35] Results reported in our study demonstrated the correlation between high expression of Eag and the activation of p38 MAPK in MG-63 cells. It has been proposed recently that

perinuclear localization of Eag could lead to the activation of MARP pathway resulting in increased cell proliferation.[36] Therefore, it is possible that overexpression of Eag induces the activation of p38 MAPK which then promotes the proliferation of osteosarcoma cells because we showed that the inhibition of p38 MAPK/p53 pathway led to reduced MG-63 cell proliferation .

Interestingly, our results showed a significant increase of p53 protein level in MG-63 cells treated by p38 MAPK inhibitor or siRNA, suggesting that p53 acts downstream of p38 MAPK in MG-63 cells. Furthermore, p53 could modulate the expression of Eag in MG-63 cells as shown by the use of p53 activator and inhibitor. These data suggest that Eag and p38 MAPK may form a positive feedback loop to maintain the high expression of Eag in osteosarcoma cells. When Eag expression is high, it activates p38 MAPK, which in turn inactivates p53 and relieves the inhibition of Eag expression by p53. This is consistent with a recent report that Eag expression is negatively regulated by p53 through p53-miR34-E2F1 pathway in human neuroblastoma cells SHSY5Y.[37]

It is important to note that Eag contributes to tumor progression independently of its primary function as an ion channel. Previous study has shown that Eag interferes with oxygen homeostasis by increasing HIF-1 activity and VEGF secretion, thus promoting tumor vascularization.[6] In this study our *in vitro* data using imipramine, a nonspecific blocker of Eag conduction activity, indicated that the proliferation of osteosarcoma cells depends on the K^+ conducting activity of Eag. However, our *in vitro* and *in vivo* data using virus mediated Eag silencing to inhibit Eag expression suggest that other oncogenic role of Eag independent of its K^+ conducting activity could not be excluded. Further studies are necessary to characterize the role of Eag in osteosarcoma development, especially angiogenesis and metastasis.

In summary, our results showed that Eag channel functions as an oncogene to promote the proliferation of human osteosarcoma cells. Furthermore, the high expression of Eag in osteosarcoma cells is regulated by p38 MAPK/p53 pathway. These findings suggest that the inhibition of p38 MAPK pathway or Eag overexpression is a promising approach for osteosarcoma therapy.

Acknowledgment

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research article

Hypofractionated stereotactic radiotherapy for large or involving critical organs cerebral arteriovenous malformations

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Background. The treatment of large arteriovenous malformations (AVMs) or AVMs involving eloquent regions of the brain remains a challenge. For inoperable lesions, observation, volume-staged radiosurgery or hypofractionated stereotactic radiotherapy (HFSRT) are proposed. The aim of our study was to assess the safety and efficiency of HFSRT for large AVMs located in eloquent areas of the brain.

Materials and methods. An analysis of records of 49 patients irradiated for cerebral AVMs with a mean dose of 19.9 Gy (12-28 Gy) delivered in 2-4 fractions with planned gap (at least one week) between fractions. Actuarial obliteration rates and annual bleeding hazard were calculated using Kaplan-Meier survival analysis and life tables.

Results. Annual bleeding hazard rates were 4.5% and 1.6% after one and two years of the follow-up, respectively. Actuarial total obliteration rates were 7%, 11%, and 21% and total response rate (total and partial obliterations) 22%, 41%, and 55% after one, two and three years of the follow-up, respectively. There was a trend towards larger total obliteration rate in patients irradiated with fraction dose ≥ 8 Gy and total dose > 21 Gy for lesions of volume ≤ 8.18 cm^3 which was not observed in case of partial obliterations.

Conclusions. HFSRT results with relatively low obliteration rate but is not associated with a significant risk of permanent neurological deficits if both total and fraction doses are adjusted to size and location of the lesion. Predictive factors for total and partial obliterations can be different; this observation, however, is not firmly supported and requires further studies.

Key words: arteriovenous malformations; hemorrhage; hypofractionated stereotactic radiotherapy; obliteration; stereotactic radiosurgery

Introduction

Treatment of large arteriovenous malformations (AVMs) is still a challenge for clinicians. The available options include surgery in rare cases, embolization, usually as a part of the combined treatment, stereotactic radiotherapy, and eventually, observation. Single dose radiosurgery in case of large-volume lesions is associated with a significant risk of morbidity.[1,2] To reduce the risk of complications, volume-staged radiosurgery, repeated radiosurgery or hypofractionated stereotactic

radiotherapy (HFSRT) are implemented. The reported results, however, are below expectation if compared with the results of stereotactic radiosurgery for selected, small and low-grade lesions. The most common system used for grading AVMs is the Spetzler-Martin grading system. It was originally developed for assessing the risk of surgery and the grade is the sum of points assigned for the size of the nidus (<3 cm – 1, 3-6 cm – 2, >6 cm – 3), eloquence of the adjacent brain (non eloquent – 0, eloquent – 1), and type of venous drainage (superficial only – 0, deep – 1).[3] If one thoroughly analyzes these reports, it can be easily seen that the best results of radiosurgery are reported in series with a great proportion of low-grade (Spetzler-Martin I and II) lesions, sometimes exceeding 60% of the whole group.[4] When high-grade AVMs constitute a large proportion of the treated patients, the results are strikingly worse and for grade IV lesions obliterations of the order of 20% are reported.[1] Some authors claim that large size of an AVM is not a risk factor for hemorrhage and in their series a higher risk of bleeding from smaller lesions was reported.[5] On the other hand, there is an increasing evidence that large volume of an AVM can be an independent risk factor of bleeding after diagnosis, especially in case of high-grade AVMs with bleeding as a presenting syndrome.[6,7] This observation is the rationale for stereotactic irradiation for otherwise untreatable large AVMs. The aim of our study was to evaluate the early outcome of patients treated with HFSRT for large AVMs or malformations involving structures prone to radiation damage like optic pathway or brainstem.

Materials and methods

The analysis is based on records of 49 patients (25 women and 24 men) of age ranging between 14 and 71 years (mean 36), irradiated with HFSRT between 2003 and 2009. Mean volume of the lesion was 25.07 cm^3, median 18.05 cm^3 and ranged between 0.36 and 153.98 cm^3. Most of the lesions were Spetzler-Martin grade III AVMs (36.7%), the detailed distribution of the lesions according to Spetzler-Martin grade is shown in Table 1.

The proportion of grade II AVMs in our series is quite significant. These patients were referred to radiosurgery by neurosurgeons because of comorbidities that increased the risk of anesthesia or surgery or because of the risk of severe complications, *e.g.* if the lesion was in an eloquent area or had both multiple deep draining veins and deep arterial feeders. Some of the patients preferred a non-invasive treatment and did not agree for neurosurgical intervention. Overall, all the patients were consulted by a neurosurgeon and either refused surgery or were referred to our department for treatment due to a high risk associated with the potential surgical intervention. Modified AVM score ranged between 0.95 and 16.11 with the mean and median value of 3.29 and 2.61, respectively. AVM score calculated according to the original formula was within the same range with mean and median values of 3.45 and 2.61, respectively. Eighteen (37%) patients presented with hemorrhage, 17 (34.7%) with epilepsy, 17 (34.7%) with paresis and 31 (63%) with headaches. At least one embolization before radiosurgery was performed in 28 (57%) (Table 2).

All radiosurgical procedures were performed using a linear accelerator (Clinac 2300 C/D, Varian Medical Systems, Palo Alto, USA) equipped with a micro-multileaf collimator (m3, BrainLab AG, Feldkirchen, Germany). Radiosurgery planning was based on the fusion of computed tomography (CT), magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) images. The target was defined as an AVM nidus without draining veins and outlined on the fused images. The PTV was not outlined separately but the leaves of the micro-multileaf collimator were moved 1-2 mm away from the target to account for positioning inaccuracy and possible intrafraction movement. The

TABLE 1. Distribution of Spetzler-Martin grades

Grade	Number	Percent
II	15	30.6
III	18	36.7
IV	12	24.5
V	4	8.2

TABLE 2. Number of embolizations before stereotactic radiotherapy

Number of procedures	Patients	Percent
None	21	43.6
1	9	18.7
2	5	10.4
3	7	14.5
4	3	6.2
5	2	4.1
6	1	2

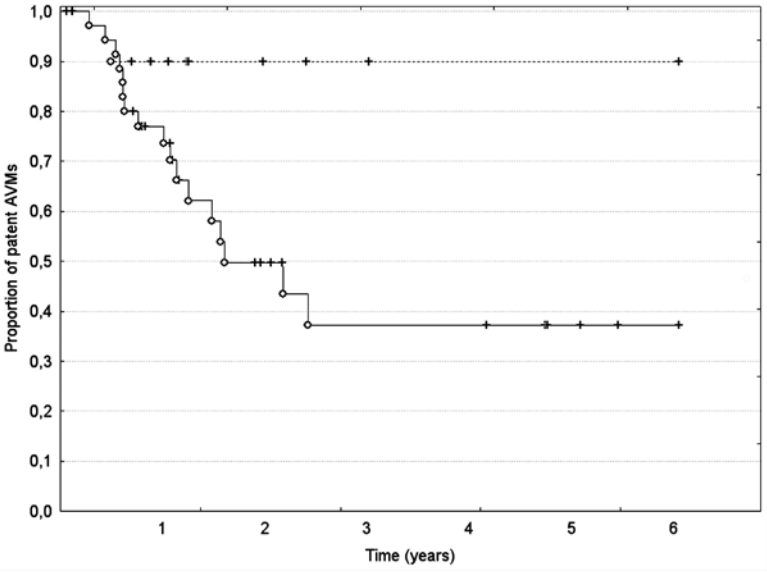

FIGURE 1. Actuarial obliteration rates for lesions with radiosurgery-based AVM score < 4 (solid line) and ≥ 4 (dashed line). The difference, although apparent, did not reach statistical significance (p = 0.06). O = complete; + = censored

width of the margin was modified according to proximity of organs at risk. Thermoplastic masks were used for immobilization of the patients. To verify the position of the radiation isocenter the Winston-Lutz test was performed before irradiation. The treatment position was verified with electronic portal image device (EPID) generating MV images or with an on-board imaging device (OBI) acquiring orthogonal kV images used after the treatment unit upgrade to assure a better image quality precision of localization.[8,9] If any shift was recorded, the treatment couch was moved and the imaging was made again to ensure the correct position of the patient. The patients were irradiated with 2-4 fractions of 6-11 Gy to total doses of 12-28 Gy. Mean and median total doses were 19.9 and 21 Gy, respectively. Mean and median fraction doses were 8.1 and 8 Gy, respectively. The most frequently used fractionation regimen was 3x7 Gy (14 patients) and 3x8 Gy (9 patients). The dose was prescribed according to lesion size and proximity of organs at risk and was specified at the isocenter. The dose constraint for the optic chiasm and optic nerves is 8 Gy for single fraction radiosurgery in our institution. The tolerable doses for hypofractionated regimens are not reliably defined. Consequently, we chose the fractionation scheme which allowed maintaining the dose for optic apparatus within 6-6.5 Gy per fraction in multifraction regimens. Several static conformal fields or fixed-angle intensity-modulated radiosurgery were used for irradiation whichever yielded a better dose distribution. The radiosurgical procedures are performed only once a week (Saturdays) in our center due to very high workload (linear accelerator used for radiosurgery operates on weekdays form 7 am. to 10 pm. and is used for conventional fractionated radiotherapy). Consequently, the overall treatment time varied according to the number of fractions and length of intervals between them. The mean and median overall treatment time was 7.8 and 6 weeks, respectively. Median and mean follow-up time was 23.8 and 28.9 months, respectively.

The investigators strictly followed recommendations of the Helsinki Declaration (1964, with later amendments) and of the European Council Convention on Protection of Human Rights in Bio-Medicine (Oviedo 1997).

The Kaplan-Meier analysis was used to determine actuarial obliteration rates. The annual bleeding hazard rate was calculated with Kaplan-Meier life tables. The log-rank test was used to compare outcomes of different groups defined according to selected patient- and treatment-related factors (sex, age, AVM volume, number of hemorrhages and embolizations, Spetzler-Martin grade, AVM score, fraction dose, total dose, number of fractions). All statistical evaluations were performed with Statistica 7.0 PL software.

Results

Actuarial total obliteration (TO) rates after one, two, and three years of observation were 7, 11, and 21%, respectively. If partial obliterations (PO) were added, the cumulative response to the treatment (partial and total obliterations) was 22, 41, and 55%, respectively. According to Spetzler-Martin grade TO was seen in 4/15 (26.6%) grade II, 1/18 (5.5%) grade III, and 1/12 (8.3%) grade IV AVMs, whereas PO in 3/15 (20%), 6/18 (33.3%), and 4/12 (33.2%), respectively. None of grade V AVMs obliterated. Mean and median time to PO was 12.5 and 12.3 months, respectively, whereas mean and median time to TO was 16 and 12.8 months, respectively. The statistical analysis did not reveal significant differences in probability of obliteration (TO + PO) according to sex, age, Spetzler-Martin grade, number of fractions and hemorrhages. There was, however, a trend towards higher obliteration rates in patients with AVM score less than 4 (Figure 1).

Similar differences were observed when total obliterations were analyzed. AVMs irradiated with total dose >21 Gy, fraction dose ≥8 Gy, and of volume ≤ 8.18 cm^3 which is an equivalent of a lesion

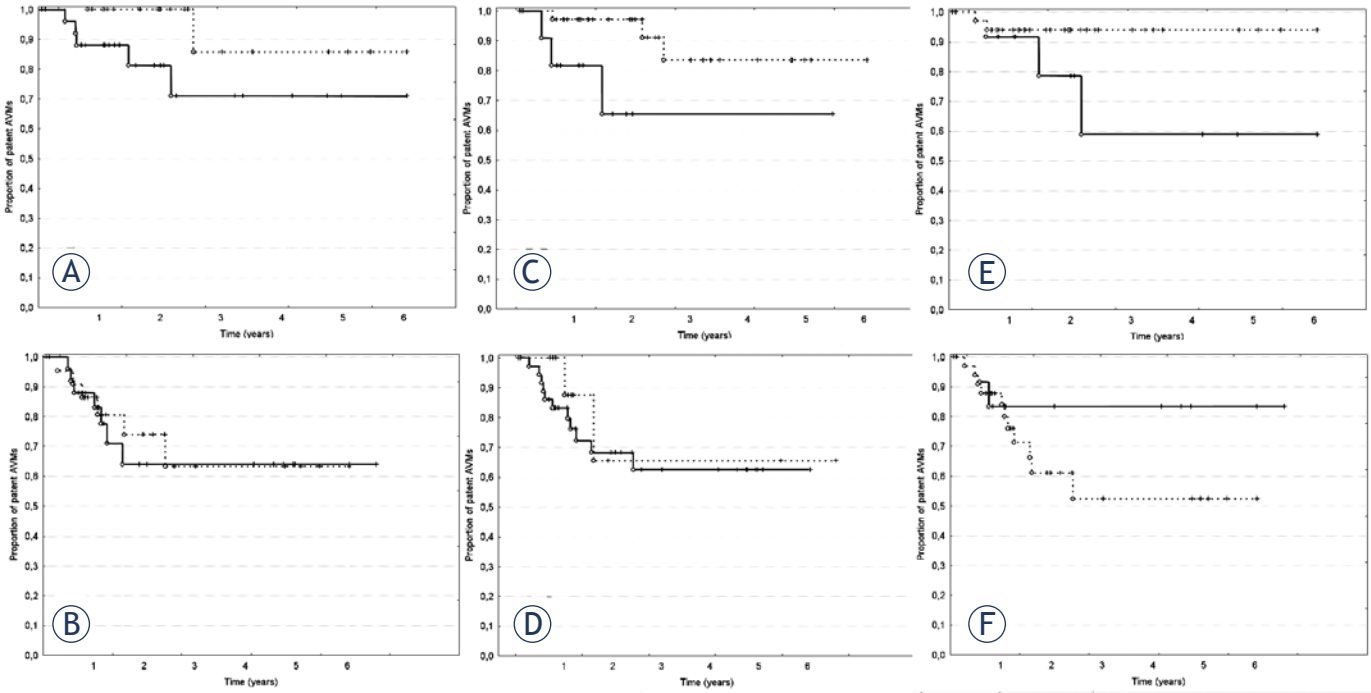

FIGURE 2. Actuarial obliteration rates for lesions treated with fraction dose ≥ 8 Gy (solid line) and < 8 Gy (dashed line); graphs A and B: A – complete obliterations; B – partial obliterations; total dose > 21 Gy (solid line) and ≤ 21 Gy (dashed line); graphs C and D: C – complete obliterations, D- partial obliterations; and of volume equal or smaller than 8.18 cm^3 (solid line) and larger than 8.18 cm^3 (dashed line); graphs E and F: E – complete obliterations, F- partial obliterations. O – complete; +- censored

of 2.5 cm diameter tended to have higher TO rates (p = 0.0518, p = 0.0937, and p = 0.0867, respectively, log-rank test). The differences, although apparent, did not reach the level of statistical significance. Interestingly, these factors did not show a tendency to influence the response rate in case of PO (p = 0.5895, p = 0.8357, and p = 0.2604, respectively, Figure 2).

In the group of partially obliterated AVMs the lesions that had previously been partially embolized tended to respond better than those treated *de novo* but this difference was also not statistically significant (p=0.0949). This trend, however, was not observed when TO were analyzed (p=0.3382).

Two patients bled after the treatment which resulted in annual bleeding hazard rate of 4.5 and 1.6% in the first and second year of the follow-up, respectively. Both hemorrhages occurred within the first four months after the treatment, before any treatment response could be seen. None of the patients bled before the completion of the treatment. The first patient with hemorrhage was a 40-years old male with a giant, grade IV AVM localized in the left thalamus and extending into the third ventricle. He had an episode of bleeding and one embolization before radiotherapy. He was treated with 18 Gy in two fractions. The other moved abroad and did not show-up for the scheduled follow-up visit. The information about bleeding was mailed from neurosurgery department taking care of the patient at that time. The patient was a 23-years-old female with a grade II AVM localized in the parasagittal, fronto-parietal region. She received 20 Gy in 2 fractions and was neither previously treated with endovascular methods nor had a hemorrhage before radiosurgery. The bleeding was diagnosed with CT imaging which revealed an intraventricular hemorrhage. Bilateral external ventricular drains were placed to relieve the symptoms of hydrocephalus. The patient finally recovered and was able to return to work. Apart from the AVM the patient had multiple aneurysms that could also have bled and because the source of bleeding was not clearly identified in the documentation we received, it should be in fact considered undetermined. If we assume that an aneurysm was the source of hemorrhage in this patient, the calculated annual hazard ratios for bleeding in our series would be 2.2 and 1.6% in the first and the second year of follow-up, respectively.

We observed transient neurological deterioration in six patients after the treatment. In most cases these were aggravated symptoms of paresis or epileptic seizures and headaches that resolved

during the follow-up. In two patients an episode of sudden, severe headache and neurological deterioration occurred which was the reason for hospitalization and intensive diagnostic workup. In none of them a hemorrhage was diagnosed, in both, however, no blood flow through the AVM nidus was seen. In both patients the symptoms finally resolved to the level from before the treatment. No symptomatic necrosis after HFSRT was observed. We did not record any apparent signs of edema or demyelination asymmetric or shifted in relation to the target volume suggestive of geographical error. Consequently, we can assume that the position verification procedures were sufficient for dose delivery according to the treatment plan.

Discussion

In our series the number of obliterated AVMs is low with only 21% of total obliterations and additional 34% of partial obliterations after three years of the follow-up. Nevertheless, if one takes into account very large median volume of the lesions treated, the results compare favorably with those presented in other papers. The obliteration rate depends mainly on the size and grade of the AVMs and also on the total and fraction dose applied.[1,10,11] In publications showing the results of the treatment of exclusively high grade lesions the results are discouraging. Xiao *et al.* reported the results of the treatment with HFSRT a series of 20 AVMs greater than 5 cm. No lesion obliterated, nevertheless, they observed a significant decrease in the volume of patent AVM with residual volume ranging from 1.5-98% of initially treated lesion.[12] The annual risk of bleeding after the treatment was 2.06% which is in line with our results and indicates that HFSRT does not increase the risk of subsequent hemorrhage.[12] In a series of AVMs larger than 15 cm^3 reported by Lee *et al.* they observed 25% of complete and 50% of partial obliterations after the mean follow-up of 41.2 months.[13] In patients treated with proton beam radiosurgery for high-risk inoperable AVMs at the median follow-up of 56.1 months 15% of TO and 34% of PO were observed.[14] Moreover, a significant actuarial rate of hemorrhage of 22% at 5 years was reported with large volume of the lesion being the risk factor for bleeding.[14] Our data did not confirm the high risk of bleeding in the latency period. This may be the result of several factors like higher total doses applied in our series, shorter follow-up and patient selection. Some authors consider partially occluded AVMs more prone to bleeding and advocate a further treatment to reduce the risk of hemorrhage. This is certainly the case after partial embolization or partial resection of the lesion which causes sudden increase of blood pressure in the remaining and defective vessels of the nidus.[15] Partial obliteration of an AVM after radiosurgery is a slower and gradual process. Histological changes in the vessels of an irradiated AVM make them thicker and more resistant to changes in blood pressure and thus, to rupture.[16] There is also some evidence that in a long-term perspective, even partial treatment of an AVM can eventually be more advantageous than no treatment at all.[6,17] Moreover, partially obliterated lesions can be further treated with stereotactic radiosurgery aimed at obliteration of the patent portion of the nidus or can become suitable for surgery.[12,18,19]

A relatively short follow-up can be considered a shortcoming of this paper. The increasing number of obliterations with extending follow-up time is well appreciated. We observed only 8% of TO after the 1st year of the follow-up, whereas after 3 years it was already 21%. Lindvall *et al*. observed a raise in actuarial obliteration rates from 56% to 81% for lesions of volume 4-10 cm^3 and from 50% to 70% for lesions larger than 10 cm^3 after two and five years of the follow-up, respectively.[20] Zabel du Bois *et al*. irradiated with HFSRT lesions of median volume of 27 cm^3 which resulted in obliteration rate of 17% after three years. The proportion of obliterated lesions rose to 33% after four years of the follow-up.[10] In the series reported by Chang *et al*. the 3-year, 5-year and 6-year actuarial obliteration rate for AVMs treated with HFSRT was 32, 61 and 71%, respectively.[21] Taking into account much larger median volume of the AVMs in our series (18.5 cm^3, whereas in their series only 28% AVMs had diameter >2.5 cm which results in volume of 8.18 cm^3 assuming spherical shape of a lesion, the median volume was not given) the results after three years of the follow-up are comparable.[21] Veznedaroglu *et al*. reported 22% obliteration rate after the 5-year follow-up in the group of patients irradiated with 30 Gy in 6 fractions. Irradiation with 7 Gy per fraction instead of 5 Gy resulted in total dose of 42 Gy and a high – 81% 5-year obliteration rate but at the cost of significant treatment-related morbidity. As a result, the dose of 7 Gy was considered too high to assure a safe treatment.[22] We did not implement a fixed protocol of hypofractionation like in most other studies which assumed a fractionation schedule of 5-6 x 5 Gy, 5 x 6 Gy or 5 x 7 Gy irrespective of volume and localization of the lesion.[12,22,23] Instead,

we often used fraction doses higher than 7 Gy to maximize the biological effect of a single fraction. Nevertheless, along with the increase of the fraction dose we reduced the number of fractions to decrease the total dose and thus, the probability of complications. We expect, therefore, a higher 5-year total obliteration rate with 21% of total obliteration rate and another 34% of partial obliterations just after three years of the follow-up. On the other hand, Aoyama *et al*. reported a much higher three-year obliteration rate of 53% after HFSRT but with a higher total dose of 28 Gy given in 4 fractions. In their series however, 12/26 AVMs were grade I or II lesions and there was the same number of AVMs with maximum diameter <2.5 cm and >2.5 cm (mean 2.26 cm).[24]

An interesting observation is the difference between factors that may influence the total and partial obliteration rates. Although the level of statistical significance was not reached, there was a clear tendency towards higher probability of complete obliteration of AVMs of smaller size (≤2.5 cm) irradiated with fraction doses at least 8 Gy and total doses greater than 21 Gy which is in good agreement with other studies.[1,20,24] Factors influencing a partial response for the treatment seem to be somewhat different. We observed a trend towards greater probability of PO only in patients who had at least one embolization before irradiation whereas the dose and size of the lesion seemed to play a lesser role. These observations, however, require further studies because of borderline statistical significance resulting from low number of obliterations and limited number of patients in our series.

Larger than usually reported intervals between fractions did not influence negatively the treatment results in any obvious way. The obliteration and bleeding rates appear slightly superior to those reported by the Boston group.[14] We suppose that elongation of time between consecutive fractions can reduce the probability of complications. In our series we did not observe any serious treatment-related damage. The treatment schedule with elongated intervals between fractions is very scarcely represented in the literature. Irradiation with protons with at least one week interval between fractions was not associated with any complications that could be linked to the fractionation schedule. Moderate success of this treatment, like in our series, was rather associated with large volumes of the lesions and relatively low doses applied.[14] Doses in the range reported in the current series proved to be safe and perhaps there is still some possibility to increase the total dose by adding fractions or increase dose per fraction.

The treatment of large AVMs still remains a challenge, especially in light of conflicting results of the treatment reported in the literature. Many clinicians advocate observation for patients with large AVMs following the results published by Han *et al*.[25] In newer series, however, these results were not confirmed. Moreover, recent comprehensive analyzes suggest that large AVMs can be particularly prone to bleeding resulting with morbidity and mortality larger than usually reported for AVMs, especially if they presented with hemorrhage. Consequently, the active treatment is proposed in these patients to reduce the risk of subsequent bleeding with often debilitating or even fatal outcome.[6]

Conclusions

HFSRT should be offered only to patients with AVMs unsuitable to surgery or single fraction procedure because of relatively low probability of obliteration after the fractionated irradiation. A low risk of hemorrhage after the treatment and a low rate of complications if the fraction and total dose are adjusted to the size and location of the lesion indicate that hypofractionated stereotactic radiotherapy can still be an attractive option for patients with otherwise untreatable AVMs. The probability of total and partial obliteration may be associated with distinct factors and possibly depend on more variables than radiation dose and size or volume of the lesion. Further studies are necessary to confirm these observations.

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research article

Epirubicin and docetaxel as neoadjuvant treatment of hormone receptor positive, HER-2 negative breast cancer: findings from two successive phase II studies

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Background. We report on the activity of the combination of epirubicin and docetaxel given in neoadjuvant setting for 4 and 8 cycles respectively in 2 successive series of patients with large operable or locally advanced, hormone receptor positive, HER-2 negative breast cancer.

Patients and methods. Patients were treated from 2002 to 2006 with epirubicin 90 mg/m^2 and docetaxel 75 mg/m^2 intravenously, every 3 weeks for 4 cycles before and 4 cycles after surgery (Series I – 13 patients), and from 2006 to 2010 with the same regimen administered for 8 cycles preoperatively (Series II – 37 patients), plus hormonal therapy for 5 years and radiation therapy if indicated. All Series I and 32 Series II patients were able to complete the preoperative chemotherapy.

Results. A complete response was found in 1 patient from Series I and 13 patients from Series II and the partial remission in 10 patients from Series I and 21 patients from Series II. Two Series I and 3 Series II patients did not respond clinically. Response rate (Series I/Series II) was 84/92%. All 50 patients underwent surgery. In Series I patients, 3 pCR occurred in the breast and the axilla was histologically negative in 2 cases. No evidence of disease both in the breast and in the axilla was achieved in 7.6% (1/13) of patients. In Series II patients, 8 pCR occurred in the breast and axilla was histologically negative in 15 patients. No evidence of disease both in the breast and in the axilla occurred in 10.8% (4/37) of patients. G3-G4 toxicity included myelosuppression in 3 patients from Series I and all patients from Series II, and mucositis in 1 patient from Series I and 4 patients from series II. No other G3-4 toxicities or toxic deaths occurred. Five-year progression free survival was 38% and 90% in Series I and Series II patients respectively.

Conclusions. The incidence of pathologic complete remissions was lower in our patient population, compared to reported data. A longer duration of the preoperative treatment might be associated with a longer progression-free survival.

Key words: breast cancer; docetaxel; epirubicin; neoadjuvant chemotherapy

Introduction

The identification of subgroups of breast cancers with different behaviour and different response to therapy has profoundly modified the approach to breast cancer, both in the adjuvant[1] and in the advanced settings.

Preoperative or neoadjuvant chemotherapy is a standard of care for cases of breast cancer not amenable to conservative surgery.[2,3] The achievement of a pathological complete response (pCR) has a

prognostic value, regardless of the hormone receptor status of the tumour.[4] Advantages of such approach include the possibility to perform smaller resections with better cosmetic outcome[5,6] and an early assessment of response to chemotherapy.[3,7] The pathological complete response rate is in the order of 20-25%, with a considerable degree of interstudy variability.[3,8]

There is an agreement on the use of the two most active classes of drugs (anthracyclines and taxanes) in the neoadjuvant setting. The combination of epirubicin and docetaxel (ET) is widely accepted in this situation.[9,10]

Patients with hormone receptor positive, HER-2 negative disease, defined as Luminal A and B cases[11], have been found to respond less well to neoadjuvant chemotherapy, particularly in terms of achieving pCR compared to patients with HER-2 positive or triple negative disease.[12-14] For example, in a large pooled analysis of 7 neoadjuvant trials, pCR rates were 9% in Luminal A and B cases, 32% in HER-2 positive cases and 34% in triple negative cases.

In March 2002, we started a study that evaluated the activity of four cycles of neoadjuvant ET in patients with breast cancer. Patients were not selected according to the molecular subtype. The results of that study have been reported previously.[15] Since 2006, the neoadjuvant treatment was changed and for the subsequent study hormone receptor positive, HER-2 negative patients were selected only. They were treated with ET at the same dosages as in the previous study, but received 8 instead of 4 neoadjuvant chemotherapy cycles.

In this paper we descriptively present the updated outcome of the group of hormone receptor positive, HER-2 negative cases (selected cases from previous study) treated with 4 cycles of neoadjuvant ET (Series I) and the outcome of patients treated with 8 cycles of neoadjuvant ET that were included in the subsequent study (Series II). No formal comparison was made throughout.

Patients and methods

Eligibility criteria for Series I and II were the same and included: histologically confirmed invasive breast cancer, oestrogen (ER) and/or progesterone receptor (PR) positive, HER-2 negative, operable T2-T4 disease, unsuitable for conservative surgery, no evidence of distant disease, no previous antineoplastic treatment, adequate bone marrow (absolute neutrophil count $\geq 2.0 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l), renal, liver and cardiac (left ventricular ejection fraction $\geq 50\%$ by echocardiography) functions. Pre-treatment evaluation included mammography and breast ultrasound, routine blood tests, chest X-ray, abdominal CT scan or ultrasound, electrocardiogram and echocardiogram. Bone scan was performed in the presence of symptoms. All patients provided written informed consent prior to the initiation of treatment. ER, PR, HER2 and Ki-67 status was evaluated by immunohistochemistry (IHC). IHC analysis was performed on formalin-fixed, paraffin-embedded breast cancer tissue using specific primary rabbit monoclonal antibodies to ER (SP1, Ventana, ready to use), PgR (1E2, Ventana, ready to use), ki-67 (30-9, Ventana, ready to use) and HER2 (4B5, Ventana, ready to use) with an autostaining system (Ventana Medical System, Tucson, Arizona). ER, PR and Ki-67 immunostaining results were recorded as the percentage of immunoreactive cells over at least 2000 tumor cells randomly selected from the periphery of invasive carcinoma in surgical specimens. HER2 positivity was defined as 3+ overexpression by IHC and/or as 2.2 or greater HER2-toCEP17 ratio by SISH according to American Society Oncology/College of American Pathologist Guidelines.

Chemotherapy consisted of epirubicin 90 mg/m^2 and docetaxel 75 mg/m^2 administered intravenously every 3 weeks for 4 cycles preoperatively in Series I and for 8 cycles in Series II. Prednisone 25 mg orally was administered every 6 hours 3 times before and 3 times after chemotherapy. 5-HT3 based antiemetic treatment was provided. Complete blood counts were prescribed weekly. Prophylactic oral antibiotics were recommended in cases with neutrophil count below 0.5×10^9/l. Therapy was administered every three weeks, provided that neutrophil count was $>2.0 \times 10^9$/l and the platelet count was $>100 \times 10^9$/1 on the day scheduled for the retreatment. G-CSF support (30 MU/day subcutaneously from day 2-11) was initiated in individual patients at the subsequent cycle in cases with febrile neutropenia or failure of neutrophil count recovery by the day of the retreatment. The epirubicin dose was decreased to 75 mg/m^2 in instances of grade 4 thrombocytopenia, grade >3 non-haematological toxicity (except for alopecia, nausea/vomiting, musculoskeletal pain), persistence of grade ≥ 2 non-hematologic toxicity at scheduled retreatment or febrile neutropenia despite G-CSF support. Treatment was discontinued in instances of congestive heart failure of any grade and/or of a significant reduction in left ventricular ejection fraction (≥10 % decrease from baseline as-

TABLE 1. Patient characteristics

	SERIES I	SERIES II
Total number of patients	13	37
Median age (range)	46 years (23-65)	46 years (27-67)
Menopausal status		
Premenopausal	7	28
Postmenopausal	6	9
Stage: T2	4	24
T3	5	10
T4	4	3
Inflammatory signs	3	1
N0/N1	9/13	15/22
Histology		
Ductal	12	29
Lobular	1	8
Hormone receptor status		
ER+/PgR+	9	23
ER+/PgR-	3	14
ER-/PgR +	1	0
Ki-67 (%)		
< 14	-	14
> 14	-	9
Unknown	13	14

sociated with a decline to a level <50 %) confirmed by an echocardiogram performed at one week interval.

Patients in Series II underwent mammography after 4 cycles to rule out the disease progression. At completion of preoperative chemotherapy (4 cycles in Series I, 8 cycles in Series II), repeat mammography and breast ultrasound were performed. Modified radical mastectomy or breast sparing surgery was performed. After surgery, 4 additional chemotherapy cycles as above were administered to Series I patients in the event of clinical response to pre-surgery chemotherapy. Patients in Series II received postoperatively Cyclophosphamide, Methotrexate and Fluorouracil (classical CMF) for 3 cycles. At completion of chemotherapy, hormonal therapy (tamoxifen plus LHRH analogue in premenopausal, aromatase inhibitor in postmenopausal patients) was prescribed to all patients. Radiation therapy was indicated after chemotherapy according to institutional guidelines. In the event of breast sparing surgery, the breast was irradiated with tangential fields at the dose of 50 Gy in 25 sessions, plus a 10 Gy in 5 sessions boost to the scar. After radical mastectomy, 40 Gy in 20 sessions were delivered to the tumour area in cases with of pT4 disease. The axillary region was irradiated if more than 3 lymph nodes were involved at histological examination.

The RECIST criteria for response evaluation were used[16], while toxicity was classified according to the Common Toxicity Criteria of the National Cancer Institute.[17] According to RECIST criteria, a 30% reduction in the longest diameter of the target lesion was required to qualify it for partial response. The pathological complete response (pCR) was defined as the absence of infiltrating and/or in situ carcinoma in the surgical specimen (breast and lymph nodes). Overall survival and progression free survival were calculated from the start of therapy until death or progression of disease, respectively. Progression free survival and overall survival curves were plotted by the Kaplan-Meier method.[18]

From March 2002 to May 2006, 13 Series I patients and from May 2006 to May 2010, 37 Series II patients were entered into the studies. The patient characteristics are reported in Table 1.

Results

Series I patients (n=13)

Out of the 13 patients, 12 received the planned 4 cycle of preoperative chemotherapy. Surgery was anticipated in one case due to the patient's preference. No patient progressed during the preoperative phase.

Clinical responses, evaluated before planned surgery, included complete response in 1 patient, partial response in 10, stable disease in 1, progression in 1. Response rate (responding/entered patients) was 84% (95% confidence limits, 64-100%). All 13 patients underwent surgery (radical mastectomy in 10, quadrantectomy in 3). The histological examination of the breast revealed no signs of disease in 3 patients, infiltrating carcinoma in 10. Pathological T classification was T0 in 3 patients, T1b in 1, T1c in 2, T2 in 3, T3 in 1, T4 in 3. In 2 patients with small residual primary tumour the disease was multifocal, precluding conservative surgery. Pathological tumour downstaging occurred in 3/4 T2, 4/5 T3, 3/4 T4 cases. The median number of examined axillary lymph nodes was 23 (range 1-29). In 1 patient less than 5 lymph nodes could be identified in spite of meticulous search. Lymph nodes were pathologically negative in 2 patients, positive in 11 patients. The median number of positive lymph nodes was 6 (range 2-22). Eight patients had more than 3 lymph nodes involved. A pCR (no evidence of disease both in the breast and in the axilla) was achieved in 7.6% (1/13) of patients. All 13 patients started with postoperative chemotherapy

TABLE 2. Toxicity of chemotherapy (Series I vs. Series II)

Toxicity	All grades (%)	G1 (%)	G2 (%)	G3 (%)	G4 (%)
Neutropenia	12 (92) vs 37 (100)	0 vs 0	1 (7) vs 0	1 (7) vs 2 (5)	10 (77) vs 35 (95)
Thrombocytopenia	0 vs 0	0 vs 0	0 vs 0	0 vs 0	0 vs 0
Anaemia	6 (46) vs 19 (51)	5 (38) vs 11 (29)	1 (7) vs 7 (18)	0 vs 1 (2)	0 vs 0
Nausea and vomiting	7 (53) vs 13 (35	3 (23) vs 2 (5)	4 (30) vs 11 (29)	0 vs 0	0 vs 0
Mucositis	8 (61) vs 20 (54)	4 (30) vs 9 (24)	3 (23) vs 7 (18)	1 (7) vs 3 (8)	0 vs 1 (2)
Diarrhoea	2 (15) vs 4 (10)	2 (15) vs 1 (2)	0 vs 3 (8)	0 vs 0	0 v s0
Onicopathy	1 (7) vs 5 (13)	0 vs 2 (5)	1 (7) vs 2 (5)	0 vs 0	0 vs 0
Asthenia	7 (53) vs 18 (48)	6 (46) vs 7 (18)	1 (7) vs 11 (29)	0 vs 0	0 vs 0

as planned and 8 of them received all 4 planned cycles (8 cycles altogether). One patient did not receive postoperative epirubicin and taxotere because of poor clinical/pathological response to neoadjuvant treatment. This patient received CMF postoperatively.

Twelve patients received adjuvant hormonal therapy and 9 underwent radiation therapy. Six events (5 distant metastases, 1 loco-regional relapse and 4 deaths, all due to progressive disease) occurred at a median follow up of 44.1 months.

Series II patients (n=37)

All patients completed the 4 pre-evaluation cycles. Anticipated surgery was offered to 3 patients due to lack of response and in 2 due to the patient's preference. Thirty-two patients were able to complete the 8 cycle preoperative phase. No patient progressed during preoperative chemotherapy.

Clinical responses, evaluated before planned surgery, included complete remission in 13 patients, partial remission in 21, stable disease in 3. Response rate (responding/entered patients) was 92% (95% confidence limits, 84-100%). All 37 patients underwent surgery (radical mastectomy in 22, quadrantectomy in 15). The histological examination of the breast revealed no signs of disease in 8 patients, infiltrating carcinoma in 29. Pathological T classification was T0 in 8 patients, T1a in 6, T1b in 4, T1c in 11, T2 in 5, T3 in 2, T4 in 1. In 1 patient with small residual primary tumour the disease was multifocal, precluding conservative surgery. Pathological tumour downstaging occurred in 21/24 T2, 10/10 T3, 2/3 T4 cases. The median number of examined axillary lymph nodes was 14 (range 1-32). Lymph nodes were negative in 15 patients, positive in 22 patients. The median number of positive lymph nodes was 3 (range 1-10). Ten patients had more than 3 lymph nodes involved. A pCR (no evidence of disease both in the breast and in the axilla) occurred in 10.8% (4/37) of patients. Thirty-four patients received postoperative CMF for 3 cycles as planned, 3 declined further chemotherapy.

Thirty-six patients received adjuvant hormonal therapy and 25 underwent radiation therapy. Two events (1 distant metastasis, 1 loco-regional relapse and no deaths) occurred at a median follow up of 37.5 months.

Altogether, only one of 9 patients with a lobular histology and none of 14 patients with a low proliferation index (Ki-67 < 14%) achieved a pCR. The toxicity encountered in the neoadjuvant phase in the 2 patient series is reported in Table 2. Overall, toxicity was acceptable and no toxic deaths occurred. Progression free survival curves are presented in Figure 1. In Series I, median progression free survival was 51 months and 5-year progression free survival was 44% (95% confidence interval: 15%- 71%). Median overall survival has not been reached yet.

In Series II, median progression free survival has not been reached yet and 5-year progression free survival was 93% (95% confidence intervals, 75%- 98%).

Discussion

Several studies have reported high clinical response rates following preoperative chemotherapy, but the pCR rates have remained relatively low. The results of studies large enough to permit a subtype analysis have shown that pCRs tend to concentrate in patients with HER2 overexpressing or triple negative tumours.[12-14] Moreover, lobular carcinoma has been associated with poor response to chemotherapy.[19]

In addition, an early response to and the duration of chemotherapy has been shown to increase the pCR rate. For instance, the combination of docetaxel, Doxorubicin and Cyclophosphamide for 2 cycles followed by either 4 additional cycles of the same regimen or by 4 cycles of vinorelbine and capecitabine yielded a 7.3 and 3.1% respectively pCR rate in patients who did not respond clinically to the first 2 chemotherapy cycles, while in initially responding patients the final pCR rate was 22.6%.[7] In the large NSABP B-27 trial, it was shown that the addition of 4 docetaxel cycles after 4 cycles of Doxorubicin plus Cyclophosphamide alone increased the pCR rate from 13.7% to 26.1%.[20]

In a previous series of 45 evaluable patients treated with 4 cycles of neoadjuvant ET at our Institution, 7 (16%) showed no signs of disease and 2 additional patients presented only carcinoma in situ at histological examination of the breast [15]. However, some of these patients had persistent nodal disease, leaving only 3 patients with pCR. Although numbers of patients were small, pCR occurr more often in ER negative (9%) than in ER positive patients (4%). These unsatisfactory results might have been related to the limited number of preoperative chemotherapy courses (only 4 cycles were administered). We added, therefore, in a subsequent study 4 additional chemotherapy cycles in the preoperative setting and limited inclusion criteria to hormonal receptor positive, HER2 negative cases.

With the aim to gain insight in the niche of ER positive, HER-2 negative cases, we report on the updated outcome of cases treated with 4 chemotherapy cycles and on the outcome of patients included in the subsequent study, treated with 8 cycles of neoadjuvant chemotherapy. The present study adds to the body of evidence showing that preoperative chemotherapy of breast cancer is of limited value in hormone receptor positive, HER2 negative cases. No obvious increase in the pCR rate by increasing the number of chemotherapy cycles was achieved (10.8% *vs*. 7.6%), but numbers are too small for comparison and differences in the distribution of prognostic factors, starting from tumour size, in the two series of patients should be taken into account.

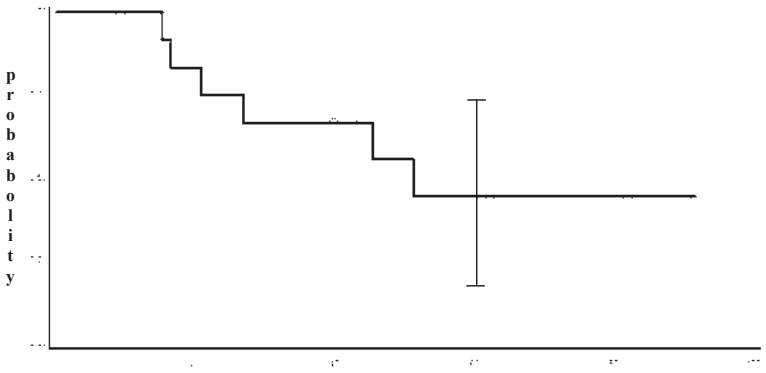

FIGURE 1. Progression free survival of 13 patients of the Series I.

The impact of other parameters possibly influencing pCR rate in these patients, i.e. proliferation index and histological type was in agreement with the reported chemoresistance of tumours with lobular histology and/or low proliferation rate.[12,19] the progression free survival was longer (90% *vs*. 38% at 5 years) in patients treated with 8 preoperative cycles than in those receiving 4 only, but again the nature of the study precludes comparisons.

Based on the analysis of large, randomised studies[14], once a decision is taken, based on several considerations such as patient's age, histology, tumour grade and proliferation, to offer an ER positive, HER2 negative patient preoperative chemotherapy, the latter should be given for a sufficient period of time, probably in the order of 6 months.[3] Our findings reassure us on the benefit of a longer chemotherapy duration with the inherent toxicities. The hormonal therapy has emerged as a viable alternative to chemotherapy in patients with a little chance of chemotherapy response, particularly in postmenopausal women.[21]

The continuing evaluation of biologic features should permit a treatment tailoring aimed to offer cytotoxic chemotherapy only to patients who have a substantial chance of deriving benefit from it.

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research article

Analysis of new N-category on prognosis of oesophageal cancer with positive lymph nodes in a Chinese population

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Background. The 7th edition of the new TNM classification system for oesophageal cancer (EC) has been published. N-category is now divided into N0, N1, N2 and N3. In this study, we aimed to validate the prognostic ability of the new N classification system in EC with positive lymph nodes in a Chinese population, and evaluate whether the new N classification system can help the decision-making for postoperative adjuvant therapy.

Patients and methods. From 2002 to 2008, thoracic EC who underwent oesophagectomy were retrospectively analysed. Patients pathological stage 6th edition of the American Joint Committee on Cancer / Union International Against Cancer (AJCC/UICC) TNM classification were switched to pathological stage 7th edition for this analysis. Patients with pathological stage T1-4N1-3M0 EC were selected. Kaplan-Meier method and Cox regression analysis were employed to compare overall survival (OS).

Results. A total of 545 patients met the inclusion criteria: 346 (63.5%) received oesophagectomy alone, 199 (36.5%) received oesophagectomy and adjuvant radiotherapy, and 36.1% (197/545) received oesophagectomy and adjuvant chemotherapy. Univariate analysis and multivariate analysis revealed significant difference in OS among patients at different postoperative pN-category ($p<0.001$). This was also present in patients receiving postoperative radiotherapy ($p<0.001$) and those undergoing postoperative chemotherapy ($p<0.001$). There was no marked difference in OS between patients receiving postoperative adjuvant therapy and surgery alone at the same postoperative pN-category, except that postoperative radiotherapy marginally improved OS in patients with pN2 and pN3 disease.

Conclusions. Our results validated the prognostic ability of new N classification system. The N-category is an independent prognostic factor in patients with resectable thoracic EC who were positive for lymph nodes in a Chinese population. Further studies are required to clarify the role of new N classification system in the decision-making for postoperative adjuvant therapy.

Key words: oesophageal cancer; prognostic factor; radiotherapy; oesophagectomy; chemotherapy

Introduction

Oesophageal cancer (EC) is the eighth most common cancer worldwide. In China, EC is the fourth most common cause of death and frequently found in the thorax, and 95% of EC is pathologically diagnosed as squamous cell carcinoma.[1] Surgery, like in other thoracic malignancies[2,3], is the a preferred therapeutic strategy for EC patients. But, most of patients still die of recurrence or distant metastases even in the presence of radical resection and extended lymph node dissection. Many factors have been found to affect prognosis of EC, including age, gender, tumour location, local tumour stage, degree of cell differentiation, lymph node metastasis. Among the various prognostic factors of EC, lymph node metastasis is thought to be one of the most important prognostic determinants. The

overall 5-year survival rate after surgical resection is between 70% and 92% for patients without nodal involvement, but only 18-47% for patients with lymph node metastasis.[4,5]

The 6th edition of the American Joint Committee on Cancer / Union International Against Cancer (AJCC/UICC) TNM classification for EC only defines the pathologic nodal status based on the lymph node metastasis. The 7th edition of the AJCC/UICC TNM classification was published in 2010.[6] Different from the 6th edition, N-category of the 7th edition were divided into pN0: no lymph node metastasis; pN1: metastasis in 1~2 lymph nodes; pN2: metastasis in 3~6 lymph nodes; pN3: metastasis in ≥7 lymph nodes. Increasing numbers of reports show that the numbers of positive lymph node was positively related to the prognosis.[7-11]

However, there are several problems to be considered before this new staging system is used to assess the prognosis of patients in Asia population. For example, most EC in Western countries is diagnosed as adenocarcinoma at the lower oesophagus or gastroesophageal junction but EC is squamous cell carcinoma in the majority of patients in Asia. Previous studies have shown that, when compared with adenocarcinoma patients, those with oesophageal squamous cell carcinoma have worse prognosis and a distinct pattern of lymphatic spread, and are susceptible to spread locally rather than systemically.[12,13] In addition, lymph node dissection for EC is less performed in Western countries, whereas extended lymph node dissection is usually carried out in Asia. Thus, it is imperative to evaluate the application of this new classification system in an Asia population.

In addition, the 7th edition TNM classification system for EC was revised based on the retrospective analysis of pathologic data from patients treated with primary surgical resection alone. However, patients with positive lymph nodes receiving surgery alone usually have a poor prognosis, and thus adjuvant chemotherapy and/or radiotherapy have been introduced for the therapeutic strategies. Can new N classification be used to identify patients who may require adjuvant chemotherapy and/or radiotherapy? To our best knowledge, the prognostic impact of the 7th edition TNM classification system has been not evaluated in detail in EC patients undergoing postoperative adjuvant therapy.

The present study aimed to validate the prognostic ability of the new N classification system in patients with EC who were positive for lymph nodes in a Chinese population, and evaluate whether the new N classification system can help the decision-making for postoperative adjuvant therapy in this population.

Patients and methods

Patients

From 2002 to 2008, thoracic EC who underwent oesophagectomy were retrospectively analysed. Patients pathological stage 6th edition of the American Joint Committee on Cancer / Union International Against Cancer (AJCC/UICC) TNM classification were switched to pathological stage 7th edition for this analysis. Patients with pathological stage T1-4N1-3M0 EC were recruited into the present study. Other essential conditions: no distant metastasis, no preoperative chemotherapy and/or radiotherapy, no invasion to cervical oesophagus and cardiac part of the stomach.

In addition, only patients who survived for more than 3 months after surgery were included in the present study. This was done to remove possible bias in favour of the adjuvant treatment group, because some of the patients who received surgery alone may have died in the perioperative period before receiving adjuvant therapy. Thus, patients treated with oesophagectomy with or without adjuvant therapy were enrolled.

Staging

Tumour size and extent was coded primarily according to the operative medical record and pathological findings. Number of lymph node metastasis was determined based on pathological findings. This information was used for the tumour, node, metastasis (TNM) classification according to the American Joint Committee on Cancer (AJCC) classification system (7th edition). The stages of EC in these patients are shown in Table 1.

Treatment

All patients were treated with radical resection. The standard surgical approach consisted of a limited thoracotomy on the right side and intrathoracic gastric tube reconstruction (Ivor Lewis procedure) for lesions at the middle/lower third of the oesophagus. Upper third lesions were treated by cervical anastomosis (Mckeown procedure). Most of patients underwent two-field lymphadenectomy. The number of lymph nodes harvested per case ranged from 6 to 96 (median 28). Pyloroplasty and feeding jejunostomy were not routinely done.

TABLE 1. Patient characteristics based on TNM classification and AJCC stage grouping

	Stage grouping	PORT	POCT	Surgery alone
T1-2N1	II B	13	16	41
T1-2N2	IIIA	16	12	11
T3N1	IIIA	70	68	132
T3N2	IIIB	54	51	91
T4N1-3	IIIC	46	50	71
Total No. of patients		199	197	346

TNM = tumour, node, metastases based classification; AJCC = American Joint Committee on Cancer; PORT = postoperative radiation therapy; POCT = postoperative chemotherapy

A nasogastric tube was placed in each patient until anastomotic wound closed as assessed by oesophagography on post-operative day 14.

In this study, all patients were recommended to receive some adjuvant treatment. As the role of postoperative adjuvant radiotherapy and/or chemotherapy in the treatment of oesophageal squamous cell carcinoma was controversial at the time of treatment for these patients, the postoperative adjuvant therapy was not mandatory. The utilization of postoperative adjuvant radiotherapy and /or chemotherapy was per individual physicians' preference and the general physical conditions. A total of 197 patients received postoperative adjuvant chemotherapy (>2 cycles). Cisplatin and 5-fluorouracil were used most frequently (67%), although several other chemotherapeutics were also used. Postoperative adjuvant radiotherapy, if given, was initiated at 4~5 weeks after surgery. Large T-shaped field encompassing bilateral supraclavicular fossa, mediastinum and tumour bed was used. Radiation was given through anteroposterior field first to 36 Gy at 2 Gy per fraction followed by parallel opposing oblique fields to 14 Gy to avoid the spinal cord. Ten MV photons were used to deliver the radiation to the mediastinum through the anteroposterior and oblique fields. The radiation dose in all cases was prescribed to the isocenter. The bilateral supraclavicular fossas were treated with 9-12 MeV electrons. In some cases, targets were reduced on the basis of patient's condition or physician's judgment.

Statistical analysis

Overall survival (OS) was determined as the time (in months) from the date of surgery to last follow-up or to September 1, 2010 for patients alive or to the date of death. Survival probability was calculated with Kaplan-Meier method and compared with log-rank test. Multivariate analysis was performed with Cox regression model. Variables in the analysis included gender, age, tumour size, pathologic T-stage, pathologic N-stage, tumour differentiation, postoperative radiotherapy and postoperative chemotherapy. Statistical analysis was performed using SPSS Version 13.0 (SPSS Inc., Chicago, IL). A value of two-sided $P<0.05$ was considered statistically significant.

Results

General data

A total of 545 patients were included in the present study: 346 (63.5%) received surgery alone, 199 (36.5%) postoperative radiotherapy and 197 (36.1%) postoperative chemotherapy. The average number of dissected lymph nodes was 31.2±17.4 (mean±SD) nodes per case (median 28, range 6-96). The mean number of metastatic nodes was 4.1±1.9 (median 2, range 1-36). According to the 7th edition of TNM classification system, pN1 EC was found in 270 cases, pN2 EC in 188 and pN3 EC in 87 cases. The median age was 57 years (range: 36~86 years). Median follow-up period for survived patients was 51 months (range 4~93 months). The patients' characteristics are presented in Table 2. Males, and those aged <65 years, or with tumor length >5 cm or having more positive lymph nodes are more likely to receive postoperative adjuvant therapy.

Overall survival

The median survival time was 26.5 months, and the 3-year OS rate was 41.0%. The postoperative radiotherapy was found to be significantly associated with improved OS ($p = 0.006$). The median survival time was 31 months in patients receiving postoperative radiotherapy and 21 months in those un-

TABLE 2. Comparison of patient characteristics by treatment assignment (N=545)

Variable	All patients (%)	PORT			POCT		
		Yes	No	p[a]	Yes	No	p[a]
Gender				0.024			0.012
Female	55(10)	13(7)	42(12)		12(6)	43(12)	
Male	490(90)	186(93)	304(88)		185(94)	305(88)	
Age				0.018			<0.001
<65	421(77)	164(82)	257(74)		175(89)	246(71)	
≥65	124(23)	35(18)	89(26)		22(11)	102(29)	
Tumour length				0.030			0.020
<5cm	274(50)	89(45)	185(53)		87(44)	187(54)	
≥5cm	271(50)	110(55)	161(47)		110(56)	161(46)	
T-stage				0.784			0.764
T1-2	93(17)	33(16)	60(17)		34(17)	59(17)	
T3	407(75)	149(75)	258(75)		149(76)	258(74)	
T4	45(8)	17(9)	28(8)		14(7)	31(9)	
N-stage				0.042			0.015
N1	270(50)	85(13)	185(53)		86(44)	184(53)	
N2	188(34)	79(40)	109(32)		71(36)	117(34)	
N3	87(16)	35(17)	52(15)		40(20)	47(13)	
Tumour differentiation				0.428			0.342
High (G1)	78(14)	33(16)	45(13)		24(12)	54(16)	
Moderate (G2)	347(64)	123(62)	224(65)		124(63)	223(64)	
Low (G3)	120(22)	43(22)	77(22)		49(25)	71(20)	

[a] = X^2 *p*-value; PORT = postoperative radiation therapy; POCT = postoperative chemotherapy.

TABLE 3. Overall survival based on 7th AJCC N-stage grouping

Variable	All patients (%)	PORT				POCT			
		Yes/No	No. (%)	3-yr OS (%)	p	Yes/No	No. (%)	3-yr OS (%)	p
N1	270(50)	Yes	85(43)	53.3	0.507	Yes	86(44)	50.9	0.768
		No	185(53)	50.8		No	184(53)	52.8	
N2	188(34)	Yes	79(40)	45.7	0.080	Yes	71((36)	31.2	0.570
		No	109(32)	31.4		No	117(34)	40.6	
N3	87(16)	Yes	35(17)	16.9	0.064	Yes	40(20)	15.4	0.131
		No	52(15)	8.6		No	47(13)	10.2	

PORT = postoperative radiotherapy; POCT = postoperative chemotherapy; OS = overall survival

dergoing surgery alone. The postoperative radiotherapy dramatically improved OS at 3 years from 38.3 to 45.8% when compared with surgery alone. However, the median survival time was decreased from 28 months to 23 months in patients receiving postoperative chemotherapy as accompanied by a decrease in 3-year OS from 43.1 to 37.1%, but without significant differences ($p = 0.508$).

Overall survival by (7th edition) AJCC N classification grouping

In pathologic lymph nodes positive patients (pN1, pN2 and pN3), significant difference in OS were found among patients at different postoperative pN-category. The median survival time for pN1, pN2 and pN3 patients were 41 months, 23 months

and 13 months, respectively ($p < 0.001$) and the 3-year OS rate was 51.5%, 37.5% and 13.8%, respectively ($p < 0.001$) (Figure 1). Subgroup analysis indicated that there was significant difference in OS among patients at different postoperative pN-category who received postoperative adjuvant radiotherapy. In these patients, the median survival time for pN1, pN2 and pN3 patients were 41 months, 34 months and 16 months, respectively ($p < 0.001$) and the 3-year OS rate was 53.3%, 45.7% and 16.9%, respectively ($p < 0.001$) (Figure 2). Furthermore, marked difference was also noted in OS among patients at different postoperative pN-category who underwent postoperative adjuvant chemotherapy. The median survival time for pN1, pN2 and pN3 patients were 38 months, 19 months and 16 months, respectively ($p < 0.001$) and the 3-year OS rate was 50.9%, 31.2% and 15.4%, respectively ($p < 0.001$) (Figure 3).

There was no marked difference in OS between patients receiving postoperative radiotherapy and surgery alone at the same postoperative pN-category. For patients undergoing surgery alone and those receiving concomitant postoperative radiotherapy, the 3-year OS was 50.8% and 53.3%, respectively, in patients at pN1 ($p = 0.507$); 31.4% and 45.7%, respectively, in those at pN2 ($p = 0.080$); 8.6% and 16.9%, respectively, in those at pN3 ($p = 0.064$), only marginally improved OS for pN2 and pN3 disease. This was also noted between patients receiving postoperative chemotherapy and surgery alone. For patients receiving surgery alone and those undergoing concomitant postoperative chemotherapy, the 3-year OS was 52.8% and 50.9%, respectively, in patients at pN1 ($p = 0.768$); 40.6% and 31.2%, respectively, in those at pN2 ($p = 0.570$); 10.2% and 15.4%, respectively, in those at pN3 ($p = 0.131$) (Table 3).

Univariate and multivariate analyses

On unvariate analysis, the number of positive lymph nodes (pN2/pN1: hazard ratio [HR] 1.56, 95% confidence interval [CI] 1.22-1.99, $p < 0.001$; pN3/pN1: HR 3.19, 95% CI 2.40-4.23, $p < 0.001$) was associated with survival. Postoperative radiotherapy was associated with improved survival (HR 0.79, 95% CI 0.63-0.99, $p = 0.045$). Male gender and high T stage predicted a decrease of OS (Table 4).

On multivariate analysis, the number of positive lymph nodes was also associated with survival (pN1/pN3: HR 1.55, 95% CI 1.20-2.00, $p = 0.001$; pN2/pN3: HR 2.27, 95% CI 1.45-3.54, $p < 0.001$). Postoperative radiotherapy was associated with

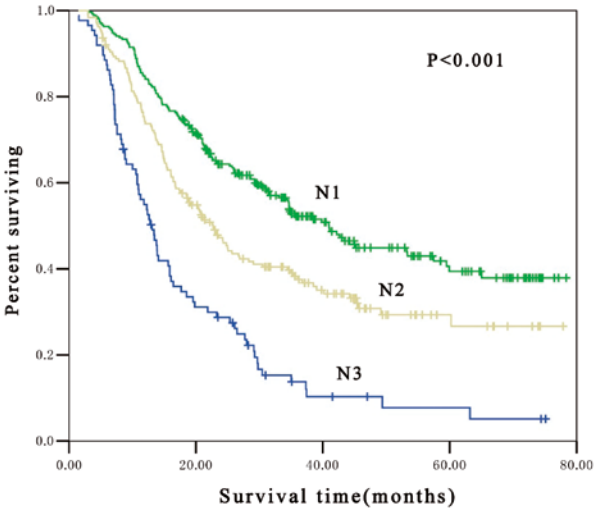

FIGURE 1. Kaplan-Meier estimates for overall survival of 545 patients stratified by N classification. The median survival time was 41 months, 23 months and 13 months for pN1, pN2 and pN3, respectively ($p < 0.001$).

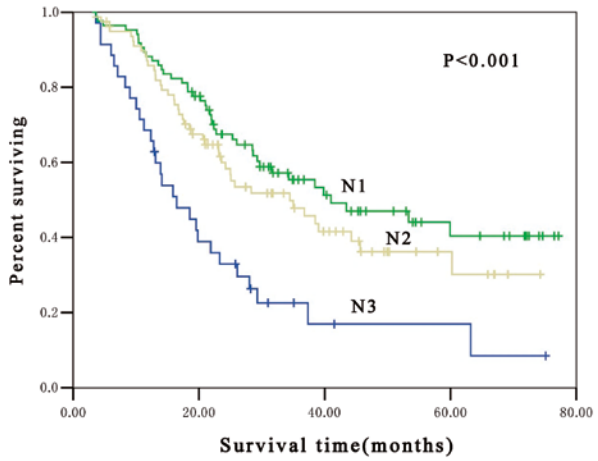

FIGURE 2. Kaplan-Meier estimates for overall survival of 199 postoperative adjuvant radiotherapy patients stratified by N classification. The median survival time was 41 months, 34 months and 16 months for pN1, pN2 and pN3, respectively ($p < 0.001$).

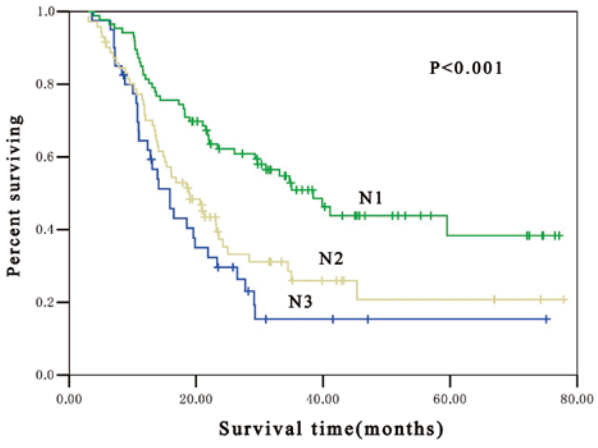

FIGURE 3. Kaplan-Meier estimates for overall survival of 197 postoperative adjuvant chemotherapy patients stratified by N classification. The median survival time was 38 months, 19 months and 16 months for pN1, pN2 and pN3, respectively ($p < 0.001$).

TABLE 4. Univariate analysis for survival

Variable	CHR	95% CI	p
Gender			
Female	1		
Male	2.04	1.31-3.17	0.002
Age			
<65	1		
≥65	1.16	0.90-1.48	0.254
Tumor length			
<5cm	1		
>5cm	1.06	0.85-1.31	0.600
T-stage			
T1-2	1		
T3	1.37	1.00-1.86	0.049
T4	2.60	1.69-4.01	<0.001
N-stage			
N1	1		
N2	1.56	1.22-1.99	<0.001
N3	3.19	2.40-4.23	<0.001
Tumor differentiation			
High (G1)	1		
Moderate (G2)	0.87	0.63-1.19	0.369
Low (G3)	1.19	0.83-1.70	0.339
PORT			
Yes	0.79	0.63-0.99	0.045
No	1		
POCT			
Yes	1.08	0.86-1.35	0.509
No	1		

CHR = Cox hazard ratio; 95% CI = 95% Confidence Interval; PORT = postoperative radiation therapy; POCT = postoperative chemotherapy

TABLE 5. Mutivariate analysis for survival

Variable	CHR	95% CI	p
Gender			
Female	0.56	0.36-0.88	0.012
Male	1		
Age			
<65	1		
≥65	1.04	0.80-1.34	0.783
Tumor length			
<5cm	1		
≥5cm	0.97	0.78-1.21	0.785
T-stage			
T1-2	1		
T3	1.35	0.99-1.85	0.059
T4	2.15	1.37-3.36	0.001
N-stage			
N1	1		
N2	1.55	1.20-2.00	0.001
N3	2.27	1.45-3.54	<0.001
Tumor differentiation			
High (G1)	1		
Moderate (G2)	0.85	0.62-1.17	0.319
Low (G3)	1.00	0.70-1.44	0.993
PORT			
Yes	0.71	0.55-0.87	0.001
No	1		
POCT			
Yes	1.20	0.92-1.55	0.163
No	1		

CHR = Cox hazard ratio; 95% CI = 95% Confidence interval; PORT = postoperative radiation therapy; POCT = postoperative chemotherapy

improved survival (HR 0.71, 95% CI 0.55-0.87, $p = 0.001$). Male gender and high T stage predicted a decrease of OS (Table 5).

Discussion

The oesophageal cancer TNM classification system was recently revised in the 7th edition of AJCC/UICC) staging system. The major differences between the 6th and 7th editions include: (1) T is re-defined and T4 subclassified as T4A and T4B; (2) the regional lymph nodes are re-defined. N is subclassified according to the number of positive regional lymph nodes; (3) M is re-defined. In addition, prognostic staging, including histological grade and cancer location, are defined for T1-3N0M0 patients. However, the reliability and accuracy of the nodal portion of the TNM staging system still remains the much debate. Several studies have evaluated the influence of the number of lymph node metastasis on the prognosis of patients with esophageal cancer and have found significant differences in prognosis between the patients with different numbers.[7-10] However, several authors have criticized the new N classification, claiming that it omits some potentially critical prognostic information relating to lymph node status.[14] The present study showed that, according to the 7th edition TNM classification system, N classifica-

tion independently affected the prognosis of thoracic EC patients with positive lymph nodes after radical surgery in Chinese population. This was also present in patients receiving postoperative radiotherapy ($p < 0.001$) and those undergoing postoperative chemotherapy ($p < 0.001$). Lymph node involvement is a significant prognostic indicator of overall survival, and as the number of involved nodes increases, it may suggest a greater tumour burden or more aggressive tumour biology, and so the likelihood of locoregional or systemic recurrence could be assumed to be higher.

Cancer staging systems aim to predict survival and then provide information for the selection of therapeutic strategies. The high incidence of regional recurrence of EC suggests that surgery alone is not effective enough in such cases and adjuvant therapy is indicative. However, up to now, whether postoperative radiotherapy and chemotherapy affect the therapeutic outcomes remains controversial.[15-20] Therefore, in those for whom the primary treatment is surgery, there is no clear indication for adjuvant therapy. It is necessary to generate criteria to help the decision-making for postoperative adjuvant therapy in this population. In the 7th edition, the postoperative pN-categories are re-defined based on the number of positive regional lymph nodes. Many studies have addressed the impact of postoperative radiotherapy on the prognosis of oesophageal squamous cell carcinoma patients based on the number of positive lymph nodes. Xiao *et al*.[17] randomized 495 patients with oesophageal squamous cell carcinoma to radical resection alone vs. postoperative radiotherapy (a total of 50~60 Gy). There was no survival benefit with addition of postoperative radiotherapy (a 5-year OS of 31.7% for surgery alone *vs.* 41.3% for postoperative radiotherapy; $p = 0.4474$). However, when patients were stratified based on the number of positive lymph nodes, obvious survival benefit was noted in patients receiving postoperative radiotherapy with an improvement in 5-year OS from 17.6 to 34.1%, ($p = 0.0378$). In patients with 1~2 positive lymph nodes, the 5-year survival rate was 23.5% in the surgery alone group and 45.1% in the postoperative radiotherapy group ($p = 0.1286$). In patients with ≥3 positive lymph nodes, the 5-year survival rate was 0% and 20.6% in the surgery alone group and postoperative radiotherapy group, respectively ($p = 0.0265$). Chen *et al*.[21] retrospectively evaluated patients with thoracic oesophageal squamous cell carcinoma, their results showed the postoperative radiotherapy was significantly associated with improvement in survival, which was only observed in patients with ≥3 positive lymph nodes. Similar to the findings above mentioned, our study revealed that postoperative radiotherapy marginally improved OS of patients with thoracic EC at pN2 and pN3 disease. Adjuvant radiotherapy can theoretically treat microscopic disease left behind after an incomplete surgery and increase local control. Therefore, we postulate that the new N classification system may potentially help the decision-making for postoperative adjuvant radiotherapy. Given the insufficient evidence, adequately powered prospective randomized trials are required to confirm these findings. Thus, caution should be taken before applying these findings in clinical practice.

Ando *et al*.[20] conducted a randomized multicenter trial to determine whether postoperative adjuvant chemotherapy improves the outcome of patients with esophageal squamous cell carcinoma who underwent radical surgery. Their results showed that postoperative adjuvant chemotherapy with cisplatine and fluorouracil significantly prevented relapse of EC when compared with surgery alone. The theoretical advantages of adding adjuvant chemotherapy to the treatment of esophageal cancer are for potential targeting micrometastatic disease, thus decreasing the risk of distant spread. Nevertheless, our study showed that the therapeutic efficacy of adjuvant chemotherapy was unsatisfactory, even for patients with pN3 patients. This might be attributed to the fact that our study was retrospective and the chemotherapy regimens were not quite the same among these patients. Therefore, the therapeutic efficacy of postoperative adjuvant chemotherapy in these patients is still no clear indication regardless of EC at pN1, pN2 or pN3 based on our data. More studies are required to confirm the role of adjuvant chemotherapy.

The weakness of this study can be summarized as follows: First, only patients with squamous cell carcinoma were recruited in this study. In contrast, the incidence of adenocarcinoma is dramatically increasing in Western countries. Therefore, the results of this study may be not generalized to be applied in North American and European patients. In addition, on univariate and multivariate analysis, male gender and increased number of positive lymph nodes were significantly associated with worse outcome (Tables 4, 5). However, males and those having more positive lymph nodes were more likely to receive postoperative radiotherapy and chemotherapy (Table 2), likely providing a further bias against improved survival in receiving postoperative adjuvant therapy patients. Moreover, in a phase Ⅱ non-randomized trial which evalu-

ated postoperative concurrent chemoradiation with cisplatin and 5-fluorouracil in patients with poor prognosis oesophageal and gastroesophageal junction (EGJ) cancers, the projected rates of 4-year overall survival, freedom from recurrence, distant metastatic control and locoregional control were 51%, 50%, 56% and 86% respectively for patients with lymph node positive (T3 or T4) tumours, which are better than the historical outcomes with surgery alone.[22] However, the efficacy of postoperative chemoradiation has not been compared to surgery alone in a randomized trial in patients with EC. Therefore, evaluation of postoperative chemoradiotherapy for these patients may be important. Further development of postoperative adjuvant therapy in EC is warranted.

In summary, the results of our study demonstrate that the new N-category in the 7th edition of AJCC/UICC TNM classification system are an independent prognostic factor in lymph node positive patients with thoracic EC in a Chinese population. However, the evidence is not powerful enough to support that the new N classification system can help the decision-making for postoperative adjuvant therapeutic strategy in these patients.

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case report

Radiotherapy for inverted papilloma: a case report and review of the literature

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Background. Sinonasal inverted papilloma (IP) is a rare, usually benign tumor arising from the respiratory mucosa of the sinonasal tract. Surgical resection is the treatment of choice. In histologically overt benign IPs (*i.e.* without associated malignancy) irradiation was employed only anecdotally. The patient with gross residual of benign IP after up-front surgery that was subsequently treated with irradiation is presented and the literature reports on the use of radiotherapy (RT) in this tumor type are reviewed.

Case report. After the surgical treatment the residuum in the region of the sphenoid and adjacent cavernous sinus was irradiated to 54 Gy in 1.8 Gy daily fractions. No recurrence or deterioration of olfaction, hearing or vision was observed 2.6 years post-RT.

Review of the literature. In the literature, six reports were identified with 16 patients describing necessary details on RT and outcome. Twelve of 14 cases (our case included) with gross or subtotal tumor resection and postoperative RT were locally controlled. The lowest and the median irradiation doses were 47.15 Gy and 56.5 Gy, respectively, and the follow-up period ranged between 0.5-20.5 years (median 7.8 years).

Conclusions. RT is safe and valuable treatment option in histologically overt benign IPs. It is indicated when the risk of tumor recurrence after surgery is increased and in inoperable tumors.

Key words: inverted papilloma; local control; radiotherapy; surgery

Introduction

Sinonasal inverted papilloma (IP) is by far the most frequent histologic type of Schneiderian papillomas, a group comprising also of fungiform and oncocytic variants, which altogether represent 0.4-4.7% of all sinonasal tumors.[1] From an etiological perspective, the formation of IPs have been linked to human papillomavirus (HPV) infection, although there are other opinions suggesting that HPV more likely represents incidental colonization than being an important etiological factor.[2,3] Associated malignancy, most often of squamous cell histology, may arise from IPs or may appear concomitantly with IPs in 11% of cases.[1]

A typical patient with IP is male in his fifth to seventh decade of life presenting with a unilateral nasal obstruction. Clinical examination usually reveals an unilateral polypoid tumor that originates from the lateral nasal wall in the region of the middle turbinate or ethmoid recesses although extensive lesions destroying neighboring structures could also be seen at the initial presentation.[1] Surgical resection is the treatment of choice for benign IPs. Endoscopic treatment is preferred if the attachment site, preoperatively identified by the CT finding of focal hyperostosis, could be confirmed intraoperatively and adequately reached by the instrument.[4,5]

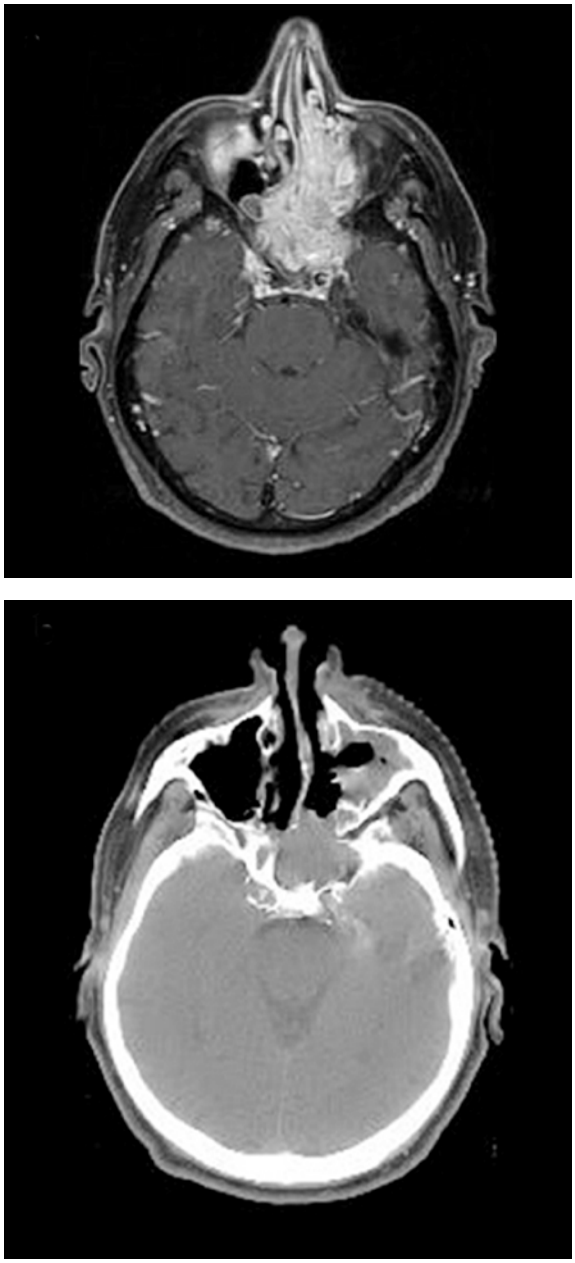

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FIGURE 1. A. Before therapy (post-contrast MRI T1 SE WI with fat suppression). B. After subtotal resection of tumor (native CT scan).

For tumors with associated malignancy such as IP with squamous cell carcinoma (SCC) there is a generally accepted view that RT adjuvant to surgical resection contributes to better local control.[6] In benign IPs, however, irradiation has been employed only anecdotally. In this report, we present our experience with RT for gross residual benign IP after up-front surgery and a summarization of literature reports on the use of RT in this tumor type.

Case report

A 69-year old male, heavy smoker and abstainer, was presented in January 2009 with a 5-year history of progressive nasal obstruction, anosmia lasting one year and diplopia lasting two weeks. Thirty years prior to referral he suffered from an acute myocardial infarction with a cardiac by-pass inserted 5 years ago; since that time he has been under the regular surveillance of a cardiologist.

Upon clinical examination, extensive polyps occluding the middle nasal meatus bilaterally were seen. In addition, double vision was recorded when the patient looked to the left and inferiorly along with a left eye small visual field defect temporally. Rinne's test was positive on the right and negative on the left ear with lateralization to the left upon Weber testing. The remainder of the physical examination and the results of laboratory tests were unremarkable.

Contrast enhanced magnetic resonance imaging (MRI) revealed a 7.5x4.5x5.5 cm tumor mass involving the sphenoid sinuses, nasal cavity and ethmoidal cells bilaterally. Cranially the tumor extended to the optic chiasm pushing apart optic nerves; it infiltrated the cavernous sinus on the left and the clivus posteriorly but no dural or brain invasion were documented. The tumor was also present in the posterior part of the left maxillary sinus and the nasopharynx (Figure 1A). A biopsy was taken and a histological examination was consistent with the diagnosis of IP with no dysplastic or malignant changes present (Figure 2). In situ hybridization for HPV 16/18 and 6/11 was negative.

The patient underwent Caldwell-Luc surgery on the left side with tumor being removed from both nasal cavities, the nasopharynx and left maxillary, ethmoid and part of the sphenoid sinuses. Due to the vicinity of the pituitary gland and infiltration of the left cavernous sinus, no attempt was made to resect intracranial part of the tumor. A residuum of 3.5 x 3 cm was left behind in the region of the sphenoid and adjacent cavernous sinus with minimal extension into the posterior part of the nasal cavity as was documented on a postoperative computer tomography (CT) scan (Figure 1B). At the same time, revision of the left frontal sinus did not reveal a visible tumor which was confirmed with biopsy specimens being negative for IP. Postoperatively, the patient was irradiated using CT-based 3-dimensional computer planning and five 6 MV linear accelerator photon beams. The clinical target volume encompassed the residual tumor and a margin of 3 mm was added to create a planning target

volume. A tumor dose of 54 Gy was delivered in 30 daily fractions of 1.8 Gy over 45 days.

Three months after finishing irradiation, a control MRI did not demonstrate any significant change in tumor size when compared to postoperative CT. Three months later, however, a substantial reduction in tumor mass was observed on an MRI with a tumor residue of 3 x 2 cm encompassing the sphenoid and left cavernous sinus (Figure 3A) that remained unchanged on a control MRI 9 months post-therapy. Additional shrinkage of the tumor was documented at radiologic controls one and two years later with residual changes in the area of the left sphenoid sinus appearing more homogenous when compared to previous scans (Figure 3B). No deterioration of olfaction, hearing or vision was observed in the patient at the last follow up examination in January 2012.

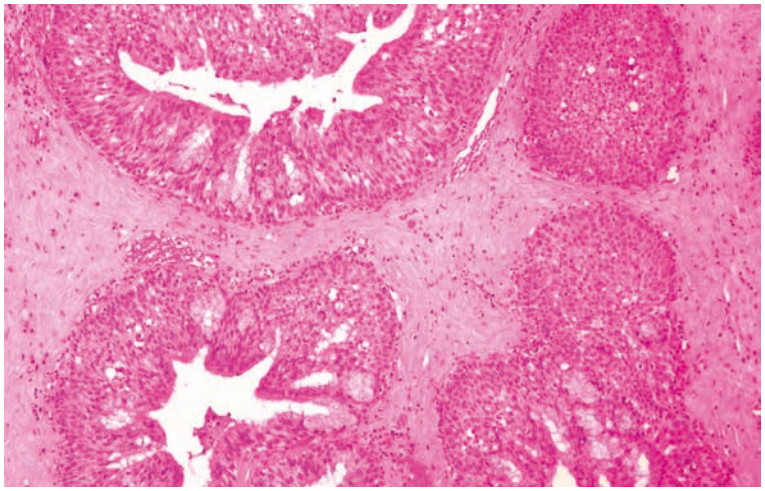


FIGURE 2. Inverted papilloma: invagination of nonkeratinizing squamous and pseudostratified columnar ciliated epithelial cells into the subepithelial stroma (H&E, orig. magnification x 100).

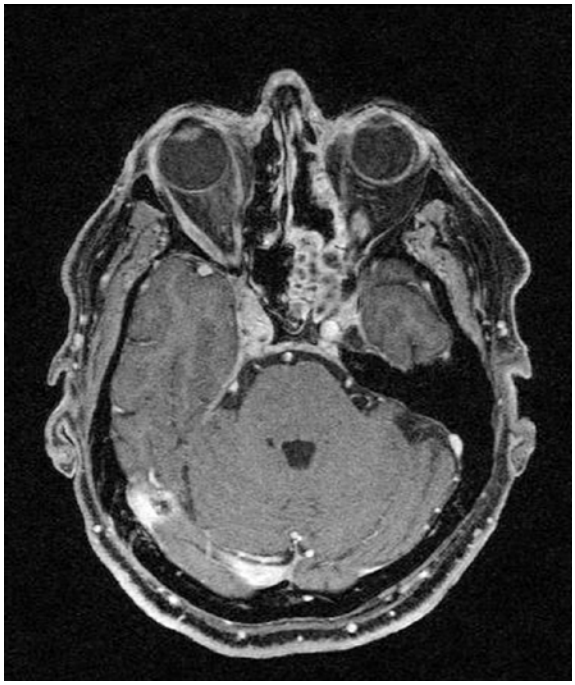
TABLE 1. Radiotherapy for sinonasal inverted papilloma: review of the literature

Author, (year)[Ref]	Sex/Age (years)	Prior surgery	Tumor extent	Surgery	Radiotherapy	Post-radiotherapy status
Fechner & Sessions (1977)[7]	F/22	No	Rt medial canthal area & Rt neck mass	Primary tumor – no & excision of neck mass	50 Gy	PD locally at 1.8 years →NED, 3 years (after SURG)
Hug *et al.* (1993)[8]	7 patients, M (all 7)/ 30-70	Yes, ≤8 (in 5 patients) No (in 2 patients)	ES 72%, MS 68%, SS 36%, FS 20%, nasopharynx 16%; Neighboring structures[a] 52%	Gross total resection, 3 patients	51.2 Gy, bid 54.6 Gy, qd 56.6 Gy, qd	NED, 6.3 years NED, 7.2 years NED, 8.4 years
				Subtotal resection, 3 patients	56.4 Gy, bid 57.4 Gy, bid 64.8 Gy, qd	NED, 0.5 years PD→NED, 5.2 years (after repeated SURG) NED, 12.9 years
				No, 1 patient	68.4 Gy, qd	NED, 3.5 years
Miller *et al.* (1996)[9]	F/42	Yes, 1	Lt-FS, IC, dura	Gross total resection	70 Gy	NED, 3 years
Gomez *et al.* (2000)[10]	M/40	Yes, 3	Lt-NC, Lt-ES, Lt-SS, cribriform plate, Lt&Rt-FS, IC	Subtotal resection, gross residual tumor	47.15 Gy, 32 fx, qd (sc, over 78 day)	DOC (lung carcinoma), 20.5 years
	M/56	Yes, 2	Lt-MS, ES, cribriform plate	Gross total resectio equivocal margins	67 Gy, 60 fx, bid	NED, 9 years
	M/47	No	Lt-NC, Lt-MS, ES	Gross total resection microscopic residual	61.3 Gy, 32 fx, qd (sc, over 58 days)	DOC (lung carcinoma), 9 years
	F/32	Yes, 1	Lt-ES, Lt-MS, cribriform plate, medial orbital wall	Gross total resection	60 Gy, 33 fx, qd	NED, 8.5 years
	F/84	Yes, 1	Lt-NC, Lt-MS, Lt-ES, Lt-orbit, Lt-FS	No	65 Gy, 36 fx, qd	DOD, 1.4 years
Acevedo-Henao *et al.* (2010)[11]	M/63	Yes, 2	Rt-MS, Rt-middle ear, temporal fossa, n.VII	Gross total resection	50 Gy, 25 fx, qd	DOD[b], 2.2 years
Kainuma *et al.* (2011)[12]	M/63	Yes, 4	Lt-middle ear	Gross total resection	54 Gy	NED, 0.8 years
Present report	M/69	No	NC, EC, SS, Lt-MS, Lt-cavernous sinus	Subtotal resection, gross residual tumor	54 Gy, 30 fx, qd	NED, 2.6 years

M = Males; F = Females; ES = Ethmoid sinus; MS = Maxillary sinus; SS = Sphenoid sinus; FS = Frontal sinus, IC = Intracranial; NC = Nasal cavity; n.VII = Facial nerve; Lt = Left; Rt = Right; bid = twice-a-day irradiation; qd, = once-a-day irradiation; fx = fraction; sc = split-course

[a] Neighbouring structures: orbit, cribriform plate, infratemporal fossa, clivus, pterygomaxillary space, palate, or cheek.

[b] At the time of RT, histologic diagnosis was benign IP; subsequently, associated squamous cell carcinoma was found during the course of the disease



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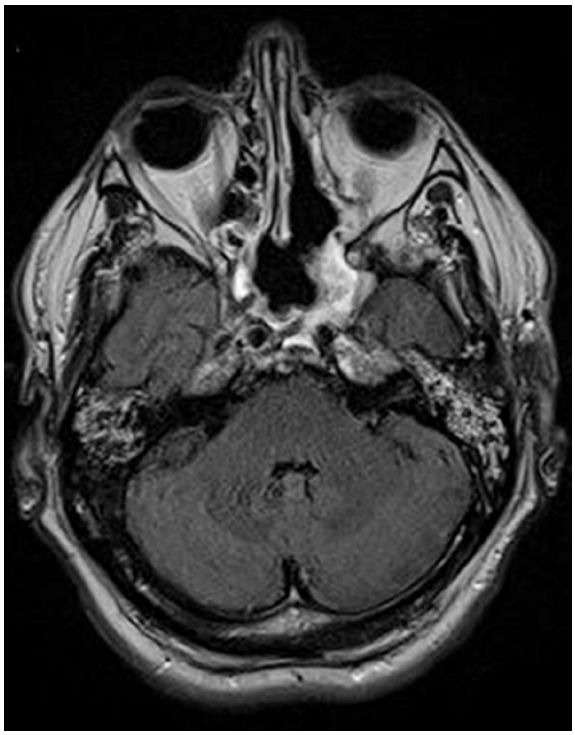

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FIGURE 3. A. Six months after radiotherapy (post-contrast MRI T1 GE WI with fat suppression). B. Three years after diagnosis (post-contrast MRI T1 SE WI with fat suppression).

Review of the literature

A systematic review of the English literature was accomplished by using the PubMed database and the search terms *inverted papilloma, radiotherapy* and *irradiation.* In the publications displayed, content was reviewed for possible inclusion and references were checked for additional relevant reports. The criteria for inclusion of the article in the present review were availability of data on RT procedures, survival times and status at the last follow-up of reported IP patients.

Altogether, six reports were identified with 16 patients that met the inclusion criteria (Table 1).[7-12] According to the volume of tumor that was irradiated, patients (including our case) were grouped as follows: (*i*) no residual tumor visible after gross tumor resection – 9 cases; (*ii*) macroscopic residual tumor after subtotal resection – 5 cases; and (*iii*) macroscopic tumor with no surgery performed – 3 cases. RT doses in the first two groups ranged from 50 Gy to70 Gy (median 56.6 Gy) and from 47.15 to 68 Gy (median 56.4 Gy), respectively, whereas in three patients who only had a biopsy prior to irradiation, the doses were 50 Gy, 65 Gy and 68 Gy. Local recurrence occurred in four cases, one from each group with surgery, (after 50 Gy, 57.4 Gy) and in two cases that were only irradiated (after 50 Gy and 65 Gy). Salvage surgery was successful in two patients[7,8] and associated malignancy was documented subsequently during the course of the disease in another patient.[11]

Discussion

Radiotherapy has only been used on exception in the treatment of histologically overt benign IPs. One reason is historical: in 1965, Mabery *et al*. identified in the literature four out of 14 patients with a malignant transformation in papillomatosis who had a history of RT.[13] Sporadic cases of assumed RT-induced malignancy in previously benign IPs were also reported by others.[14-16] Due to the risk of anaplastic transformation after treatment with irradiation, many authors in the past advocated avoiding the use of RT in this type of tumor. However, this argument should be considered non-relevant in the context of more recent data as no such relationship could be confirmed in the majority of IP/SCC cases.[8,10,17]

The second argument, also originated in the past when imaging and RT were much less sophisticated and effective than nowadays, would be the opinion that RT is ineffective in preventing recurrences.[4,18,19] Favorable results, however, were also described with RT by others.[8-10,12,17,20] Lastly, high local control rates achieved by surgery eliminate

the need for adjuvant therapy.[5,21] Recently, modern radiologic techniques (*e.g.* CT, MRI) allow even better visualization of the extent of tumors and more effective planning of surgical procedures.

In reviewing the literature for reports on irradiation of benign IPs, we found only six studies with, in total, 16 patients describing necessary details on therapy and outcome (Table 1).[7-12] Indications for irradiation were inoperability, due to the extent of a tumor or medical comorbidities, incomplete resection or history of multiple recurrences of otherwise benign tumor. When only those patients, after gross or subtotal tumor resection from Table 1 are considered, RT failed in 2 out of 14 cases (our case included). The two were irradiated to 57.4 Gy and 50 Gy.[8,11] The first patient was treated in 1970s, when imaging and RT technologically inferior to the present standards were employed.[8] In the latter case, however, a malignant component was identified 12 months later when the patient was re-operated on due to a subsequent recurrence. This may provoke speculation on preexisting but originally overlooked malignant features in the tumor that require doses well above 50 Gy.[6] In patients who were locally controlled, the lowest and the median dose were 47.15 Gy (gross residual, split-course RT) and 56.5 Gy, respectively, and the follow-up period ranged between 0.5-20.5 years (median 7.8 years). It seems that in overt benign IPs RT with doses ranging from 50 Gy to well below 60 Gy effectively prevent tumor re-appearance when there is no residual or only a small residual left behind after surgery. If tumor burden is extensive, *e.g.* when no surgical debulking is carried out, irradiation doses in the range of 70 Gy appear to be indicated.

In the presented patient, infiltration of the cavernous sinus prevented gross resection of the tumor and subsequent RT resulted in persistent local control of 2.6 years post-therapy. Importantly, the RT dose that was used (54 Gy in 30 fractions of 1.8 Gy per day) is still in the range of doses closer to a "near zero" incidence of RT-induced optic neuropathy,[22] which makes RT acceptable also from a morbidity point of view. Hyperfractionation of RT dose and modern RT techniques with increased dose conformity (*e.g.* intensity-modulated RT with image guidance) can further reduce the risk of optic neuropathy.[23,24] Because only two cases with neck metastases of benign-appearing IPs have been reported so far[7,25], no elective irradiation of regional lymphatics is needed.

In our patient, a reduction in tumor mass was seen on an MRI scan no earlier than six months post-RT, and this trend continued at radiologic controls one and two years after treatment. This observation points to the need for a sufficient post-RT interval before it is found to be ineffective.[10] To date, no late recurrences have been described among IP patients after irradiation although this is obviously not the case in the IP/SCC group.[6,10,17]

To conclude, in histologically overt benign IPs, RT is safe and is indicated when the risk of tumor recurrence after surgery is increased, either due to subtotal resection or a history of recurrent disease, and in inoperable tumors. Moderate RT doses well below 60 Gy can effectively prevent recurrence after gross or subtotal resection whereas for inoperable tumors doses in the range of 70 Gy are indicated. Tumor response after irradiation should be assessed not earlier than 3-6 months after treatment.

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case report

Thoracobiliary fistulas: literature review and a case report of fistula closure with omentum majus

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Background. Thoracobiliary fistulas are pathological communications between the biliary tract and the bronchial tree (bronchobiliary fistulas) or the biliary tract and the pleural space (pleurobiliary fistulas).

Review of the literature. We have reviewed aetiology, pathogenesis, predilection formation points, the clinical picture, diagnostic possibilities, and therapeutic options for thoracobiliary fistulas.

Case report. A patient with an iatrogenic bronchobiliary fistula which developed after radiofrequency ablation of a colorectal carcinoma metastasis of the liver is present. We also describe the closure of the bronchobiliary fistula with the greater omentum as a possible manner of fistula closure, which was not reported previously according to the knowledge of the authors.

Conclusions. Newer papers report of successful non-surgical therapy, although the bulk of the literature advocates surgical therapy. Fistula closure with the greater omentum is a possible method of the thoracobiliary fistula treatment.

Key words: thoracobiliary fistula; bronchobiliary fistula; treatment; omentum majus

Introduction

In the case of bronchobiliary (BBF) and pleurobiliary fistulas (PBF), there is an existing pathological communication between the biliary tract and bronchial tree in the first case and pleural space in the second.

The literature indicates several possible *causes* for the conditions, but all potential causes can be summarized in five groups:[1-5]

1. Congenital bronchobiliary or pleurobiliary fistulas;

fistulas that are the result of

2. hepatic hydatid disease or liver abscess (echinococcic, amoebic, pyogenic),
3. biliary tract obstruction (iatrogenic cause or trauma excluded),
4. injury (blunt or penetrant) and
5. iatrogenic fistulas (liver resection, radiofrequency ablation - RFA, bile duct stricture, irradiation, thoracic drainage, etc…).

Pathogenesis may be, except in the case of the congenital form of the disease, explained by two mechanisms.[2,4,6] In the first case biliary tract obstruction is the primary reason for fistula formation. Causes may be scars (trauma, surgery, after radiation, etc…), inflammatory diseases, foreign bodies, primary tumours, metastases or granulomas of different aetiologies, which obstruct the bile ducts. The result is the retention of bile proximal to the barrier, the formation of a liver biloma and subsequently the abscess formation. By increasing, the abscess gradually erodes the diaphragm. In case of adhesions between the lower lung lobe and the diaphragm (due to previous pleural or lung pathology), the abscess erodes directly into the lung

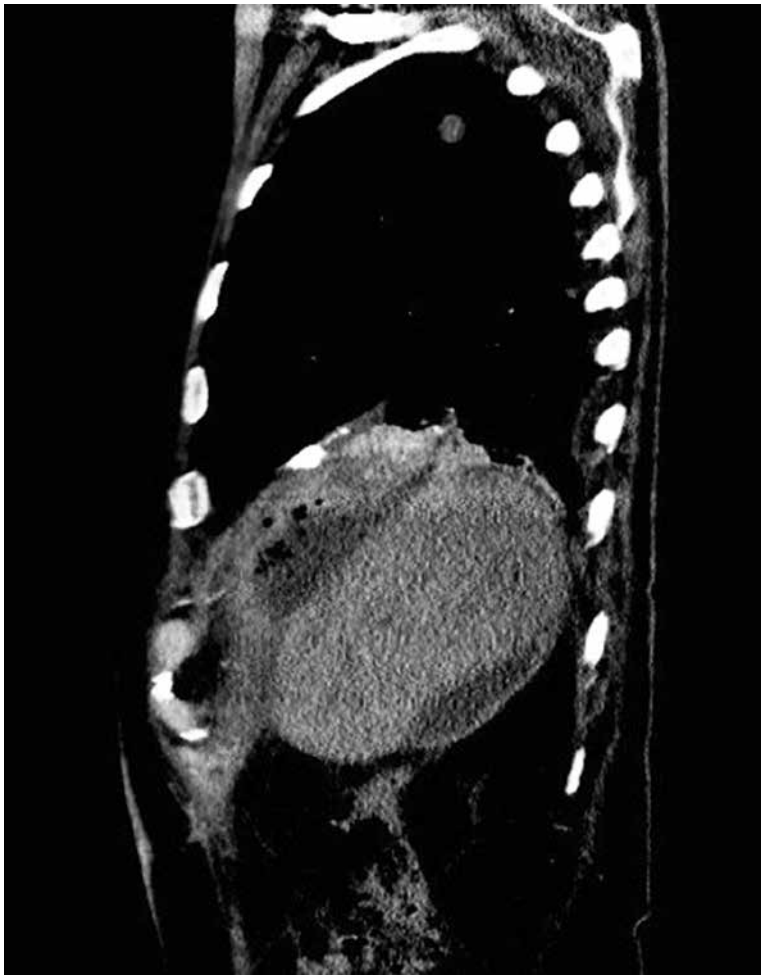

FIGURE 1. Bronchobiliary fistula (BBF) demonstrated by contrast enhanced CT.

parenchyma until it reaches the nearest bronchus and a BBF is formed. When no previous pleural pathology is present, the abscess gradually erodes into the pleural space and a PBF with pleural empyema is formed.

In the second case, the formation of a thoracobiliary fistula (TBF) takes place without biliary tract obstruction. In this case a hydatid *cyst* or a *liver abscess* is the primary reason for the fistula formation. The abscess can be echinococcic (most often), amoebic, or pyogenic in origin. As described above, the cyst or the abscess gradually enlarge and erode the diaphragm. Depending on the previous state of the pleural space, a BBF or a PBF are formed. The *predilection point* for the fistula formation is the posteromedial part of the right hemidiaphragm- *i.e.* the part which is in a direct contact with the area nuda hepatis.[7]

Because BBF and PBF are rare phenomena, larger studies on their frequency and the most common causes do not exist. In the literature, liver abscess of echinococcic origin or hydatid disease of the liver are stated as the most frequent causes in the developing countries and also globally.[2,3,5,6,8,9] Information about the most common cause in the developed world is contradictory (Table 1).[1,2,4,5,7,10-25]

The clinical picture of patients with BBF and PBF may be present either in the acute or chronic form.[2,7] Symptoms result from the underlying disease of the liver, biliary tract, and lung pathology. Bilioptysis, whenever encountered, is pathognomonic for bronchobiliary fistula.[2,3,7,8,10,21] In the case of the acute form[2,7], the patient is in distress, with elevated body temperature, and complains of pain in the lower right part of the chest. In the case of BBF, bilioptysis is present and during physical examination, inspiratory crackles over the lower-right parts of the lung are noticed. The fulminant disease presents in the form of acute respiratory distress syndrome (ARDS). When the patient has a PBF, the cough is dry and irritating, with the absence of respiratory phenomena above the right bottom of the lungs. A chronic TBF[2,7] presents with dry, irritating cough, occasional yellowish sputum, intermittent fever with malaise, and weight loss. The clinical picture mimics recurrent pneumonia.

Beside the pulmonary symptoms there are also symptoms due to the underlying disease of the liver and biliary system.[2,7] There might be upper right quadrant abdominal pain that irradiates to the right shoulder. Jaundice in the case of bile duct obstruction. Sometimes a biliocutaneus fistula can also be encountered.

When examining the laboratory results elevated white cell blood count and C-reactive protein (CRP) is noticed. Anaemia and hypoalbuminaemia are signs of chronic TBF. In the case of bile duct obstruction, elevated levels of direct and total bilirubin are present.[2] The *microbiological analysis* of sputum reveals the presence of the following microbes: E. coli, Klebsiella spp., Pseudomonas aerug., Enterococcus spp. and Enterobacter cloacae.[3,8,17]

On chest x-ray a raised right hemidiaphragm, shadowing in the area of the lower and middle right lobus, atelectasis and pleural effusion might be seen.[6,9] Occasionally, gas-fluid levels are present on abdominal x-ray images.[4,8]

In cases when a clinical suspicion for BBF or PBF exists, the first dilemma is which diagnostic method should be used to either confirm or refute our suspicion. The possible diagnostic methods listed in the literature are bronchoscopy[2,21], bronchogram[2,4], CT[3,21] (Figure 1) and MRI[21] that are routinely used

TABLE 1. Case and retrospective studies of thoracobiliary fistulas (TBF)

Authors	Year	No. of patients	Aetiology	Fistula type	Initial therapy	Second line therapy	Outcome	Special recommendations
Ferguson and Burford[1]	1967	7	Trauma (blunt) 4x Abscess (pyogenic) 2x Biliary obstruction 1x	PBF BBF BBF	**Surgical**		No recurrence of TBF	The paper summarizes the basic steps for a successful surgical treatment of TBF
Saylam *et al.*[10]	1974	6	Echinococcosis 2x Abscess (pyogenic) 2x Not known 2x	TBF	**Surgical**: Thoracic approach, 2 patient refused surgery		1 patient died of septic shock	Advocates thoracotomy. Biliary obstruction if present, should be resolved first
Boyd[7]	1977	16	Iatrogenic bile duct stricture	PBF and BBF	**Surgical**: Stricture correction, only 1 lobectomy		Not clearly stated	Stricture correction and subdiaphragmatic drainage are needed for TBF healing
Tierris *et al.*[11]	1997	3	Echinococcosis	BBF	**Surgical**	**Repeated surgery** in 1 patient	1 patient died due to massive PE	A case of left liver and left lung BBF in described.
Oparah and Mandal[12]	1978	4	Trauma (penetrant)	PBF	**Surgical**: Thoracotomy, +/- laparotomy. In 2 instances only a chest tube was inserted.		No recurrence. Patients with only tube thoracostomy had a prolonged hospital stay	Tube thoracostomy without surgery is indicated only if instituted early in combination with adequate subphrenic drainage
Wei *et al.*[13]	1982	2	Bile duct obstruction (stones)	BBF	**Surgical**: Abdominal approach		No recurrence of BBF	
Warren *et al.*[2]	1983	15	Bile duct obstruction: Iatrogenic stricture 10x Congenital 1x Trauma 1x Stones 2x	BBF 13x PBF 2x	**Surgical**: Abdominal approach. Only 1 lobectomy	**Repeated surgery** in 9 patients	63 operations in total. Eventually all recovered.	Advocates abdominal approach for TBF that are the result of biliary tract disease
Caporale *et al.*[14]	1987	30	HDL	TBF Uses a different classification	**Surgical**: Thoracotomy, laparotomy or thoracophreno-laparotomy, with lung resection, cyst and pericyst removal, TBF resection, suturing of the diaphragm and subphrenic drainage for 2 to 4 weeks.	**Repeated surgery** in 2 patients	3 patients died – 10.3% (2 of haemorrhagic hock, one PE) 2 patients had a recurrence of TBF	Thoracotomyifpreoperativestudiesshowirreversiblelungimpairmentandthelivercyst is single. Laparotomy if the abdominal disease is prevalent. Forextensivediseaseperformtwoseparateincisionsratherthenperformingthoracophreno-laparotomy.
Gugenheim *et al.*[4]	1988	16	Iatrogenic bile duct obstruction 8x Echinococcosis 7x Abscess (amoebic) 1x	BBF	**Surgical**: Abdominal approach	**Repeated surgery**	42 operations total Eventually all recovered	Abdominal approach for TBF that result from biliary tract disease. Thoracic approach for traumatic TBF and if lung resection is planned
Yilmaz *et al.*[15]	1996	11	Complicated liver hydatidosis (previously operated) 8x Iatrogenic bile duct stricture 1x HDL + bile stones 1x Abscess (amoebic) 1x	BBF	**Conservative**: NBD in 4 patients EST + NBD in 7 patients	**Repeated Conservative treatment** in 3 cases. Prolonged NBD + stent insertion in 1 case	All recovered	First successful case series of nonsurgical treated BBF

Authors	Year	No. of patients	Aetiology	Fistula type	Initial therapy	Second line therapy	Outcome	Special recommendations
Senturk *et al.*[16]	1998	3	Alveolarhydatid disease (AHD) 1x AHD (after surgical cyst removal) 1x AHD (after surgical cyst removal)+ TBC 1x	BBF	**Conservative**: ERCP +EST after unsuccessful surgery (1st case) ERCP +NBD (2nd case) Anti-tuberculotics only (3rd case)	**Repeated Conservative treatment**: Octreotid (1st case) Stent (2nd case)	Recurrence in all cases. At the time of publication one patient was bed-ridden	Treatment of BBF due to AHD is unsatisfactory by either surgery or nonsurgical therapy. The reason is probably the more invasive nature of AHD
Chua *et al.*[17]	2000	2	Iatrogenic	BBF	**Conservative**: Abscess and biliary drainage (1st case) **Surgical** with thoracotomy, fistula and lung resection (2nd case)	**Surgical**: after BBF recurrence in 1st case	Both patients recovered	Describes the use of a vascularized intercostal muscle pedicle and a pericardial fat pad as a way of fistula closure.
Kabiri *et al.*[18]	2001	123 (cases of thoracic rupture of HDL)	HDL	BBF – confirmed in 50 cases by biliopytisis	**Surgical**: Postero-lateral thoracotomy with trans diaphragmatic cyst removal.		11 patients died (8.9%), only 1 recurrence	Advocatestransthoracicsurgerywithpreoperativeendoscopicsphincterotomy
Singh *et al.*[19]	2002	8	Abscess : Amoebic 3x Pyogenic 1x Trauma 3x Iatrogenic 1x (after PTC)	 BBF BBF BBF(1x), PBF (2x) PBF	**Conservative** (7 cases) EST+ Abscess or pleural drainage + octreotide **Surgical**(1 case) Iatrogenic biliary stricture repair	**Surgical** (2 cases)	All recovered	TBF may be successfully managed conservative. Surgery reserved for failure of this approach. Routinely uses octreotide
Gerazounis *et al.*[20]	2002	21	Echinococcosis	BBF	**Surgical**: Right postero-lateral thoracotomy.		2 patients died - 9.5%. All remaining patients recovered	Advocates surgery in cases of complicated echinococcosis with BBF
Uchikov *et al.*[21]	2003	3	HDL 2x Abscess (echinoccocic) 1x	BBF	**Surgical**: Right thoraco-phrenotomy		All recovered	
Ong *et al.*[22]	2004	2	Bile duct obstruction Stones 1x Iatrogenic 1x	BBF	**Conservative**: ERCP +EPT + stent+ octreotide	**Surgical**: in 2nd patient	1 death due to v. cava inf. laceration	Advocates the use of octreotide
Peker *et al.*[23]	2007	4	HDL	BBF	**Surgical**(2cases) **Conservative** (2 cases)		All recovered	Proposes a BBF treatment algorithm
Tocchi *et al.*[24]	2007	31	HDL	BBF (23 histologically con-firmed)	**Surgical** Lung resection was required in 25 cases		3 patients died (9.6%) 26 patients recovered	Advocates thoracoabdominal incision (approach)
Eryigit *et al.*[5]	2007	3	Abscess (echinoccocic) 2x Trauma (penetrant) 1x	BBF	**Surgical** with thoracotomy and lung resection in two cases		No recurrence reported	
Aydin *et al.*[25]	2009	3	Abscess (echinoccocic) 1x Iatrogenic 2x	BBF	**Conservative**: Percutaneous drainage with EPT + stent placement or NBD.		No recurrence reported	Advocates conservative approach. Embolization of the fistula is described.

BBF = bronchobiliary fistulas; PBF = pleurobiliary fistulas; HDL = hydatid disease of the liver; ERCP = endoscopic retrograde cholangiopancreatography; EPT = endoscopic papilotomy; NBD = nasobiliary drainage; PE = pulmonary embolism

in every clinical praxis[26,27], cholescintigraphy[4,28], magnetic resonance cholangiopancreatography (MRCP)[3,28], percutaneous transhepatic cholangiography (PTC)[2,3], endoscopic retrograde cholangiopancreatography (ERCP)[2,3,28], and fistulography in the case of a biliocutaneus fistule.[2,4] Most authors agree that bronchoscopy and bronchogram are not sensitive enough. From the remaining methods, PTC, ERCP and fistulography of the biliocutaneus fistula (if present) are stated as the most sensitive by most of the articles reviewed.[2-4,28] The latter being described as the method of choice for TBF confirmation by some articles.[2,4] The advantage of ERCP is the additional therapeutic possibility in the case of the biliary tract obstruction. Cholescintigraphy (hepatobiliary iminodiacetic acid – HIDA, paraisopropyl iminodiacetic acid – PIPIDA or diisopropyl iminodiacetic acid – DISIDA scan), CT, and MRI are less sensitive methods. Intraoperatively, a fistula can be demonstrated with the intraoperative cholangiography.

Regarding the therapy there are three possible approaches to treat TBF: surgical, conservative, and the combined approach. The bulk of the literature advocates the surgical approa ch.[1,2,4,5,7,10-14,17,18,20,21,23,24,29] In 1967, Ferguson and Burford[1] published an article on TBF, summarizing the basic steps necessary for a successful TBF treatment:

early aggressive treatment by thoracotomy,

adequate subcostal drainage of the hepatic bed under direct vision,

secure closure of the diaphragmatic perforation by non-absorbable sutures,

decortication for pleurobilia, if necessary lobectomy for bronchobiliary fistula and

the awareness of the need for prophylactic decompression of the biliary tree.

In the next decades other authors have published their experience in treating TBF. The studies reviewed included from 2 to 123 cases. [1,2,4,5,7,10-14,16,18-25] By comparing these studies, one can notice that non-traumatic TBF requires more operations for the successful treatment than traumatic TBF. This statement is best illustrated by the publications of Warren *et al*.[2] and Gugenheim *et al*.[4] In the first case 15 patients have been cumulatively operated 63 times and in the second case 16 patients have been operated 42 times. In contrast, Oprah and Mandal[12] report of only 17 operative interventions needed for the successful treatment of 14 patients with traumatic TBF. The common denominator in TBF treatment is to remove or solve the abdominal pathology that caused TBF formation. Additionally, adequate drainage of the subphrenic space and a secure closure of the diaphragmatic defect are of importance. Regarding the need for decortication or lobectomy, the authors do not share the same opinion. A lot of them think that lobectomy is necessary only occasionally, if the lung tissue is chronically inflamed or damaged[2,4,7] to the point that it could cause problems in the future.

According to the literature the possible ways of TBF closure are: non-absorbable sutures[1], the use of a mesh[30,31], AlloDerm®[3], pericardial fat tissue flap[17], pleural flap[5], and the use of a vascularized pedicel of intercostal muscle.[17]

In the last decade of the 20th century reports of conservative ways of TBF management started to appear. In these cases the treatment was comprised of biliary drainage using PTC (percutaneous transhepatic biliary drainage – PTBD) or ERCP and percutaneous drainage of the subphrenic, subhepatic or intrahepatic abscess if it existed. When performing ERCP there is the additional option of endoscopic sphincterotomy (EST) and stent placement or nasobiliary drainage (NBD).[32] The goal of these interventions is to minimize the pressure in the biliary tree, drain an abscess if present, prevent the flow of bile through the TBF and enable the healing of the TBF. In 1996 Yilmaz *et al*.[15] published a series of 11 cases of BBF after hydatid cyst operation. In all 11 patients an ERCP with nasobiliary drainage was performed, which lead to fistula closure. In some articles histoacyrl embolization of the TBF is stated as an additional option to the procedures listed above. Richter *et al*.[33] report of successful histoacyrl occlusion of a BBF after a selective duct cannulation during ERCP. Kim *et al*.[34] report of a successful histoacyrl embolization of BBF under bronchoscopic guidance. According to some authors, there is also the option of applying octreotide – a somatostatin analogue which is thought to reduce the secretion through enteral and biliary fistulas and thus promote their healing.[19,22,35] But it should be kept in mind that there were reports of an increased number of septic and thrombotic complications while using octreotide.[36]

The combined approach uses biliary drainage (via ERCP or PTC) and abscess drainage (US or CT guided) in the first step, followed by a delayed surgical intervention. The reason for such a course of action are patients who are initially not candidates for surgery (ARDS, sepsis, comorbidity, etc…)[3] and patients where non-surgical interventions have failed.[8,17,19,22,28]

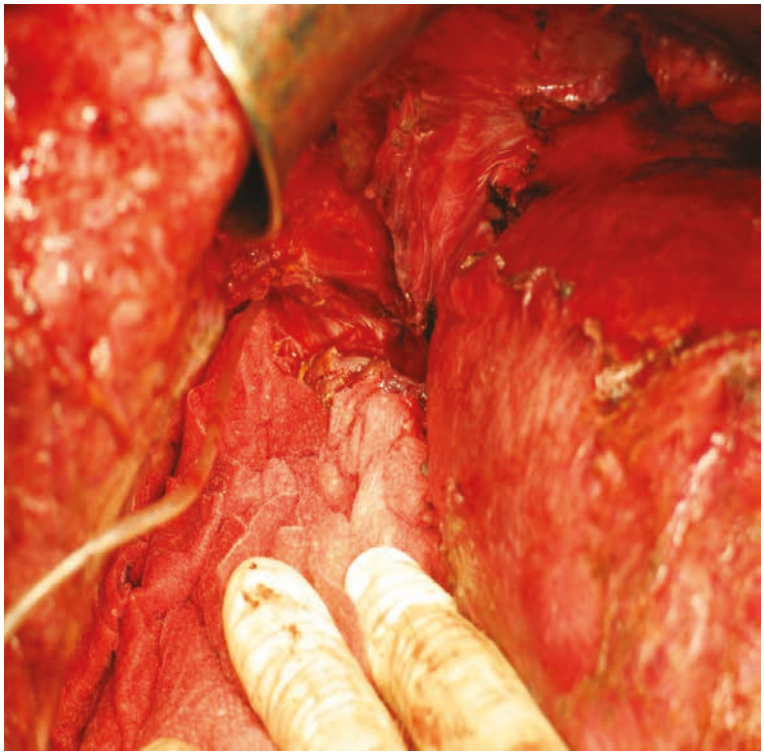

FIGURE 2. Placing of the catheter through the defect diaphragm.

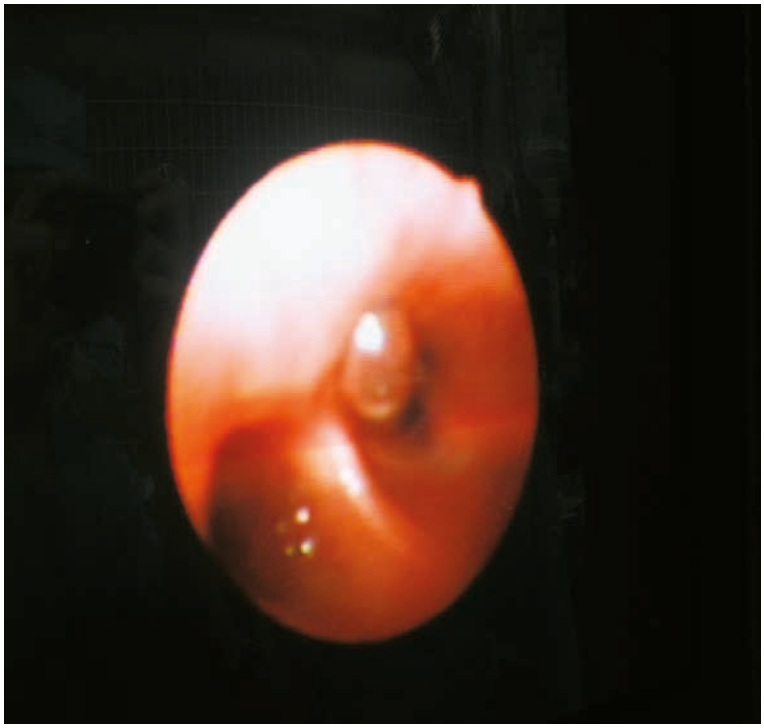

FIGURE 3. Visualizing the tip of the catheter with a bronchoscope in the 8th segment of the right lung.

Case report

The patient was referred to our medical centre in September 2009. At the time he was 73 years old. In the past an elective cholecystectomy for cholecystolithiasis was performed. He had essential arterial hypertension (regulated), suffered from a myocardial infarction, and in his youth he also had pulmonary TBC. In July 2009 a right hemicolectomy for colon carcinoma (stage T3N0M0) was performed at a regional general hospital. Preoperatively, pleural thickening in the left apical and right basal part of the lung was described on chest x-ray (due to TBC during his youth).

On follow-up examinations, the progression of the disease (using US and CT) with two metastases in the right hemiliver was diagnosed. One was located in the deep of the right anterior section (border of 5th and 8th segment) and was 4 cm in size. The other was located in the 7th segment and was 2 cm in size. There were also two metastases found in the lungs; one in the 2nd left and the other in the 6th right segment. At the multidisciplinary team meeting it was decided that liver surgery should be attempted first. A formal anatomical right hemihepatectomy was planned. In the beginning of October 2009 the patient was operated. With intraoperative inspection and US we found the two tumours in the right hemiliver as depicted by CT. Unexpectedly we also found two metastases in the left hemiliver, located in the 3th and 4th segment. Both were 1 cm in diameter and were not visible on the preoperative CT scan. A metastasectomy in the 3rd and 4th liver segment was performed. The remaining two metastases in the 7th segment and right anterior liver section were treated with radiofrequency ablation (RFA). A few days after the surgery the patient reported malaise and increasing pain in the right upper part of the abdomen. His body temperature and inflammatory parameters were elevated. A control US showed a hypoechogenic fluid collection in the area of the liver where RFA had been performed. ERCP demonstrated a biliary leak from the right anterior section into the cavity after RFA, endoscopic papillotomy (EPT) was performed and US-guided puncture and drainage was done, which revealed the fluid to be an infected biloma. Despite drainage and the broad spectrum antimicrobial therapy, the patient's state deteriorated. Pneumonia of the right lower and middle lung lobes and a biliocutaneus fistula developed. A reoperation was performed, which included the evacuation and lavage of the abscess cavity, sutures at the area of biliary leakage and the placement of two drains. After the inter-

vention the patient's state promptly improved. At the end of November 2009 he was discharged from our hospital, with the intention of the further oncological and surgical therapy.

In January 2010 the patient was again urgently admitted to our department. He was complaining of pain in the upper right part of the abdomen and pleuritic pain in the lower right part of the thorax. His body temperature was 39°C and he had a productive cough with yellowish sputum. White cell blood count and CRP were elevated and shadowing was identified in the lower right part of the lungs on chest x-ray. A CT scan of the thorax and abdomen was performed, showing a subphrenic abscess with a BBF. Again an operative evacuation, lavage and drainage of the abscess were performed. Sutures were placed at the site of biliary leakage and at the abdominal ostium of the BBF. A right hepatectomy was not performed, because the remaining liver volume would have been too small. Instead, a right portal vein embolization (PVE) was planned. We speculated that this would lead to atrophy of the right hemiliver, cessation or limitation of biliary leakage and compensatory left liver hypertrophy. The patient was discharged with a drain. The right PVE mentioned followed and his case was presented at an oncological council. Because the number of pulmonary metastases increased, the conclusion of the council was that he should receive palliative oncological therapy.

In August 2010 he was again admitted with malaise, intermittent fever, and cough with yellowish sputum. On CT scan the abscess in the remaining right liver was again present. As it was speculated, the right hemiliver was atrophic and the left liver was hypertrophic as a result of right PVE. The BBF seen on the previous CT scan was still present. Despite the pulmonary progression, we decided to perform a right hepatectomy with the intention to eliminate the inflammatory focus which was the reason for the BBF. Decisive for this step was the left liver hypertrophy taking place after the right PVE. After the right hepatectomy, we intraoperatively confirmed the presence of the BBF by placing a small diameter catheter through the abdominal ostium (Figure 2) and visualizing the tip of the catheter with a bronchoscope (Figure 3). Because of the pulmonary metastatic progression we did not decide to excise the fistula and the right lower lung lobe. Instead we closed the abdominal ostium of the BBF with a part of the omentum majus (Figure 4).The omentum was sutured over the abdominal opening of the BBF and was also used to cover the resection surface of the remaining left hemiliver, but was not sutured to it. After lavage a drain was placed. Postoperatively, a prompt decrease of the white cell blood count and CRP, cessation of bilioptysis and the improvement of the patient's general status was recorded. On follow-up examinations there was no sign of BBF recurrence. The patient expired in March 2011 due to the pulmonary metastatic progression of the disease.

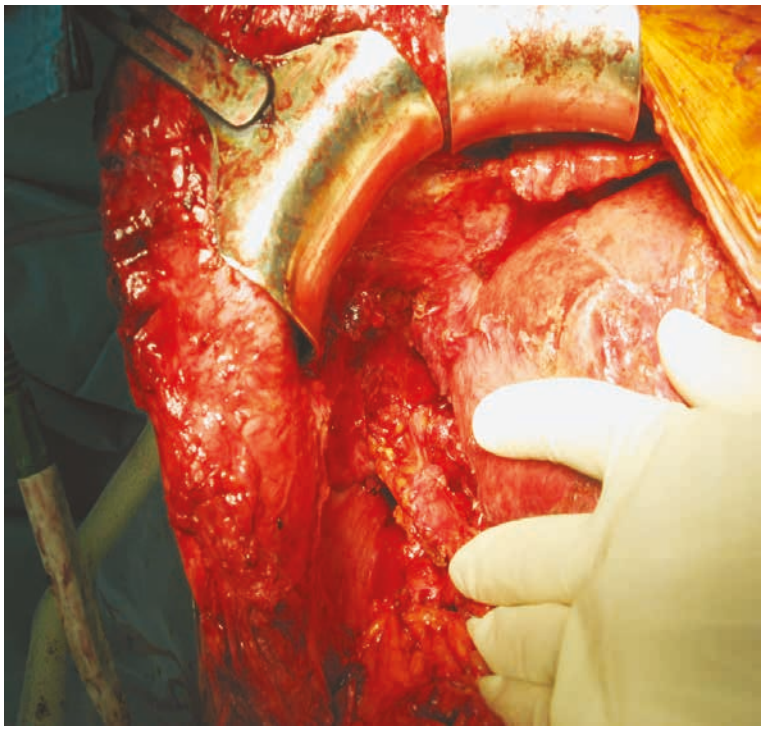

FIGURE 4. Placing of the omentum majus into the ostium of the BBF and closing the defect.

Discussion

By comparing our case to the literature reviewed, one can notice several parallels. Our case describes an iatrogenic BBF. In fact more cases of TBF in the developed world are probably due to trauma and iatrogenic causes than due to liver infection (echinococcic amoebic or pyogenic). Pathogenesis and the formation point of BBF described, coincides with the reviewed papers. The clinical suspicion was in our case confirmed with contrast enhanced CT of the thorax and upper abdomen. According to the literature this is not the most sensitive method. ERCP offers better results and has the additional advantage of biliary tree decompression as shown above. Intraoperative, TBF can be confirmed with cholangoigraphy. In the case of the liver resection,

as it was shown in our clinical case, it can also be confirmed by visualizing a small diameter catheter put through the abdominal ostium with a bronchoscope.

The biggest dilemma regarding TBF is the proper treatment. Although the bulk of the literature advocates surgery, non-surgical or conservative therapy is a good alternative in some cases. As shown by Yilmaz *et al*.[15], Singh *et al*.[19] and Ertugrul *et al*.[37], the conservative therapy with biliary and abscess drainage can be effective in cases where TBF is the result of liver abscess, complicated liver hydatidosis after surgical cyst removal, biliary tract obstruction due to stones or short strictures and in selected cases of posttraumatic TBF.

For the rest, surgery is probably the best solution. The basic steps outlined by Ferguson and Burford[1] are a good basis but need some comment. Thoracotomy is necessary if the preoperative investigation indicates the need for the lung resection[4,14] or in the case of posttraumatic TBF.[4] Laparotomy is mandatory if biliary tract obstruction which cannot be managed conservative is the cause of TBF.[2,4] In cases of extensive disease two separate incisions (approaches) are probably superior to thoracophrenolaparotomy.[14] Regarding the diphragmal defect closure, we can add our report of BBF closure with omentum majus to those listed above. In our opinion preoperative biliary decompression would act beneficial on TBF therapy outcome in all TBF cases not just those caused by the biliary tract obstruction.

In our case two operations were needed for a successful BBF treatment. Despite the palliative nature of the treatment a surgical approach was chosen. The reason was the persistence of biliopytisis and recurrent septic bursts, although the conservative treatment with ERCP, EPT and US guided abscess drainage had been done first. We think that biliary leakage into the cavity produced by RFA was the reason for failure of the conservative measures. The only possible solution was the right hepatectomy, which removed the inflammatory focus and closure of the fistula as described above. Though this might seem an extensive treatment for a patient with a poor prognosis, it was the only way of improving his quality of life.

Conclusions

Thoracobiliary fistulas are pathological communications between the biliary tract and the bronchial tree or the biliary tract and the pleural space. The first are termed bronchobiliary and the second pleurobiliary fistula. Etiologically, they can be divided into congenital TBF, TBF resulting of liver hydatid disease or liver abscess, biliary tract obstruction, traumatic TBF, and iatrogenic TBF. The article summarizes the characteristics of the clinical picture and laboratory findings of BBF and PBF. The most sensitive methods for TBF confirmation are ERCP, PTC, and fistulography of a biliocutaneus fistula, if it exists. Opinions regarding the therapy differ. Although the bulk of the literature advocates surgical therapy, newer papers report of successful non-surgical therapy. These interventions are comprised of a biliary drainage via ERCP or PTBD, percutaneous US or CT guided drainage of a subdiaphragmatic, subhepatic or intrahepatic abscess if it exists and ERCP or bronchoscopic guided fistula embolization. The authors presented the fistula closure with the greater omentum as a possible method of TBF closure which was according to the authors' knowledge not reported on previously.

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research article

Distance deviation measure of contouring variability

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Background. Several methods that are currently used for contouring analysis have problems providing reliable and/or meaningful results. In this paper a solution to these problems is proposed in a form of a novel measure, which was developed based on requirements defined for contouring studies.

Materials and methods. The proposed distance deviation measure can be understood as an extension of the closest point measures in such a way that it does not measure only distances between points on contours but rather analyse deviation of distances to both/all contours from each image point/voxel. The obtained result is information rich, reliable and provided in a form of an image, enabling detailed topographic analysis. In addition to image representation, results can be further processed into angular representation for compact topographic analysis or into overall scalar estimates for quick assessment of contour disagreement.

Results. Distance deviation method is demonstrated on a multi observer contouring example with complex contour shapes, *i.e.*, with pronounced extremes and void interior. The results are presented using the three proposed methods.

Conclusions. The proposed method can detect and measure contour variation irrespective of contour complexity and number of contour segments, while the obtained results are easy to interpret. It can be used in various situations, regarding the presence of reference contour or multiple test contours.

Key words: contouring; contour comparison; distance transform

Introduction

Medical treatment often involves planning or analysis based on delineated structures defined on 3D images such as MRI or CT.[1-3] One typical area is three dimensional image guided radiotherapy, which, on the basis of delineated structures, enables individualized irradiation, applying high doses to the target volume while respecting organs at risk dose constraints. The structures are defined by contouring regions of interest on 2D image slices, such that multiple contours on many slices may be required to delineate one structure of interest. The contouring requires highly skilled experts, because the boundary line of the structure may not be clearly visible or multiple edges may appear in the region. Furthermore, even small changes in contour position may have high impact on the success of the treatment and consistent reporting. Certain contouring analysis is therefore required to quantify inter and intra-observer variability, automatic contouring algorithms error or to compare contouring using different imaging technologies. The contouring error is difficult to assess, because the ground truth is generally not known. In some cases reference contours can be provided by an experienced (and eminent) expert or by consensus of a panel of experts. Otherwise, the magnitude of contouring variation between observers can be used as an indicator of contouring quality. The technical problem in evaluating contour variability is the absence of generally accepted method for contour comparison. Several methods were proposed, however certain limitations usually prevent their widespread use.

In this paper we focus on this problem and propose a novel measure of contouring differences, *i.e.*, a distance deviation measure. Two different purposes of contour analysis need to be distinguished. The first one is analysis of contouring variation, *e.g.*, for studying the contouring process. The metric for these studies must assess the difference between contours, which are drawn on image slices and are, thus, two-dimensional (2D) objects. Consequently, the analysis should also be performed in two dimensions only. On the other hand, if the purpose of the analysis is to study the effects of contouring variation, the whole delineation of a three-dimensional (3D) structure must be evaluated, which requires analysis based on three-dimensional metrics. Of course, both of the study types are interrelated; however, the relation between them may not be straightforward.

A review of methods for contouring analysis was made by Jameson *et al.*[4] The most widely used method is comparison of structure volume. The major problem of this method is that even if two structures have the same volume, they may have different shape or location, which is not taken into account. The second method, with the opposite drawback, is comparison of the centre of volume (COV). It defines a single point that represents the whole structure and cannot distinguish structure size or shape. The next, very popular metric is a conformity index (CI), also called concordance or Jaccard index. It is defined as the percent ratio of the volume of intersection and volume of the union. To take into account more than two delineations, its generalized version was proposed (CIgen).[5]

The limitation of those measures is in their relativity. They do not provide any information of absolute variation in size, shape or location. All these metrics have an additional problem, that different implementation, *i.e.*, using different volume computation methods, may yield different results.[4] These measures are rarely used individually and are often combined to detect different kinds of contouring variations.[6-10]

The solution to the previously mentioned problems was often searched by methods that quantify shape or surface variations. The shape and surface differences were most often analyzed by mapping to cylindrical or spherical parametric space and observing the edges of structures from the origin.[11] The problem here is in the definition of coordinate system origin and in exaggerated results when structures have complex non-circular or non-spherical shapes.

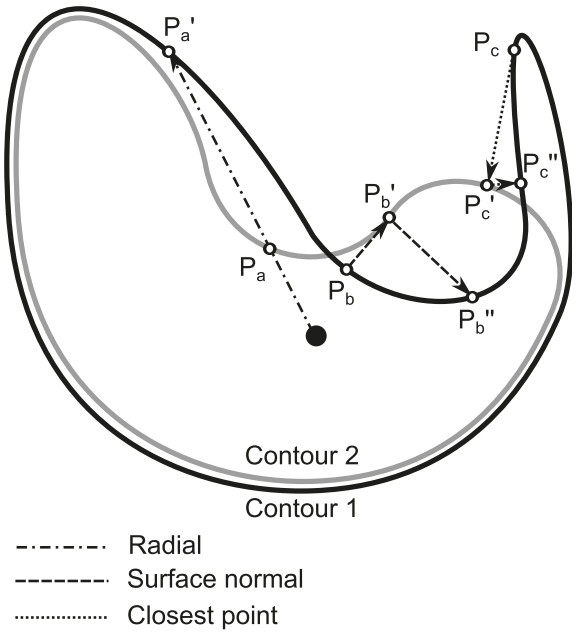

FIGURE 1. Illustration of limitations of methods based on origin, surface normal and closest points. The radial and surface normal methods may overestimate the distance between the contours. The surface normal and closest point methods are not symmetric, which means that distance may be different when measuring from contour 1 to contour 2 than it is in the opposite direction. Asymmetry also means that certain differences cannot be detected in certain direction, as some points on the contour may never get matched, e.g., point *Pc* when measuring distance from contour 2 to contour 1.

Especially difficult is comparison of structures if the surface is not 'visible' from the origin. There are two alternative methods, which measure distance between shapes or surfaces according to the surface normal[12,13] or according to closest points.[14,15] The problem here is the lack of symmetry; interchanging the first and the second structure yields different results. The most important drawback of asymmetric methods is that they cannot detect all the differences, especially in the case of complex shapes (Figure 1). The symmetrisation of the closest point method to obtain more reliable statistics could be made by applying it in both directions.[16] However, due to differences between results obtained in both directions, spatial information about the contour correspondence is not obtained. These inconsistencies were reduced by ComGrad method[17], which searches for the point pairs according to gradients of edges of both compared structures. However, the one-to-one mapping of all points on two different contours is in general not possible and limits the reliability of all these methods.

To design a new method for analysis of contour variations, the requirements of such measure need to be clearly defined. First, the measure must be expressed absolutely in standard distance units, *e.g.*, millimetres, in order to enable meaningful interpretation of results, as the opposite to relative methods that provide some ratio that is less informative in the aspect of contouring differences. It needs to be consistent and therefore symmetric. Second, it must enable topographic analysis, *i.e.*, measuring of local discrepancies irrespective of the complexity of contours. Third, it must be general enough to be usable for various kinds of contouring studies, *i.e.*, for studies with or without the reference contour and for arbitrary number of test contours. The same principle must be appropriate for measuring in 2D or 3D space.

Materials and methods

The proposed measure of contouring variations is based on the well-known closest point measure, however extended from measuring distances between contours, *i.e.*, distances between points on contours only, to measuring deviation of distances from each image point/voxels to all contours, *i.e.*, for the whole image. This makes it symmetric and capable of meaningful measurement of arbitrary complex contour shape differences. The core of the measure is the Euclidean distance transform that has already proved its applicability in implementations of the closest point measure. The principle is explained in 2D space; however, the extension to 3D is straightforward as the only required difference is in the dimensionality of the distance transform used.

Application of Euclidean distance transform

The well-known closest point measure compares two contours by measuring distances from points in analysed contour to closest points in the other, reference contour. Due to the asymmetry it was proposed to apply it in both directions.[18] Such symmetrisation makes problems in localization of contour differences and does not fulfil our requirements. In spite of that, the closest point measure inspired the proposed measurement methodology by its implementation using the Euclidean distance transform.

The general idea was to measure distances between contours by computing Euclidean distance images.[19,20] A distance image covers the same region as the original image on which the contours were drawn. It is created from contours defining the delineated structure such that the value of each image voxel equals the Euclidean distance from the voxel centre to the closest point on the contour. Its computation starts with drawing a delineation image **I**, which is a binary image with voxels inside the delineated structure represented by logical '1' and voxels outside the structure represented by logical '0'. Then the distance image **D** is computed by application of the Euclidean distance transform. Here, each voxel is a real number representing a distance to the closest edge of the structure. Voxels outside the structures obtain positive values, while inside values are negative.

When used for the closest point measure, the distance image **D** needs to be computed only for the first contour, *i.e.*, the reference. The closest point distances are computed for points in the other, *i.e.*, analysed contour. For a point x, the closest point distance equals the absolute value of the (reference) distance image in this point, $|\mathbf{D}(\mathbf{x})|$. Due to the discrete nature of the distance image, some interpolation may be required. The accuracy of the obtained results equals the distance image voxel size. Generally, it is recommended to use the same voxel size as in the original image, because it also implies limitations of the contouring accuracy. Smaller voxels may be used if higher accuracy is required. See the illustration of computation and use of the distance transform (Figure 2).

When contours of 3D structures are compared, the distance transform is performed on each individual image slice separately, to preserve the 2D nature of contours. Alternatively, 3D distance transform could be used to compare 3D delineation volumes.

There are several techniques for effective computation of the Euclidean distance transform.[21-23] However, their description or comparison is out of scope of this paper.

Distance deviation measure

Our approach to avoid asymmetry and one-to-one mapping of contour points is to analyse distances to both/all contours from every image voxel. The difference between contours is therefore observed not only from the points on the contours, but also from the perspective of various nearby points. Using multiple points, covering the whole region of interest, any contour difference can be detected and measured not regarding the contour complexity. In addition, such approach enables localization of contour differences in a sense of

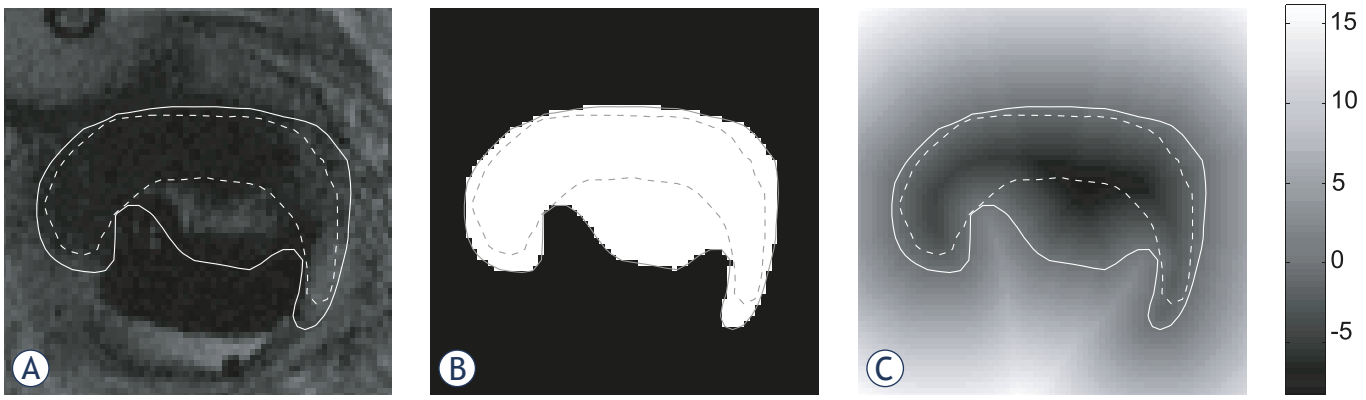

FIGURE 2. An illustration of computation and use of Euclidean distance transform for measuring closest point distances. A reference contour (solid line) and an evaluated contour (dashed line) were drawn on the original image (a). Using the reference contour a delineation image I (b) and distance image D (c) were computed. Closest point distances were obtained as an absolute value of the distance image for points in the evaluated contour. The colour scale of the distance image was provided on the right hand side and is given in millimetres.

their influence on the nearby regions. The difference of two contours $\mathbf{C}_1$ and $\mathbf{C}_2$, where one of the contours is considered as a reference for evaluating the other contour, and from the perspective of some point with coordinate $\mathbf{x}$ can be measured using the corresponding Euclidean distance images $\mathbf{D}_1$ and $\mathbf{D}_2$ as their absolute difference:

$$DD_{12}(\mathbf{x}) = \sqrt{\left(\mathbf{D}_1(\mathbf{x}) - \mathbf{D}_2(\mathbf{x})\right)^2} = abs\left(\mathbf{D}_1(\mathbf{x}) - \mathbf{D}_2(\mathbf{x})\right) \quad [1]$$

Let us call the result $DD_{12}(\mathbf{x})$ a distance deviation. It denotes a deviation of distances from an observation point to both contours. Note that distance images $\mathbf{D}$ of both contours are needed in contrast to the closest point $\mathbf{x}$ measure where distance image is computed only for one of the contours. The measure is obviously symmetric, such that $DD_{12}(\mathbf{x}) = DD_{21}(\mathbf{x}) = DD_R(\mathbf{x})$.

Furthermore, each difference between contours can be detected by selecting an appropriate observation point $\mathbf{x}$. Therefore, by analysing the distance deviations from all surrounding points, no difference between the contours remains undetected. The results denote contour differences in the absolute manner, e.g., in millimetres, and enable meaningful topographic analysis.

Contouring studies often require analysis of multiple contours, *e.g.* to find some general characteristics of contour disagreement. A reference contour may be provided to define the ground truth. In such cases distance deviation measure estimates the contour disagreement for observation point x as a RMS value of distance deviations corresponding to individual analysed contours with respect to the reference one:

$$DD_R(\mathbf{x}) = \sqrt{\sum_{i=1}^{N} \frac{\left(\mathbf{D}_i(\mathbf{x}) - \mathbf{D}_R(\mathbf{x})\right)^2}{N}} \quad [2]$$

Here, $\mathbf{D}_R$ is a reference distance image and N is the number of analysed contours. Note that distance image $\mathbf{D}_i$ must be computed for every contour $\mathbf{C}_i$ involved into analysis.

When the reference is not known, the contour error can not be measured. However, the magnitude of contouring variation can be used as an indicator of contour quality. The contouring variation can be estimated by computing distance deviations against an average contour instead of the reference one. The average contour does not need to be computed, because only the average contour distance image is needed and can be obtained by averaging distance images of all analysed contours:

$$\overline{\mathbf{D}}(\mathbf{x}) = \frac{\sum_{i=1}^{N} \mathbf{D}_i(\mathbf{x})}{N} \quad [3]$$

Distance deviations, in the case of absence of reference labelled DD_S, are then obtained using exactly the same principle as in the case of provided reference, and for some observation point $\mathbf{x}$ represent a standard deviation of distances form this point to all analysed contours $\mathbf{C}_i$:

$$DD_S(\mathbf{x}) = \sqrt{\sum_{i=1}^{N} \frac{\left(\mathbf{D}_i(\mathbf{x}) - \overline{\mathbf{D}}(\mathbf{x})\right)^2}{N}} \quad [4]$$

To summarize, every contour discrepancy can be detected by measuring deviation of distances from nearby points to all analysed contours, and when deviations for all image points are computed, no contour deviation can remain undetected, even in the case of complex and pronounced ex-

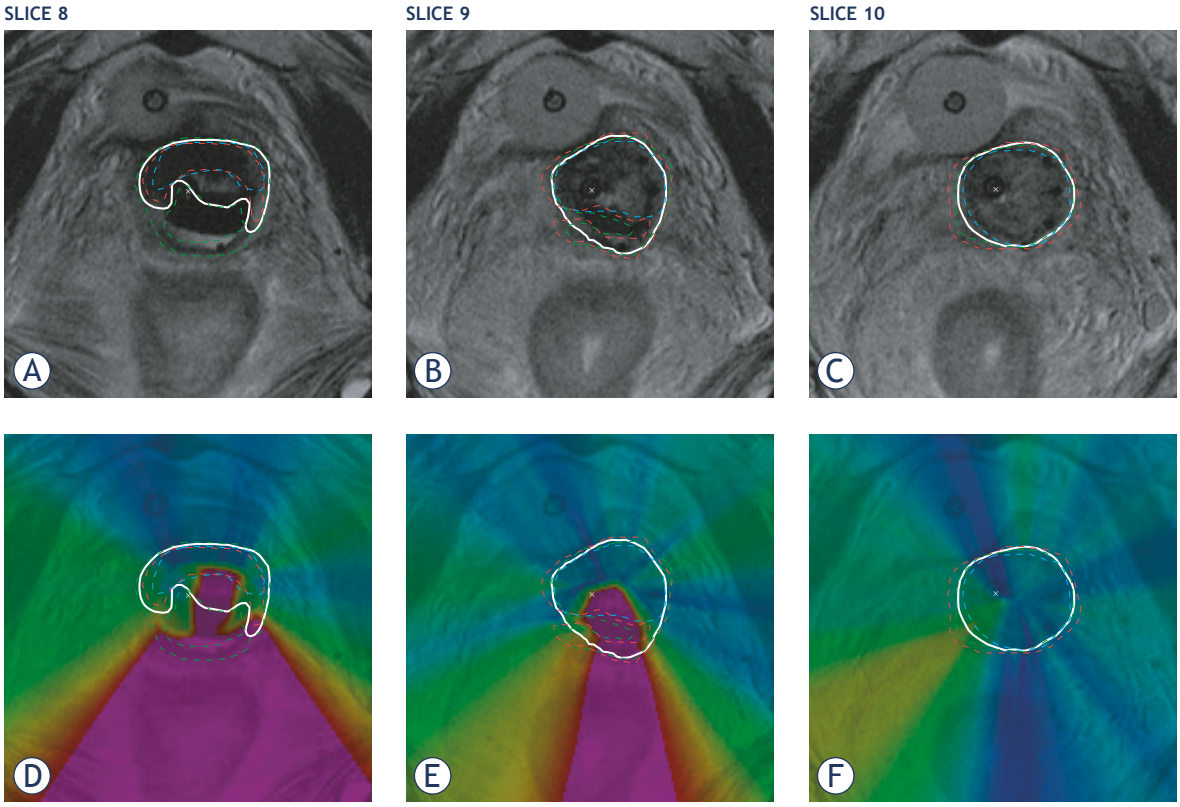

FIGURE 3. An example of contouring analysis from the field of brachytherapy. The reference contours are in images shown with thicker white line and the analyzed contours with thinner dashed ones. The small cross represents the center of the uterine tandem and is used as the contour center for the angular analysis. The top row (a-c) shows selected slices of the original image, the second row (d-f) is image representation of distance deviations and at the bottom (g) is an angular representation of distance deviations for all image slices. Here, the angle of zero degrees corresponds to the anterior direction (top of the image), negative angles to the right hand side of the body (right hand side of the image) and positive values to the left hand side of the body (left hand side of the image). The color scale of image representation equals the one of angular representation; black/purple represents contour discrepancy greater than 5mm. The overall maximal distance deviation ($DD_{R,max}$) equals 17.96 mm and the mean distance deviation ($\overline{DD}_R$) equals 2.17 mm.

tremes or void interior. It is also important that there is no need to perform any mapping of points on different contours and still obtain detailed topographic information of the contour differences. The proposed distance deviation measure can be used in various situations, not regarding the existence of the reference contours, nor the number of analysed delineations. We recommend computing distance deviations in all image points, *i.e.*, all image voxels, because the distances to contours can be obtained by the distance transform and the points are dense enough to reliably detect all contour differences. The accuracy is still limited by the accuracy of distance images and can be improved using higher resolution distance images, such as in the case of the closest point measure. Interpolation is not required, because points defined by image voxels are used instead of contour points that are used in the closest point measure.

Presentation of results

A clear representation of results that is understandable to the observer has important implications. Among other, it may have a significant positive effect on the learning process. In this way, it enables the observer to improve his contouring, which can be expected to result in improved treatment results and consistent and comparable treatment recording and reporting.

In order to interpret the obtained results, they also need to be presented in a form familiar to the observer and compliant with requirements of each particular study. We have identified three different representation methods that could satisfy all common study requirements:

- image representation,
- angular representation, and
- overall scalar estimates.

The later two methods require some statistical analysis to depict information rich results in a more compact form. Because result representation methods can be used irrespective of the presence of reference contour, we use *DD* as a general notation for distance deviations, computed either as DD_R [2] or as DD_S [4].

Image representation

The most informative way of presenting differences between contours is to visualize them on the original medical image that was used to draw the contours. The distance deviation measure provides the information of contouring variability in a form of an image and can be easily visualized in such a way. The advantage of visualizing distance deviations as an image with respect to visualizing the contours only is in explicit labeling of impact of contouring differences to nearby regions. This information enables observer not only to see the different contours but also to easily judge them from the perspective of their influence to nearby structures.

Medical images are typically provided as a grid of scalar measurements that are visualized as pixel intensities, *i.e.*, gray values. The colour spectrum is not used, except for visualizing additional data, *e.g.*, contours or labels. Thus, the extension of gray values to a colour space can be used to jointly visualize both, information of the original medical image as well as the contour variability.

There are several ways to colour code the contour disagreement as well as preserve the displayed information of the original medical image. One of them is to use a HSV (hue, saturation, and value) colour space.[24] To preserve the well known representation of medical image by intensity values, this information shall be coded as the value component (V). The contour disagreement is most clearly understandable when coded as the hue component (H), *e.g.*, representing low contour disagreement with blue and high disagreement with red color. The saturation component is not used to present any information and shall be high enough to well distinguish between the other two components.

An example of image representation of contour disagreement measured by distance deviations DD_R is shown in Figures 3d-f.

Angular representation

In order to present distance deviations in a more compact form that enables topographic analysis of a whole 3D structure from a single graph, they can be shown using angular representation in cylindrical coordinate system defined by some contour center. Angular representation is often used for topographic analysis of contouring variations.[25-27] In the case of distance deviations, the contour differences are presented by maximal distance deviation observed on certain image slice and in certain direction from the contour center. For example, an angle of 0 degrees typically represents the anterior part of the structure, +90 degrees left, −90 degrees right and ±180 degrees correspond to the posterior direction. The contour center may be defined as a center of gravity or according to position of other geometrical features, *e.g.*, applicators or needles.

Such representation is convenient for circular structures, while for more complex shapes it may blur the spatial information. However, although the spatial information may be blurred, the presented results are still correct and are not exaggerated like they are when using circular or spherical coordinate space directly for the analysis.

Converting an image of distance deviations into angular form requires some post processing. For each angle, the maximal distance deviation is searched from the contour center to the edge of the region of interest. However, it turns out that distance deviations in regions outside the most distant contour can not exceed the ones inside that region. Similarly, distance deviations inside the innermost contour are also reflections of some contour disagreement that can be detected in area between the innermost and the outermost contour, for a proof see the appendix. Thus, we can limit our analysis only to this region of contour disagreement ΔI:

$$\Delta I(\mathbf{x}) = \begin{cases} 1 & \text{if } 0 < \Sigma_{i=1}^{M} I_i(\mathbf{x}) < M \\ 0 & \text{otherwise} \end{cases} \quad [5]$$

Here, M is a total number of contours including the reference one, if available. Limiting the analysis to this region also avoids misinterpretation of angular results, because distance deviation in some point in the inner region does not necessarily represent disagreement of contours in the direction of this point from the contour center. This is especially evident in the case of eccentric or complex contour shapes. For the example see Figure 3, slice 9.

The maximal angular distance deviation in the observed region ΔI, can be obtained using cylindrical coordinate system, where r and ø denote the radial distance and the angle according to the contour center:

$$DD\angle(\phi) = \max_{\phi\in(-\pi,\pi),\ r\in(0,\infty)} \left(DD(r,\phi)\cdot\Delta I(r,\phi)\right) \quad [6]$$

Depending on the angle discretization, some interpolation of distance deviation image *DD* may be required.

Angular representation is more compact than image representation, providing only the maximal contour disagreement in certain part of the analyzed structure without detailed distribution of contour disagreement with respect to patient anatomy. However, due to compactness, results of multiple slices can be presented in a single graph to describe the contouring variations for a whole surface of the 3D structure. An example of angular representation is shown in Figure 3G.

Overall scalar estimates

For a quick estimate of contour disagreement a single scalar value representing the overall score is often required, although such representation does not enable topographic analysis.

Different statistical methods were proposed to compact complex and information rich results into a single representative value. In general, maximal and mean values are commonly used. Maximal value of a distance metrics is also known as a Hausdorff distance[28] and is popular for evaluating segmentation methods.[13,16,29] Maximal distance deviation may be obtained by searching over the whole 3D distance deviation image. The same result can be obtained by searching for maximum only in the regions of contour disagreement ΔI:

$$DD_{max} = \max_{\Delta I(\mathbf{x})=1} (DD(\mathbf{x})) \quad [7]$$

Here, the result is a maximal contour discrepancy, which equals the Hausdorff distance for M = 2. In this specific case the value in the interior of the contours cannot exceed the maximum on the contours. Alternatively, the contour disagreement could be represented by a mean value of distance deviations in some region. It can be obtained by averaging in different domains, e.g. according to area in the image representation or according to angle in the angular representation. Each approach lays stress on different properties of contour disagreement and has limitations on the others. Each one of them may be ambiguous in some perspective and may, due to information reduction, not clearly depict contour differences of complex shapes.

We have found averaging according to the area in the delineated regions to be the most balanced one. Here, the region for averaging $\hat{I}$ is defined by union of all delineated regions I_i corresponding to individual contours including the reference one:

$$\hat{I}(\mathbf{x}) = \begin{cases} 1 & \text{if } \Sigma_{i=1}^{M} I_i(\mathbf{x}) > 0 \\ 0 & \text{otherwise} \end{cases} \quad [8]$$

The union region enables balanced quantification of contour variability with respect to the whole delineated structure, without excluding eventual high contouring errors at outermost and innermost contours and parts of good contour agreement that reflects in low distance deviations in the interior of the region of contouring disagreement ΔI. Here, all the image slices must be considered in order to evaluate contours representing three dimensional structures. The overall estimate of contour disagreement in a form of an average distance deviation is

$$\overline{DD} = \frac{\sum_{\mathbf{x}} DD(\mathbf{x})\hat{I}(\mathbf{x})}{\sum_{\mathbf{x}} \hat{I}(\mathbf{x})} \quad [9]$$

Note that average distance deviation in contrast to other distance deviation indexes may violate the triangular inequality requirement of a mathematical metric, and thus cannot be used to compare contours indirectly. The obtained maximal and average distance deviations are extremely compact. They provide absolute results, and enable quick insight into contouring variation for multiple contours. They provide different information and may in some cases yield opposing results.[30] This makes them supplementary to each other.

Results

To illustrate the distance deviation measure it was applied to a manually selected complex contouring example from the field of cervix cancer brachytherapy. The contouring was performed on MR image with voxel size 0.625 × 0.625 × 3.900 mm. The con-

tours of three observers were analysed with respect to reference delineation. Contours were provided for all relevant image slices, *i.e.*, for slices 7 to 16. The contours corresponding to three successive image slices 8, 9 and 10 are shown in a top row of Figure 3.

The complexity of the case is high due to topology of contours that include noncircular shapes with pronounced extremes (slices 8 and 9) and void interior (slice 8). The results are presented in two graphical forms; image representation and angular representation. Furthermore, the two proposed overall scalar estimates of contour disagreement are computed; maximal and average distance deviation.

For the image representation of contour disagreement using distance deviation measure see Figures 3D-F. The colour coding follows the colour scheme used in Figure 3G. The maximal distance deviation displayed is limited to five millimeters, larger values are coded with black/purple. Distance deviations are computed for each image pixel/voxel and thus enable detailed topographic analysis, including localization of (anatomical) regions that could be highly affected by contour differences.

In the provided example a large distance deviation can be noticed in central and posterior regions of slices 8 and 9. Results for these two slices also show that the presence of void interior regions and pronounced extremes does not limit capabilities of the measure to clearly and correctly evaluate contour differences.

The angular representation of distance deviations is presented in Figure 3G. Here, the results of each slice contribute one row in the graph, which, as such, provides the results for the whole image. For slices on which contours were not drawn the graph remains empty/white.

The colour scale represents distance deviations from zero to 5mm, larger values are coded with black/purple. Focusing on the selected slices, large distance deviations can be noticed for slices 8 and 9. However, the results provided in the angular form are not that unambiguous as in the form of an image, and slice 8 is a good example in that manner. Because the axis for angular analysis is in the region of contour disagreement and not in the interior of all contours, the high distance deviation around the contour center results in high values for multiple angles, in our case angles below -40 degrees. These values are normally related to the contour parts at the left hand side of the analyzed image region, where in our case contours match considerably well. The high values therefore indicate an error not only in the given part of the contours, but also in regions close to the contour center that are not in the interior of all contours. The position of the contour center has in such complex cases high influence on the angular results, which may make its positioning difficult if not defined anatomically. When contour shapes are less complex, the angular representation is unambiguous, such as in slice 10 of our example.

The overall scalar estimates of contour disagreement are not only more compact, but also less informative. The average distance deviation ($\overline{DD}$), in our case $\overline{DD}_R$= 2.17 mm, is not optimal for detecting high local variations. A relatively low value means that contours are in good mutual agreement, but not also that there are no high local differences. On the other hand, maximal distance deviation (DD_{max}), in our case $DD_{R,max}$ = 17.96 mm, does not give a good insight into the overall contour agreement, but gives a clear warning when large local discrepancies are present. Thus, our example demonstrates the importance of both scalar estimates and their complementarity.

Discussion and conclusions

In comparison to other measures, see the introductory section, the distance deviation measure satisfies all the requirements of contouring variability studies. The contour differences are measured absolutely with results given in millimeters enabling straightforward interpretation. The symmetry is assured by equal treatment of all analyzed contours and the reliability by observing contour differences from the perspective of contour surrounding, *i.e.*, all image points/voxels, such that any kind of contour differences can be detected and measured.

The proposed novel measure of contour discrepancy was developed as a solution to problems identified in other measures. It extends the measurement of distance between two contours to the measurement of deviation of distances to both/all contours from points in nearby regions. The basic idea to follow the relation between contours and the image has a physical background; contouring errors influence the dose delivered to imaged regions, *i.e.*, to the target as well as organs at risk. In radiotherapy, for example, contouring uncertainties result in uncertainties of dose distribution in nearby tissues and, therefore, have clinical consequences. Distance deviation measure does not tend to model the real influence of contouring errors to treatment of nearby regions. Instead, it uses this principle to gain robustness and reliability of

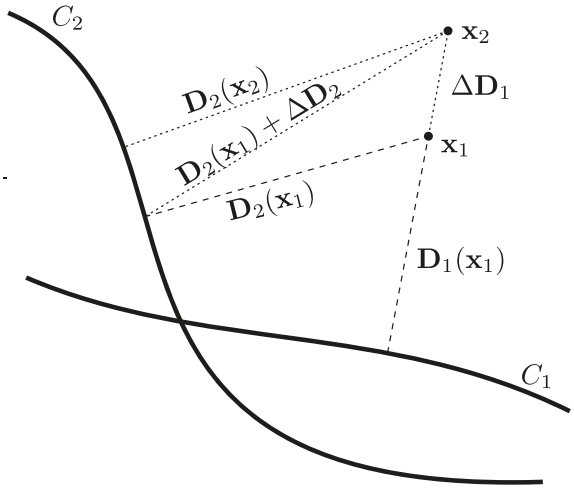

FIGURE 4. Illustration of distances for points outside the outermost contour.

detecting and measuring contour differences irrespective of contour complexity. Furthermore, this principle enables results to be easy to interpret, also because all contour differences are measured in standard distance units (millimeters). The impact of the image and angular representation of the index proposed here is in its capacity to allow the observer to appreciate the delineation uncertainties on anatomical images in the actual contouring plane, instead of a virtual 3D volume.

The distance deviation measure is suitable for diverse measurement tasks, not regarding the number of compared contours or presence or absence of a reference contour. The core of the measure is the Euclidean distance transform, which converts each analyzed contour into a distance image. Distance deviations are then obtained by basic statistical analysis (e.g. computing standard deviation) of obtained distances for each image voxel. The results can be presented clearly using different presentation methods tailored to the common needs of different contouring studies; from detailed topographic analysis to the compact scalar representation of the overall contour discrepancy.

Planning target volume (PTV) is a geometrical concept, derived from the clinical target volume (CTV) by applying margins around it to compensate for the effects of organ/patient movement, and uncertainties in beam and patient setup. The CTV as an anatomical/biological concept needs to be selected and delineated by the treating physician, before selecting the treatment modality and technique. According to these definitions, generation of margins from CTV to PTV to account for delineation uncertainties may not be advocated. Instead, every effort should be made to reduce these uncertainties through the use of adequate imaging, delineation guidelines and training.

The method has already proved its applicability in different studies of contouring in radiotherapy.[31-33] Although we found no limitations in the measure itself, some precaution is needed when compact result representations are used. Compacting of results reduces the amount of information they provide and, thus, some information gets lost. Angular representation discards the information of the radial location component and makes the results sensitive to selection of the axis used for the analysis. Scalar estimates discard all the spatial information by averaging or by searching the maximum value in the region of interest. Nevertheless, the information provided by results of distance deviation measure, even in the case of the most compact scalar representations, enable reliable assessment of the overall contouring variability. As such, we believe in contribution of distance deviation measure to advancement of contouring studies.

Appendix: Distance deviations outside the outermost contour

Let us assume that we have two contours C_1 and C_2 and some point x_1 outside the contours such that distance $\mathbf{D}_1(\mathbf{x}_1)$ is smaller than $\mathbf{D}_2(\mathbf{x}_1)$, i.e., the the point $\mathbf{x}_1$ is closer to contour C_1 than C_2. For the illustration see Figure 4. Furthermore, a point $\mathbf{x}_2$ is positioned further from the contours on a line through $\mathbf{x}_1$ and its closest point on C_1, such that $\mathbf{D}_1(\mathbf{x}_2) > \mathbf{D}_1(\mathbf{x}_1)$. Let us compare distance deviations of these two points, i.e., $DD(\mathbf{x}_1)$ and $DD(\mathbf{x}_2)$. When moving form point $\mathbf{x}_1$ to point $\mathbf{x}_2$, the distance to the closest point on contour C_2 cannot increase more than distance to contour C_1, due to triangular inequality:

$$\mathbf{D}_2(x_1) + \Delta\mathbf{D}_2 \leq \mathbf{D}2(x_1) + \Delta\mathbf{D}_1 \quad [10]$$

As the distance deviation measure relies on the closest point distances, we have to be aware of the possibility that there may exsist some other point on C_2 that is even closer to x_2 than the original closest point of x1:

$$\mathbf{D}_2(\mathbf{x}_2) \leq \mathbf{D}_2(\mathbf{x}_1) + \Delta\mathbf{D}_2 \quad [11]$$

and according to [10]

$$\mathbf{D}_2(\mathbf{x}_2) \leq \mathbf{D}_2(\mathbf{x}_1) + \Delta\mathbf{D}_1 \quad [12]$$

Using the Eq. [1] and the observed relationship, we get:

$$DD_R(\mathbf{x}_2) = \text{abs}\,(\mathbf{D}_1(\mathrm{x}_2) - \mathbf{D}_2(\mathbf{x}_2)) \quad [13]$$
$$= \mathbf{D}_2(\mathbf{x}_2) - \mathbf{D}_1(\mathbf{x}_2) \quad [14]$$
$$\leq (\mathbf{D}_2(\mathbf{x}_1) + \Delta\mathbf{D}_1) - (\mathbf{D}_1(\mathbf{x}_1) + \Delta\mathbf{D}_1) \quad [15]$$
$$= DD_R(\mathbf{x}_1). \quad [16]$$

The obtained relationship $DD_R(\mathbf{x}_2) \leq DD_R(\mathbf{x}_1)$ proves that distance deviation outside the outermost contour can not exceed distance deviation inside contours. This relationship is valid irrespective of the number of contours and holds also for the DD_S measure [4]. Similar relationship could be shown for the regions inside the innermost contour, such that it can be concluded that distance deviations in these regions are reflections of distance deviations in the region of contour disagreement $\Delta I(\mathbf{x})$.

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Zdravljenje drobnoceličnega pljučnega raka s peroralnim etopozidom – dileme in rešitve

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Izhodišča. Etopozid je protitumorna učinkovina, ki jo pogosto uporabljamo za zdravljenje različnih rakavih obolenj, vključno z drobnoceličnim pljučnim rakom. Ta je agresivni rak s slabo napovedjo poteka bolezni. Peroralno vnašanje etopozida ima veliko prednosti tako z vidika kakovosti življenja bolnika kakor tudi z vidika obvladovanja stroškov. Uporaba etopozida pa je omejena zaradi nepopolne absorpcije ter velike intra- in inter-individualne spremenljivosti biološke uporabnosti. To lahko pri nekaterih bolnikih privede do zmanjšane učinkovitosti ali povečane toksičnosti.

Zaključki. Članek s pregledom in analizo literaturnih podatkov predstavlja dileme in rešitve glede peroralnega zdravljenja z etopozidom. Rezultati številnih raziskav so pokazali, da na biološko uporabnost etopozida vplivajo genetski, fiziološki in okoljski dejavniki. Poznamo številne pristope za izboljšanje biološke uporabnosti in zmanjšanje farmakokinetične spremenljivosti peroralnega etopozida. To so želene in neželene interakcije (npr. s ketokonazolom), razvoj ustreznih dostavnih sistemov, uporaba predzdravila, ki je bolj vodotopno od etopozida in vpliv na hitrost praznjenja želodca. Etopozid je primerna zdravilna učinkovina za odmerjanje na osnovi poznavanja farmakokinetike in genotipa, kar omogoča tudi prilagajanje odmerka pri posameznemu bolniku. Ugotovili so, da se učinkovitost peroralnega in intravenskega zdravljenja z etopozidom pri bolnikih z drobnoceličnim pljučnim rakom značilno ne razlikuje, medtem ko so si rezultati primerjav toksičnosti nasprotujoči. Glavno sporočilo članka je, da boljša napovedljivost farmakokinetike peroralnega etopozida omogoča večjo uporabo le-tega v rutinski klinični praksi.

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Radiopačnost umetnih krvnih strdkov na osnovi jodnega kontrastnega sredstva za preizkušanje pripomočkov ob mehanski trombektomiji

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Izhodišča. Umetno pripravljene krvne strdke (UKS) uporabljamo za preizkušanje pripomočkov ob mehanski trombektomiji (MET). Znano je, da barijev sulfat, ki ga v tovrstnih krvnih strdkih uporabljamo kot radiopačno kontrastno sredstvo, negativno vpliva na mehanske lastnosti strdka. Z in vivo in in vitro raziskavo smo ugotavljali, ali povzroča jodno kontrastno sredstvo manj negativnih učinkov na UKS kot barijevo kontrastno sredstvo.

Materiali in metode. Iz sveže krvi prašiča smo v epruvetah pripravili UKS. Nastajanje strdka smo sprožili in pospešili z dodajanjem trombina in fibrinogena. Radiopačnost strdka z jodnim kontrastnim sredstvom smo izvedli na dva načina: z mešanjem krvi s 65 % Ioheksolom ali z 2- do 24-urnim namakanjem UKS v Ioheksolu. Tako pripravljene UKS smo primerjali z UKS, ki smo jih pripravili z barijevim kontrastnim sredstvom (kontrolna skupina). V in vitro raziskavi smo UKS preizkušali z naslednjimi štirimi testi: z ročnim natezanjem, brizganjem po katetru, ugotavljanjem radiopačnosti in ugotavljanjem izplavljanja kontrastnega sredstva. V in vivo raziskavi pa smo skupno karotidno arterijo pri dveh prašičih zaprli z UKS, pripravljenim z barijevim kontrastnim sredstvom ali z UKS, ki je bil 24 ur namočen v Ioheksolu. Obstojnost radiopačnosti smo primerjali pri obeh vrstah UKS.

Rezultati. Začetna radiopačnost je bila večja pri UKS, ki so bili pripravljeni z jodnim kontrastnim sredstvom, vendar je radiopačnost nato hitro slabela z namakanjem v fiziološki raztopini in še zlasti po vstavitvi (embolizaciji) v poskusni živi živali. Mehanske lastnosti z jodnim kontrastnim sredstvom pripravljenih UKS so bile slabše od mehanskih lastnosti UKS, ki so bili pripravljeni z barijevim kontrastnim sredstvom. Z jodnim kontrastnim sredstvom pripravljeni UKS so bili manj čvrsti in manj elastični, polovica teh strdkov je razpadla že med brizganjem po katetru. Natezna čvrstost in elastičnost z jodnim kontrastnim sredstvom pripravljenih UKS je bila manjša kot pri UKS, ki so bili pripravljeni z barijevim kontrastnim sredstvom.

Zaključki. Jodni kontrast kot radiopačno sredstvo v primerjavi z barijevim kontrastnim sredstvom ne izboljša lastnosti UKS.

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Prikaz poškodb prednje kolenske križne vezi - pomen sagitalno-poševne magnetnoresonančne projekcije

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Izhodišča. Prikaz popolne raztrganine prednje križne vezi kolena s standardnim magnetnoresonnančnim (MR) protokolom ni težavno. Delno raztrganino prednje križne vezi pa zanesljive prikažemo z uporabo dodatnih usmerjenih MR tehnik. Namen raziskave je bil primerjati MR prikaz poškodb prednje križne vezi s sagitalno-poševno tehniko in fleksijsko tehniko ter standardnim protokolom.

Bolniki in metode. V prospektivno raziskavo, ki je trajala 12 mesecev, smo vključili 149 bolnikov, napotenih na MR preiskavo zaradi poškodbe mehkih delov kolena. Analizirali smo MR znake poškodbe prednje križne vezi, posebej znake delne raztrganine, število MR tomografskih rezin, potrebnih za prikaz celotnega poteka prednje križne vezi, čas slikanja z dodatnimi protokoli in ponovljivost preiskav.

Rezultati. Pri popolnih raztrganinah prednje križne vezi je bil standardni MR protokol enako zanesljiv kot obe dodatni preiskovalni tehniki. Delne raztrganine pa smo bistveno zanesljiveje prikazali z uporabo fleksijske in sagitalno-poševne tehnike ($p < 0,001$) kot s standardnim protokolom. Raziskava ni pokazala statistično značilne razlike med prikazi poškodb pri dodatnih tehnikah ($p > 0,65$). Sagitalno-poševna tehnika je bila boljša od fleksijske tehnike pri prikazu celotne prednje križne vezi in je bila časovno krajša ($p < 0.001$).

Zaključki. Dodatni tehniki (fleksijska in sagitalno-poševna) sta enako zanesljivi kot standardni MR protokol za oceno popolne raztrganine prednje križne vezi kolena, Zato naj ju uporabljamo le v primerih suma na delno raztrganino prednje križne vezi. V naši raziskavi je bila sagitalno-poševna tehnika boljša. Ni bila odvisna od zmožnosti bolnika, da natančno ponovi položaj pri slikanju v zunanji rotaciji, kot ga zahteva standardni protokol, ali da ponovi skrčenje kolena pod istim kotom, kot ga zahteva fleksijska tehnika v primeru ponovnih preiskav. Sagitalno-poševna tehnika kot dodatna preiskava ne zahteva dodatnega pozicioniranja kolena. To je časovno ugodnejše in jo zato predlagamo kot dodatno tehniko k standardnemu protokolu za prikaz delnih raztrganin prednje križne vezi.

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Učinkovitost zaščite dojk pri rentgenskem slikanju ledvenih vretenc

Mekiš N, Žontar D, Škrk D

Izhodišča. Namen raziskave je bil ugotoviti, ali se v klasični radiografiji zmanjša sevalna doza na dojke pri ženskah, če pri slikanju ledvenih vretenc uporabimo zaščitno pregrinjalo iz svinčene gume ter ali obstaja povezanost med indeksom telesne mase in dozo na dojke.

Materiali in metode. Sevalno dozo na dojke smo merili s termoluminiscentnimi dozimetri (TLD). V prvi fazi smo meritve naredili na fantomu. Dozo na dojke smo izmerili pri slikanju ledvenih vretenc v dveh projekcijah brez zaščite in s svinčeno zaščito. Uporabili smo osebno varovalno opremo (OVO) z ekvivalentno debelino svinca 0,5 mm. V drugi fazi smo meritve izvedli na 100 bolnicah, ki so bile naključno razdeljene v dve enako veliki skupini. Pacientke iz prve skupine so bile slikane brez OVO, iz druge pa z OVO.

Rezultati. Povprečno sevalno dozo, ki jo je pri slikanju ledvenih vretenc v dveh projekcijah bolnica prejela na dojke, se je z uporabo OVO zmanjšala za približno 80% ($p < 0,001$) (na desni dojki iz 0,45±0,25 mGy na 0,09±0,07 mGy, na levi pa iz 0,26±0,14 mGy na 0,06±0,04 mGy). Dobljeni rezultati kažejo, da ni linearne povezanosti med indeksom telesne mase (ITM) in dozo na dojke.

Zaključki. Na podlagi rezultatov raziskave priporočamo ščitenje dojk pri slikanju ledvenih vretenc dojke. Kljub sicer nizki izpostavljenosti dojk pri slikanju brez zaščite dozo lahko še zmanjšamo, uporaba zaščite pa ne vpliva na kakovost rentgenograma.

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Velikost tumorja in učinkovitost elektrokemoterapije

Mali B, Miklavčič D, Campana LG, Čemažar M, Serša G, Snoj M, Jarm T

Izhodišča. Elektrokemoterapija (EKT) je učinkovita in varna metoda za lokalno zdravljenje tumorjev. Domnevamo, da se zaradi različnih intrinzičnih pogojev pri zdravljenju posamezni tumorji zelo različno odzivajo na EKT. Namen naše študije je bilo raziskati zvezo med velikostjo tumorja in učinkovitostjo EKT.

Materiali in metode. Naredili smo sistematični pregled različnih bibliografskih baz in našli devet študij, ki so ustrezale namenu naše študije. Analizo podatkov smo izvedli s splošnimi statističnimi metodami in z meta-analizo.

Rezultati. Rezultati analize, ki temeljijo na rezultatih pridobljenih na 1466 tumorjih različnih histologij zdravljenih z enkratno EKT, kažejo statistično značilno nižjo učinkovitost EKT na tumorjih s premerom večjim ali enakim 3 cm (delež popolnih odzivov = 33,3%; delež popolnih in delnih odzivov = 68,2%) v primerjavi z manjšimi tumorji (delež popolnih odzivov = 59,5%; delež popolnih in delnih odzivov = 85,7%). Rezultati meta-analizo kažejo, da se verjetnost za popolni odziv tumorja na EKT značilno poveča (v povprečju za 31,0%), kadar EKT izvedemo na tumorjih manjših od 3 cm v primerjavi z večjimi tumorji. Statistično značilen rezultat dobimo tudi za združene popolne in delne odzive, kjer se verjetnost za odziv v povprečju poveča za 24,9%. Analiza surovih podatkov o velikosti in odzivu tumorjev kaže, da učinkovitost EKT značilno upada z naraščajočim premerom tumorjev. Največji padec v deležu popolnih odzivov smo zaznali med tumorji manjšimi in večjimi od 2 cm v premeru.

Zaključki. Standardne postopke za EKT bi bilo potrebno ponovno preučiti in izpopolniti za zdravljenje predvsem večjih tumorjev. Predlagamo, da bi bilo smiselno v prihodnje raziskave vključiti individualizirano načrtovanje zdravljenja in/ali zdravljenje z več zaporednimi aplikacijami EKT ter določati odziv večjih tumorjev po daljšem času po EKT.

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p38 MAPK regulira izražanje kalijevih kanalčkov eter à go-go v celicah humanega osteosarkoma

Wu X , Zhong D , Lin B , Zhai W, Ding Z, Wu J

Izhodišča. Kanalček eter à go-go (Eag) je povečano izražen pri različnih rakih. Funkcija Eag pri osteosarkomu pa je še nepoznana in mehanizmi povečanega izražanja Eag v malignih celicah še niso jasni.

Materiali in metode. Izražanje Eag v humani osteosarkomski celični liniji MG-63 smo določili z RT-PCR in analizo Western blot. Učinek inhibicije Eag na MG-63 celicah smo določili s testom proliferacije celic. Utišanje Eag s shRNA smo preverili tudi in vivo na tumorskem modelu ksenografttov. Aktivacijo MAPK in p53 poti smo ugotavljali z analizo po Westernu.

Rezultati. V MG-63 celicah je bilo povečano izražanje Eag. Imipramin in utišanje Eag s shRNA proti Eag sta statistično značilno zavrla proliferacijo MG-63 celic in rast tumorjev in vivo. Njihovo proliferacijo smo zavrli tudi z inhibitorjem MAPK poti SB203580, kar se je izražalo v zmanjšani količini proteina Eag, vendar v povečani količini p53 proteina. Aktivacija p53 z nutlin-3 j posledično zavrla celično rast in zmanjšala količino proteina Eag, medtem ko je inaktivacija p53 s pifithrinom--alpha (PFT-a) povzročila pospešeno proliferacijo celic in povečano izražanje Eag.

Zaključki. Kanalčki Eag delujejo kot onkogeni, ki spodbujajo delitev osteosarkomskih celic. Izražanje Eag v osteosarkomskih celicah je verjetno regulirano s potjo p38 MAPK/p53.

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Hipofrakcionirana stereotaktična radioterapija pri arteriovenskih malformacijah, ki so velike ali zajemajo kritične organe

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Izhodišča. Zdravljenje velikih arteriovenskih malformacij (AVM) ali takšnih, ki zajemajo kritične organe, ostaja izziv. Neoperabilne spremembe lahko spremljamo ali pa jih zdravimo z večkratno radiokirurgijo in zmanjšujemo volumen oz. hipofrakcionirano stereotaktično obsevamo (HFSRT). Namen naše raziskave je bil oceniti varnost in učinkovitost HFSRT pri velilih ali v elokventnih področjih možgan ležečih AVM.

Bolniki in metode. Analizirali smo dokumentacijo 49 bolnikiv, ki so bili obsevani zaradi AVM. Bolniki so prejeli srednji skupni odmerek 19,9 Gy (12-28 Gy) v 2 do 4 odmerkih, med odmerki načrtovan presledek pa je bil vsaj en teden. Izračunali smo delež obliteracij in letna tveganja za krvavitev. Za izračun smo uporabili Kaplan-Meierjevo analizo preživetja in preživetne tabele.

Rezultati. Letno tveganje za krvavitev po enem in po dveh letih spremljanja je bilo 4,5% in 1,6%. Delež popolnih obliteracij po enem, dveh in treh letih spremljanja je bil 7%, 11% in 21%. Delež vseh obliteracij (popolnih in delnih) pa je bil 22%, 41% in 55%. Ugotovili smo težnjo naraščanja deleža obliteracij pri bolnikih obsevanih z odmerki nad 8 Gy, skupnemu odmerku nad 21 Gy in volumnu 8,18 cm3 ali manj. Te težnje nismo opazili pri delnih obliteracijah.

Zaključki. Delež obliteracij pri HFSRT je razmeroma majhen, ravno tako pa je majhno tudi tveganje za nastanek trajnih nevroloških poškodb, če sta skupni odmerek in odmerek na frakcijo prilagojena velikosti in lokaciji AVM. Napovedni dejavniki pri delnih in popolnih obliteracijah bi lahko bili različni, česar pa nismo popolnoma potrdili. Za potrditev te domneve so potrebne nadaljnje raziskave.

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Neoadjuvantno zdravljenje z epirubicinom in docetakselom pri hormonsko pozitivnem in HER-2 negativnem raku dojk. Rezultati dveh zaporednih kliničnih raziskav druge faze

Tuzi A, Lombardi D, Crivellari D, Militello L, Perin T, La Grassa M, Massarut S, Veronesi A

Izhodišča. Poročamo o neoadjuvantnem zdravljenju z epirubicinom in docetakselom pri bolnicah s hormonsko pozitivnim in HER-2 negativnim rakom dojk. Primerjali smo bolnice v dveh zaporednih kliničnih raziskavah, ki so imele velik operabilni ali lokalno napredovali rak dojke ter so prejemale neoadjuvantno zdravljenje s 4 ali 8 krogi kemoterapije.

Bolniki in metode. Bolnice v prvi klinični raziskavi smo od leta 2002 do 2006 intravensko zdravili vsake 3 tedne z epirubicinom 90 mg/m2 in docetakselom 75 mg/m2. Prejele so 4 kroge kemoterapije pred in 4 kroge po operaciji. V raziskavo smo vključili 13 bolnic. Bolnice v drugi klinični raziskavi smo zdravili od leta 2006 do 2010 z enako kemoterapijo, vendar so prejemale vseh 8 krogov pred operacijo, po operaciji pa 5 let ob hormonsko terapijo. Nekatere smo postoperativno obsevali. V drugo raziskavo smo vključili 37 bolnic. V prvi klinični raziskavi so vse bolnice prejele načrtovano preoperativno kemoterapijo, v drugi pa 32 od 37 bolnic.

Rezultati. V prvi raziskavi smo klinično ugotovili popoln odgovor pri eni bolnici, v drugi raziskavi pa pri 13. Delni odgovor smo ugotovili pri 10 bolnicah iz prve klinične raziskave in pri 21 iz druge. Klinični odgovor pa nismo opazili pri 2 bolnicah iz prve raziskave in 3 iz druge. Stopnja odgovora je bila v prvi raziskavi 84 % in drugi 92 %. Vseh 50 bolnic smo operirali. V prvi raziskavi smo pri 3 bolnicah dosegli patomorfološko popoln odgovor v dojki in pri dveh v pazdušnih bezgavkah, le pri 1 od 13 (7,6 %) ni bilo najti bolezni ne v dojki in ne v pazdušnih bezgavkah. V drugi raziskavi smo klinično ugotovili popoln odgovor v dojki pri 8 bolnicah in pri 15 bolnicah v pazdušnih bezgavkah, pri 4 od 37 (10,8 %) pa ni bilo najti bolezni ne v dojki in ne v pazdušnih bezgavkah.

Toksičnost 3. in 4. stopnje (G) smo videli kot supresijo kostnega mozga pri 3 bolnicah iz prve raziskave in pri vseh bolnicah iz druge raziskave, mukozitis pri eni bolnici iz prve in 4 bolnicah iz druge raziskave. Drugih toksičnosti G3 ali G4 ali smrti zaradi toksičnosti ni bilo. Pet letno preživetje brez napredovanja bolezni je bilo 38 % pri bolnicah iz prve raziskave in 90 % iz druge.

Zaključki. Odstotek patomorfoloških popolnih odgovorov na zdravljenje je bil pri obravnavanih bolnicah nižji, kot je opisan v literaturi. Daljše preoperativno zdravljenje bi lahko vplivalo na daljše preživetje brez napredovanja bolezni.

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Analiza nove kategorije N za napoved poteka bolezni pri raku požiralnika s pozitivnimi bezgavkami pri kitajskih bolnikih

Xu Y, Jiang Y, Yu X, Chen Q, Zhou X, Mao W

Izhodišča. Objavljen je bil nov sistem klasifikacije TNM (sedma izdaja) za rak požiralnika. Kategorijo N po novem delimo na N0, N1, N2 in N3. Namen raziskave je bila ocena napovednih zmožnosti nove klasifikacije N pri raku požiralnika s pozitivnimi bezgavkami pri kitajski populaciji in ocena, ali bi lahko nova klasifikacija N pomagala pri odločitvi za pooperativno dopolnilno zdravljenje.

Bolniki in metode. Retrospektivno smo analizirali bolnike z rakom požiralnika, ki so imeli narejeno ezofagotomijo med letoma 2002 in 2008. Bolnike s patološkim stadijem, določenim v skladu s šesto izdajo klasifikacije TNM Ameriškega odbora za rak in Mednarodnega združenja proti raku (AJCC/UICC), smo prevedli na sistem sedme izdaje. Za to analizo smo izbrali bolnike s patološkim stadijem T1-4N1-3M0. Za primerjavo celokupnega preživetja smo uporabili Kaplan-Meierjevo metodo in analizo regresije po Coxu.

Rezultati. Vključitvenim kriterijem je ustrezalo 545 bolnikov: 346 (63,5%) je imelo samo ezofagektomijo, 199 (36,5%) je imelo ezofagektomijo in adjuvantno obsevanje, 197 (36,1%) pa je poleg ezofagektomije prejelo še dopolnilno kemoterapijo. Univariatna in multivariatna analiza sta pokazali značilno razliko v celokupnem preživetju pri bolnikih različnih pooperativnih kategorij pN ($p<0,001$). Razlika je bila prisotna tudi pri bolnikih, ki so prejeli pooperativno obsevanje ($p<0,001$) in pooperativno kemoterapijo ($p<0,001$). Med bolniki, ki so prejeli pooperativno dopolnilno zdravljenje in tistimi, ki so bili samo operirani, pa v isti pN kategoriji ni bilo značilne razlike v celokupnem preživetju, razen mejno izboljšanega celokupnega preživetja v skupini bolnikov z boleznijo pN2 in pN3, ki smo jih pooperativno obsevali.

Zaključki. Naši rezultati so potrdili napovedno zmožnost novega sistema klasifikacije N. Kategorija N se je pokazala kot neodvisni napovedni dejavnik pri bolnikih z resektabilnim rakom požiralnika prsnega koša s pozitivnimi bezgavkami pri kitajskih bolnikih. Potrebne so nadaljnje raziskave za razjasnitev vloge novega sistema klasifikacije N pri odločitvi za pooperativno dopolnilno zdravljenje.

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Radioterapija invertnega papiloma. Prikaz primera in pregled literature

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Izhodišča. Sinonazalni invertni papilom (IP) je redek, običajno benigen tumor, ki izrašča iz respiratorne sluznice sinonazalnega trakta. Največkrat ga zdravimo s kirurško odstranitvijo. Kadar je IP histološko v celoti benigen (t.j. brez pridruženega malignoma), obsevanje uporabljamo le izjemoma.

Predstavljamo primer bolnika z makroskopskim ostankom benignega IP, ki smo ga operirali in nato obsevali. Pregledali smo literature o uporabi radioterapije pri tej redki vrsti tumorja.

Prikaz primera. Ostanek bolezni v predelu sfenoida in sosednjega kavernoznega sinusa smo pooperativno obsevali z dozo 54 Gy in dnevnimi odmerki 1,8 Gy. V obdobju 2,6 let po obsevanju nismo ugotovili ponovitev bolezni, prav tako ne poslabšanja občutka za vonj, sluha in vida.

Pregled literature. V literaturi smo našli 6 poročil, ki opisujejo skupaj 16 primerov ter navajajo tudi podrobnosti o radioterapiji in izidu zdravljenja. Lokalna kontrola je bila dosežena pri 12 izmed 14 primerov (vključen je tudi naš primer), ki so bili zdravljeni z makroskopsko ali delno odstranitvijo tumorja in pooperativno radioterapijo. Najnižja in srednja doza obsevanja je znašala 47,15 Gy oz. 56,5 Gy, čas spremljanja bolezni pa 0,5-20,5 let (srednji čas 7,8 let).

Zaključki. Radioterapija je varno in učinkovito zdravljenje pri histološko v celoti benignih IP. Indicirano je v primerih povečanega tveganja za ponovitev bolezni po kirurški resekciji in pri neoperabilnih tumorjih.

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Torakobiliarne fistule. Pregled literature in prikaz primera oskrbe fistule z veliko pečico

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Izhodišča. Torakobiliarne fistule so patološke komunikacije biliarnega vejevja z bronhialnim vejevjem ali plevralnim prostorom. V prvem primeru govorimo o bronhobiliarnih fistulah, v drugem pa o plevrobiliarnih fistulah.

Pregled literature. Predstavljamo etiologijo, patogenezo, predilekcijska mesta nastanka, klinično sliko ter diagnostične in terapevtske možnosti torakobiliarnih fistul.

Prikaz primera. Opišemo klinični primer bolnika z iatrogeno povzročeno bronhobiliarno fistulo, ki je nastala po radiofrekvenčni terapiji zasevka kolorektalnega raka v jetrih. Pri operaciji smo fistulo prešili z veliko pečico. Tak način zdravljenja do sedaj v literaturi še niso opisali.

Zaključki. V novejših objavah avtorji poročajo o nekirurškem zdravljenju fistul. Večina avtorjev pa še vedno zagovarja kirurško obravnavo. Prešitje fistule z veliko pečico je ena od možnih kirurških metod za zdravljenje torakobiliarnih fistul.

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Deviacija razdalj za merjenje variabilnosti obrisovanja pri brahiradioterapiji

Rogelj P, Hudej R, Petrič P

Izhodišča. Ko ugotavljamo variabilnost obrisovanja pri brahiradioterapiji, se soočamo s težavnostjo smiselnega primerjanja obrisov. Čeprav so predlagali veliko postopkov, ima vsak od njih omejitve, ki onemogočajo njegovo širšo uporabo.

Metode. Kot rešitev težav obstoječih mer za primerjavo obrisov predlagamo novo mero, mero deviacije razdalj (Distance Deviation Measure), ki omogoča smiselno primerjanje obrisov v najrazličnejših situacijah oziroma kliničnih raziskavah obrisovanja. Temelji na merjenju razdalj od poljubne točke na sliki do najbližjih točk na vseh primerjanih obrisih. Če tovrstno analizo opravimo na vseh točkah (vokslih) slike, je rezultat slika, iz katere je razvidno lokalno ujemanje obrisov, podano v enotah razdalje (milimetrih), hkrati pa je zagotovljena robustnost merjenja. Za predstavitev informacijsko bogatih rezultatov takšne analize predlagamo tri metode: slikovno predstavitev za detajlno topografsko analizo, kotno predstavitev za kompaktnejšo topografsko analizo in številčno predstavitev ujemanja obrisov, ki omogoča hitro oceno celotnega ujemanja obrisov.

Rezultati. Metodo smo prikazali na primeru analize obrisov, ki so jih naredili različni raziskovalci in kjer so prisotne kompleksne oblike obrisanih področij, z izrastki in luknjami. Rezultate analize smo predstavili z vsemi tremi predlaganimi metodami predstavitve.

Zaključki. Predlagan postopek omogoča zaznavo in ocenjevanje neskladij med obrisi ne glede na njihovo kompleksnost, pridobljeni rezultati pa govorijo o absolutnem neskladju med obrisi in jih lahko enostavno interpretiramo. Postopek je primeren za različne situacije, ne glede na to ali so prisotni referenčni obrisi, oziroma ne glede na število primerjanjih obrisov.

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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the second half of 2012

The Dr. J. Cholewa Foundation for Cancer Research and Education is a non-profit, non-government and non-political association of experts, institutions and organisations associated with cancer research, cancer education, cancer treatment and prevention. Its principal aim is to search for and support new ideas, initiatives and forward thinking in all of the activities associated with cancer.

The Foundation plans to broaden its sphere of activity with the establishment of a number of boards of commissioners that are to further advance the planning, execution and evaluation of research and publishing projects in the future. A new program for the Foundation's activities was discussed. Among other activities, the Foundation continues to support the publication of "Radiology and Oncology", an international scientific journal that is edited, published and printed in Ljubljana, Slovenia. "Radiology and Oncology" publishes scientific articles, reviews, case reports, short reports and letters to the editor about research and studies in experimental and clinical oncology, supportive therapy, experimental and clinical research in radiology, radiophyics, and prevention and early diagnostics of different types of cancer. "Radiology and Oncology" is an open access journal available in pdf format and with a Science Citation Index impact factor. It is indexed and abstracted also by PubMed and PubMed Central. Throughout the year 2012 the Foundation continued to support a number of lay associations and organizations that are involved in education and spread of information to professionals and lay people about cancer in Slovenia.

The Dr. J. Cholewa Foundation for Cancer Research and Education continues to assist and oncological, radiological and other professional associations in Slovenia to organise scientific and other meetings of specific interest in different fields of cancer research and education. The Foundation is also firmly committed to support all non-professional organisations that are involved in cancer education of groups interested in problems associated with oncology and population in general in Slovenia.

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Lajšanje bolečine in oteklin pri vnetju v ustni votlini in žrelu, ki nastanejo zaradi okužb in stanj po operaciji in kot posledica radioterapije (t.i. radiomukozitis).

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Imetnik dovoljenja za promet
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Tantum Verde 1,5 mg/ml oralno pršilo, raztopina

Kakovostna in količinska sestava
1 ml raztopine vsebuje 1,5 mg benzidaminijevega klorida, kar ustreza 1,34 mg benzidamina. V enem razpršku je 0,17 ml raztopine. En razpršek vsebuje 0,255 mg benzidaminijevega klorida, kar ustreza 0,2278 mg benzidamina. En razpršek vsebuje 13,6 mg 96 odstotnega etanola, kar ustreza 12,728 mg 100 odstotnega etanola, in 0,17 mg metilparahidroksibenzoata (E218).

Terapevtske indikacije
Samozdravljenje: lajšanje bolečine in oteklin pri vnetju v ustni votlini in žrelu, ki so lahko posledica okužb in stanj po operaciji. Po nasvetu in navodilu zdravnika: lajšanje bolečine in oteklin v ustni votlini in žrelu, ki so posledica radiomukozitisa.

Odmerjanje in način uporabe
Uporaba 2- do 6-krat na dan (vsake 1,5 do 3 ure). Odrasli: 4 do 8 razprškov 2- do 6-krat na dan. Otroci od 6 do 12 let: 4 razprški 2- do 6-krat na dan. Otroci, mlajši od 6 let: 1 razpršek na 4 kg telesne mase; do največ 4 razprške 2 do 6-krat na dan.

Kontraindikacije
Znana preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov.

Posebna opozorila in previdnostni ukrepi
Pri manjšini bolnikov lahko resne bolezni povzročijo ustne/žrelne ulceracije. Če se simptomi v treh dneh ne izboljšajo, se mora bolnik posvetovati z zdravnikom ali zobozdravnikom, kot je primerno. Zdravilo vsebuje aspartam (E951) (vir fenilalanina), ki je lahko škodljiv za bolnike s fenilketonurijo. Zdravilo vsebuje izomalt (E953) (sinonim: izomaltitol (E953)). Bolniki z redko dedno intoleranco za fruktozo ne smejo jemati tega zdravila. Uporaba benzidamina ni priporočljiva za bolnike s preobčutljivostjo za salicilno kislino ali druga nesteroidna protivnetna zdravila. Pri bolnikih, ki imajo ali so imeli bronhialno astmo, lahko pride do bronhospazma. Pri takih bolnikih je potrebna previdnost.

Medsebojno delovanje z drugimi zdravili in druge oblike interakcij
Pri ljudeh raziskav o interakcijah niso opravljali.

Nosečnost in dojenje
Tantum Verde z okusom mentola 3 mg pastile se med nosečnostjo in dojenjem ne smejo uporabljati.

Vpliv na sposobnost vožnje in upravljanja s stroji
Uporaba benzidamina lokalno v priporočenem odmerku ne vpliva na sposobnost vožnje in upravljanja s stroji.

Neželeni učinki
Bolezni prebavil Redki: pekoč občutek v ustih, suha usta.
Bolezni imunskega sistema Redki: preobčutljivostna reakcija.
Bolezni dihal, prsnega koša in mediastinalnega prostora Zelo redki: laringospazem.
Bolezni kože in podkožja Občasni: fotosenzitivnost. Zelo redki: angioedem.

Rok uporabnosti
4 leta. Zdravila ne smete uporabljati po datumu izteka roka uporabnosti, ki je naveden na ovojnini. Posebna navodila za shranjevanje Za shranjevanje pastil niso potrebna posebna navodila. Plastenko z raztopino shranjujte v zunanji ovojnini za zagotovitev zaščite pred svetlobo. Shranjujte pri temperaturi do 25°C. Shranjujte v originalni ovojnini in nedosegljivo otrokom.

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Ime zdravila ALIMTA 100 mg prašek za koncentrat za raztopino za infundiranje in ALIMTA 500 mg prašek za koncentrat za raztopino za infundiranje **Kakovostna in količinska sestava** ALIMTA 100 mg: vsaka viala vsebuje 100 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Po pripravi vsebuje vsaka viala 25 mg/ml pemetrekseda. Pomožne snovi: Vsaka viala vsebuje približno 11 mg natrija, manitol, klorovodikova kislina, natrijev hidroksid. ALIMTA 500 mg: vsaka viala vsebuje 500 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Po pripravi vsebuje vsaka viala 25 mg/ml pemetrekseda. Pomožne snovi: Vsaka viala vsebuje približno 54 mg natrija, manitol, klorovodikova kislina, natrijev hidroksid. **Terapevtske indikacije:** ALIMTA je v kombinaciji s cisplatinom indicirana za zdravljenje bolnikov z neresektabilnim malignim plevralnim mezoteliomom, ki jih še nismo zdravili s kemoterapijo. ALIMTA je v kombinaciji s cisplatinom indicirana kot zdravljenje prvega izbora za bolnike z lokalno napredovalim ali metastatskim nedrobnoceličnim karcinomom pljuč, ki nima pretežno ploščatocelične histologije. ALIMTA je indicirana kot monoterapija za zdravljenje lokalno napredovalega ali metastatskega nedrobnoceličnega pljučnega karcinoma, ki nima pretežno ploščatocelične histologije pri bolnikih, pri katerih bolezen ni napredovala neposredno po kemoterapiji na osnovi platine. ALIMTA je indicirana kot monoterapija za zdravljenje drugega izbora bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom, ki nima pretežno ploščatocelične histologije. **Odmerjanje in način uporabe:** *Odmerjanje:* ALIMTO smemo dajati le pod nadzorom zdravnika, usposobljenega za uporabo kemoterapije za zdravljenje raka. ALIMTA v kombinaciji s cisplatinom Priporočeni odmerek ALIMTE je 500 mg/m2 telesne površine (TP), dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21-dnevnega ciklusa. Priporočeni odmerek cisplatina je 75 mg/m2 TP, infundiran v dveh urah približno 30 minut po zaključku infuzije pemetrekseda prvi dan vsakega 21 dnevnega ciklusa. Bolniki morajo prejeti zadostno antiemetično zdravljenje, pred in/ali po prejemanju cisplatina jih moramo tudi ustrezno hidrirati. *ALIMTA kot samostojno zdravilo* Priporočeni odmerek ALIMTE je 500 mg/m2 TP, dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21 dnevnega ciklusa. *Režim premedikacije* Da zmanjšamo incidenco in resnost kožnih reakcij, dajemo kortikosteroid dan pred dajanjem pemetrekseda, na dan dajanja pemetrekseda in naslednji dan. Kortikosteroid naj ustreza 4 mg deksametazona, danega peroralno dvakrat dnevno. Za zmanjšanje toksičnosti morajo bolniki dnevno jemati tudi peroralno folno kislino ali multivitaminski pripravek, ki jo vsebuje (350 do 1000 mikrogramov). V sedmih dneh pred prvim odmerkom pemetrekseda morajo vzeti vsaj pet odmerkov folne kisline, odmerjanje pa morajo nadaljevati ves čas zdravljenja in še 21 dni po zadnjem odmerku pemetrekseda. Bolniki morajo prejeti tudi intramuskularno injekcijo vitamina B12 (1000 mikrogramov) v tednu pred prvim odmerkom pemetrekseda in enkrat vsake tri cikluse zatem. Kasnejše injekcije vitamina B12 lahko dajemo isti dan kot pemetrekseda. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Dojenje. Sočasno cepljenje proti rumeni mrzlici. **Posebna opozorila in previdnostni ukrepi:** Pemetrekseda lahko zavre delovanje kostnega mozga, kar se kaže kot nevtropenija, trombocitopenija in anemija (ali pancitopenija). Mielosupresija običajno predstavlja toksičnost za omejitev odmerka. Pri bolnikih, ki pred zdravljenjem niso prejemali kortikosteroidov, so poročali o kožnih reakcijah. Uporabe pemetrekseda pri bolnikih z očistkom kreatinina < 45 ml/min ne priporočamo. Bolniki z blagim do zmernim popuščanjem delovanja ledvic naj se izogibajo jemanju nesteroidnih protivnetnih zdravil (NSAID), denimo, ibuprofena in acetilsalicilne kisline 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Vsi bolniki, ki jih lahko zdravimo s pemetreksedom, naj se izogibajo jemanju NSAID-ov z dolgimi razpolovnimi časi izločanja vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda. Poročali so o resnih ledvičnih primerih, vključno z akutno ledvično odpovedjo, s pemetreksedom samim ali v povezavi z drugimi kemoterapevtiki. Pri bolnikih s klinično pomembno tekočino tretjega prostora moramo razmisliti o drenaži izliva pred dajanjem pemetrekseda. Kot posledico toksičnosti pemetrekseda v kombinaciji s cisplatinom za prebavila so opažali hudo dehidracijo, zato moramo bolnike pred prejemanjem terapije in/ali po njej ustrezno hidrirati, prejeti morajo zadostno antiemetično zdravljenje. Občasno so v kliničnih študijah pemetrekseda, običajno ob sočasnem dajanju z drugo citotoksično učinkovino, poročali o resnih srčnožilnih dogodkih, vključno z miokardnim infarktom in možganskožilnimi dogodki. Odsvetujemo uporabo živih oslabljenih cepiv. Spolno zrelim moškim odsvetujemo zaploditev otroka v času zdravljenja in še 6 mesecev zatem. Priporočamo ukrepe proti zanositvi ali vzdržnost. Zaradi možnosti, da zdravljenje s pemetreksedom povzroči trajno neplodnost, naj se moški pred začetkom zdravljenja posvetujejo o shranjevanju semena. Ženske v rodni dobi morajo v času zdravljenja s pemetreksedom uporabljati učinkovito kontracepcijo. Poročali so o primerih radiacijske pljučnice pri bolnikih, ki so jih zdravili z radiacijo pred, med ali po zdravljenju s pemetreksedom. Poročali so o radiacijskem izpuščaju pri bolnikih, ki so se zdravili z radioterapijo pred tedni ali leti. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Sočasno dajanje nefrotoksičnih zdravil (denimo, aminoglikozidov, diuretikov zanke, spojin platine, ciklosporina) lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Sočasno dajanje snovi, ki se tudi izločajo s tubulno sekrecijo (denimo, probenecid, penicilin), lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Pri bolnikih z normalnim delovanjem ledvic lahko visoki odmerki nesteroidnih protivnetnih zdravil (NSAID-i, denimo, ibuprofen) in acetilsalicilna kislina v visokih odmerkih zmanjšajo eliminacijo pemetrekseda in tako lahko povečajo pojavnost neželenih učinkov pemetrekseda. Pri bolnikih z blagim do zmernim popuščanjem delovanja ledvic se moramo izogibati sočasnemu dajanju pemetrekseda z NSAID-i (denimo, ibuprofenom) ali acetilsalicilne kisline v visokih odmerkih 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Sočasnemu dajanju NSAID-ov z daljšimi razpolovnimi časi s pemetreksedom se moramo izogibati vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda.Velika različnost med posamezniki v koagulacijskem statusu v času bolezni ter možnost medsebojnega delovanja med peroralnimi antikoagulacijskimi učinkovinami ter kemoterapijo proti raku zahtevata povečano pogostnost spremljanja INR. **Kontraindicirana sočasna uporaba:** Cepivo proti rumeni mrzlici: tveganje za smrtno generalizirano bolezen po cepljenju. **Odsvetovana sočasna uporaba:** Živa oslabljena cepiva (razen proti rumeni mrzlici): tveganje za sistemsko, potencialno smrtno bolezen. **Neželeni učinki** Klinične študije malignega plevralnega mezotelioma *Zelo pogosto:* znižani nevtrofilci/granulociti, znižani levkociti, znižan hemoglobin, znižani trombociti, nevropatija-senzorna, diareja, bruhanje, stomatitis/faringitis, slabost, anoreksija, zaprtje, izpuščaj, alopecija, povišan kreatinin, znižan očistek kreatinina, utrujenost. *Pogosti:* dehidracija, motnje okusa, konjuktivitis, dispepsija. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija, zdravljenje 2. izbora: *Zelo pogosti:* znižan nevtrofilci/granulociti, znižani levkocitit, znižan hemoglobin, diareja, bruhanje, stomatitis/faringitis, slabost, anoreksija, izpuščaj/luščenje, utrujenost. *Pogosti:* znižani trombociti, zaprtje, povišanje SGPT (ALT), povišanje SGOT (AST), srbenje, alopecija, povišana telesna temperatura. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA v kombinaciji s cisplatinom, zdravljenje 1. izbora: *Zelo pogosti:* znižan hemoglobin, znižani nevtrofilci/granulociti, znižani levkocitit, znižani trombociti, slabost, bruhanje, anoreksija, zaprtje, stomatitis/faringitis, diareja brez kolostomije, alopecija, izpuščaj/luščenje, povišan kreatinin, utrujenost. *Pogosti:* nevropatija-senzorična, motnje okusa, dispepsija/zgaga. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija, vzdrževalno in nadaljevalno zdravljenje: *Zelo pogosti:* znižan hemoglobin, slabost, anoreksija, utrujenost. *Pogosti:* znižani levkociti, znižani nevtrofilci, nevropatija-senzorična, bruhanje, mukozitis/stomatitis, povišanje ALT (SGPT), povišanje AST (SGOT), izpuščaj/luščenje, bolečina. Občasno so v kliničnih študijah pemetrekseda poročali o primerih resnih srčnožilnih in možganskožilnih dogodkov, vključno z miokardnim infarktom, angino pektoris, cerebrovaskularnim insultom in prehodnimi ishemičnimi atakami; primerih kolitisa ter o primerih intersticijske pljučnice z respiratorno insuficience, primerih edema, o ezofagitisu/radiacijskem ezofagitisu in o primerih sepse. Redkeje pa o primerih potencialno resnega hepatitisa in pancitopenije. Po uvedbi zdravila na trg so poročali o primerih akutne odpovedi ledvic s pemetreksedom samim ali v povezavi z drugimi kemoterapevtiki, primerih radiacijske pljučnice pri bolnikih, ki so jih zdravili z radiacijo pred, med ali po njihovem zdravljenju s pemetreksedom, primerih radiacijskega izpuščaja pri bolnikih, ki so se v preteklosti zdravili z radioterapijo, o primerih periferne ishemije, ki je včasih vodila v nekrozo okončin, redkih primerih buloznih stanj, kot sta Stevens-Johnsonov sindrom in toksična epidermalna nekroliza, ki so bila v nekaterih primerih usodna in o redkih primerih hemolitične anemije. **Imetnik dovoljenja za promet** Eli Lilly Nederland B.V., Grootslag 1 5, NL 3991 RA, Houten, Nizozemska. Datum zadnje revizije besedila 24.10.2011. **Način izdaje zdravila:** H. SAMO ZA STROKOVNO JAVNOST.
Podrobnejše informacije o zdravilu Alimta, so dostopne na spletni strani Evropske agencije za zdravila EMA http://www.ema.europa.eu in na lokalnem predstavništvu.

SIALM00025

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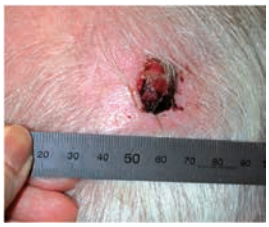
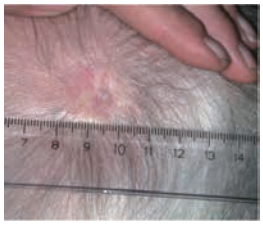
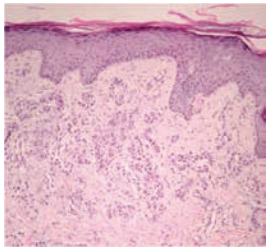


10 weeks after electrochemotherapy

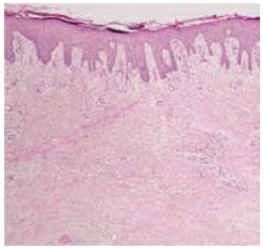


Gehl J, EJC Supplements, Volume 4, N° 11:35-37, 2006

Before electrochemotherapy



60 days after electrochemotherapy



Quaglino P, Annals Of Surgical Oncology. 15 (8):2215-2222. 2008

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Erbitux 5 mg/ml raztopina za infundiranje
Skrajšan povzetek glavnih značilnosti zdravila

Sestava: En ml raztopine za infundiranje vsebuje 5 mg cetuximaba in pomožne snovi. Cetuksimab je himerno monoklonsko IgG_1 protitelo. **Terapevtske indikacije:** Zdravilo Erbitux je indicirano za zdravljenje bolnikov z metastatskim kolorektalnim rakom z ekspresijo receptorjev EGFR in nemutiranim tipom KRAS v kombinaciji s kemoterapijo na osnovi irinotekana, kot primarno zdravljenje v kombinaciji s FOLFOX in kot samostojno zdravilo pri bolnikih, pri katerih zdravljenje z oksaliplatinom in irinotekanom ni bilo uspešno ter pri bolnikih, ki ne prenašajo irinotekana. Zdravilo Erbitux je indicirano za zdravljenje bolnikov z rakom skvamoznih celic glave in vratu v kombinaciji z radioterapijo za lokalno napredovalo bolezen in v kombinaciji s kemoterapijo na osnovi platine za ponavljajočo se in/ali metastatsko bolezen. **Odmerjanje in način uporabe:** Zdravilo Erbitux pri vseh indikacijah infundirajte enkrat na teden. Pred prvo infuzijo mora bolnik prejeti premedikacijo z antihistaminikom in kortikosteroidom. Začetni odmerek je 400 mg cetuksimaba na m^2 telesne površine. Vsi naslednji tedenski odmerki so vsak po 250 mg/m^2. **Kontraindikacije:** Zdravilo Erbitux je kontraindicirano pri bolnikih z znano hudo preobčutljivostno reakcijo (3. ali 4. stopnje) na cetuksimab. Kombinacija zdravila Erbitux s kemoterapijo, ki vsebuje oksaliplatin, je kontraindicirana pri bolnikih z metastatskim kolorektalnim rakom z mutiranim tipom KRAS ali kadar status KRAS ni znan. **Posebna opozorila in previdnostni ukrepi:** Če pri bolniku nastopi blaga ali zmerna reakcija, povezana z infundiranjem, lahko zmanjšate hitrost infundiranja. Priporočljivo je, da ostane hitrost infundiranja na nižji vrednosti tudi pri vseh naslednjih infuzijah. Če se pri bolniku pojavi huda kožna reakcija (≥ 3. stopnje po kriterijih US NCI-CTC), morate prekiniti terapijo s cetuksimabom. Z zdravljenjem smete nadaljevati le, če se je reakcija izboljšala do 2. stopnje. Zaradi možnosti pojava znižanja nivoja magnezija v serumu se pred in periodično med zdravljenjem priporoča določanje koncentracije elektrolitov. Če se pojavi sum na nevtropenijo, je potrebno bolnika skrbno nadzorovati. Potrebno je upoštevati kardiovaskularno stanje bolnika in sočasno dajanje kardiotoksičnih učinkovin kot so fluoropirimidini. Cetuksimab je treba uporabljati previdno pri bolnikih z anamnezo keratitisa, ulcerativnega keratitisa ali zelo suhih oči. **Interakcije:** Farmakokinetične značilnosti cetuksimaba ostanejo nespremenjene po sočasni uporabi enkratnega odmerka irinotekana, tudi farmakokinetika irinotekana je nespremenjena pri sočasni uporabi cetuksimaba. Pri kombinaciji s fluoropirimidini se je povečala pogostnost srčne ishemije, vključno z miokardnim infarktom in kongestivno srčno odpovedjo ter pogostnost sindroma dlani in stopal. V kombinaciji s kemoterapijo na osnovi platine se lahko poveča pogostnost hude levkopenije ali hude nevtropenije. **Neželeni učinki:** Zelo pogosti (≥ 1/10): hipomagneziemija, povečanje ravni jetrnih encimov, kožne reakcije, blage ali zmerne reakcije povezane z infundiranjem, blag do zmeren mukozitis. Pogosti (≥ 1/100, < 1/10): dehidracija, hipokalciemija, anoreksija, glavobol, konjunktivitis, driska, navzeja, bruhanje, hude reakcije povezane z infundiranjem, utrujenost. **Posebna navodila za shranjevanje:** Shranjujte v hladilniku (2 °C - 8 °C). **Pakiranje:** 1 viala z 20 ml ali 100 ml raztopine. **Način in režim izdaje:** H. **Imetnik dovoljenja za promet:** Merck KGaA, 64271 Darmstadt, Nemčija. **Datum zadnje revizije besedila:** Avgust 2012. Pred predpisovanjem zdravila natančno preberite celoten Povzetek glavnih značilnosti zdravila. **Podrobnejše informacije so na voljo pri predstavniku imetnika dovoljenja za promet z zdravilom:** Merck d.o.o., Ameriška ulica 8, 1000 Ljubljana, tel.: 01 560 3810, faks: 01 560 3830, el. pošta: info@merck.si

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Samo za strokovno javnost.

Ime zdravila: Tarceva 25 mg/100 mg/150 mg filmsko obložene tablete

Kakovostna in količinska sestava: Ena filmsko obložena tableta vsebuje 25 mg, 100 mg ali 150 mg erlotiniba (v obliki erlotinibijevega klorida).

Terapevtske indikacije: Nedrobnocelični rak pljuč: Zdravilo Tarceva je indicirano za prvo linijo zdravljenja bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč z EGFR-aktivirajočimi mutacijami. Zdravilo Tarceva je indicirano tudi za samostojno vzdrževalno zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč s stabilno boleznijo po 4 ciklih standardne kemoterapije na osnovi platine v prvi liniji zdravljenja. Zdravilo Tarceva je indicirano tudi za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč po neuspehu vsaj ene predhodne kemoterapije. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja ali drugih klinično pomembnih učinkov zdravljenja niso dokazali pri bolnikih z EGFR-negativnimi tumorji (glede na rezultat imunohistokemije). Rak trebušne slinavke: Zdravilo Tarceva je v kombinaciji z gemcitabinom indicirano za zdravljenje bolnikov z metastatskim rakom trebušne slinavke. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja niso dokazali za bolnike z lokalno napredovalo boleznijo.

Odmerjanje in način uporabe: Zdravljenje z zdravilom Tarceva mora nadzorovati zdravnik z izkušnjami pri zdravljenju raka. Pri bolnikih z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč, ki še niso prejeli kemoterapije, je treba testiranje za določanje mutacij EGFR opraviti pred začetkom zdravljenja z zdravilom Tarceva. Zdravilo Tarceva vzamemo najmanj eno uro pred zaužitjem hrane ali dve uri po tem. Kadar je potrebno odmerek prilagoditi, ga je treba zmanjševati v korakih po 50 mg. Pri sočasnem jemanju substratov in modulatorjev CYP3A4 bo morda potrebna prilagoditev odmerka. Pri dajanju zdravila Tarceva bolnikom z jetrno okvaro je potrebna previdnost. Če se pojavijo hudi neželeni učinki, pride v poštev zmanjšanje odmerka ali prekinitev zdravljenja z zdravilom Tarceva. Uporaba zdravila Tarceva pri bolnikih s hudo jetrno ali ledvično okvaro ter pri otrocih ni priporočljiva. Bolnikom kadilcem je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih manjše kot pri nekadilcih. Nedrobnocelični rak pljuč: Priporočeni dnevni odmerek zdravila Tarceva je 150 mg. Rak trebušne slinavke: Priporočeni dnevni odmerek zdravila Tarceva je 100 mg, v kombinaciji z gemcitabinom. Pri bolnikih, pri katerih se kožni izpuščaj v prvih 4 do 8 tednih zdravljenja ne pojavi, je treba ponovno pretehtati nadaljnje zdravljenje z zdravilom Tarceva.

Kontraindikacije: Preobčutljivost na erlotinib ali katero koli pomožno snov.

Posebna opozorila in previdnostni ukrepi: Pri določanju bolnikovega statusa mutacij EGFR je pomembno izbrati dobro validirano in robustno metodologijo, da se izognemo lažno negativnim ali lažno pozitivnim rezultatom. *Kadilci:* Bolnikom, ki kadijo, je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih zmanjšane v primerjavi s plazemskimi koncentracijami pri nekadilcih. Verjetno je, da je velikost zmanjšanja klinično pomembna. *Intersticijska bolezen pljuč:* Pri bolnikih, pri katerih se akutno pojavijo novi in/ali poslabšajo nepojasnjeni pljučni simptomi, kot so dispneja, kašelj in vročina, je treba zdravljenje z zdravilom Tarceva prekiniti, dokler ni znana diagnoza. Bolnike, ki se sočasno zdravijo z erlotinibom in gemcitabinom, je treba skrbno spremljati zaradi možnosti pojava toksičnosti, podobni intersticijski bolezni pljuč. Če je ugotovljena intersticijska bolezen pljuč, zdravilo Tarceva ukinemo in uvedemo ustrezno zdravljenje. *Driska, dehidracija, neravnovesje elektrolitov in ledvična odpoved:* Pri približno polovici bolnikov, ki so se zdravili z zdravilom Tarceva, se je pojavila driska (vključno z zelo redkimi primeri, ki so se končali s smrtnim izidom). Zmerno do hudo drisko zdravimo z loperamidom. V nekaterih primerih bo morda potrebno zmanjšanje odmerka. V primeru hude ali dolgotrajne driske, navzeje, anoreksije ali bruhanja, povezanih z dehidracijo, je treba zdravljenje z zdravilom Tarceva prekiniti in dehidracijo ustrezno zdraviti. O hipokaliemiji in ledvični odpovedi so poročali redko. Posebno pri bolnikih z dejavniki tveganja (sočasno jemanje drugih zdravil, simptomi, bolezni ali drugi dejavniki, vključno z visoko starostjo) moramo, če je driska huda ali dolgotrajna oziroma vodi v dehidracijo, zdravljenje z zdravilom Tarceva prekiniti in bolnikom zagotoviti intenzivno intravensko rehidracijo. Dodatno je treba pri bolnikih s prisotnim tveganjem za razvoj dehidracije spremljati ledvično delovanje in serumske elektrolite, vključno s kalijem. *Hepatisis, jetrna odpoved*: Pri uporabi zdravila Tarceva so poročali o redkih primerih jetrne odpovedi (vključno s smrtnimi). K njenemu nastanku je lahko pripomogla predhodno obstoječa jetrna bolezen ali sočasno jemanje hepatotoksičnih zdravil. Pri teh bolnikih je treba zato premisliti o rednem spremljanju jetrnega delovanja. Dajanje zdravila Tarceva je treba prekiniti, če so spremembe jetrnega delovanja hude. *Perforacije v prebavilih*: Bolniki, ki prejemajo zdravilo Tarceva, imajo večje tveganje za razvoj perforacij v prebavilih, ki so jih opazili občasno (vključno z nekaterimi primeri, ki so se končali s smrtnim izidom). Pri bolnikih, ki sočasno prejemajo zdravila, ki zavirajo angiogenezo, kortikosteroide, nesteroidna protivnetna zdravila (NSAID) in/ali kemoterapijo na osnovi taksanov, ali so v preteklosti imeli peptični ulkus ali divertikularno bolezen, je tveganje večje. Če pride do tega, je treba zdravljenje z zdravilom Tarceva dokončno ukiniti. *Kožne bolezni pri katerih so prisotni mehurji in luščenje kože:* Poročali so o primerih kožnih bolezni z mehurji in luščenjem kože, vključno z zelo redkimi primeri, ki so nakazovali na Stevens-Johnsonov sindrom/toksično epidermalno nekrolizo in so bili v nekaterih primerih smrtni. Zdravljenje z zdravilom Tarceva je treba prekiniti ali ukiniti, če se pri bolniku pojavijo hude oblike mehurjev ali luščenja kože. *Očesne bolezni:* Bolniki, pri katerih se pojavijo znaki in simptomi, ki nakazujejo na keratitis in so lahko akutni ali se poslabšujejo: vnetje očesa, solzenje, občutljivost na svetlobo, zamegljen vid, bolečine v očesu in/ali rdeče oči, se morajo takoj obrniti na specialista oftalmologije. V primeru, da je diagnoza ulcerativnega keratitisa potrjena, je treba zdravljenje z zdravilom Tarceva prekiniti ali ukiniti. V primeru, da se postavi diagnoza keratitisa, je treba skrbno razmisliti o koristih in tveganjih nadaljnega zdravljenja. Zdravilo Tarceva je pri bolnikih, ki so v preteklosti imeli keratitis, ulcerativni keratitis ali zelo suhe oči, uporabljati previdno. Uporaba kontaktnih leč je prav tako dejavnik tveganja za keratitits in ulceracijo. Med uporabo zdravila Tarceva so zelo redko poročali o primerih perforacije ali ulceracije roženice. *Medsebojno delovanje z drugimi zdravili*: Močni induktorji CYP3A4 lahko zmanjšajo učinkovitost erlotiniba, močni zaviralci CYP3A4 pa lahko povečajo toksičnost. Sočasnemu zdravljenju s temi zdravili se je treba izogibati. Tablete vsebujejo laktozo in jih ne smemo dajati bolnikom z redkimi dednimi stanji: intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze/galaktoze.

Medsebojno delovanje z drugimi zdravili in druge oblike interakcij: Erlotinib se pri ljudeh presnavlja v jetrih z jetrnimi citokromi, primarno s CYP3A4 in v manjši meri s CYP1A2. Presnova erlotiniba zunaj jeter poteka s CYP3A4 v črevesju, CYP1A1 v pljučih in CYP1B1 v tumorskih tkivih. Z zdravilnimi učinkovinami, ki se presnavljajo s temi encimi, jih zavirajo ali pa so njihovi induktorji, lahko pride do interakcij. Erlotinib je srednje močan zaviralec CYP3A4 in CYP2C8, kot tudi močan zaviralec glukuronidacije z UGT1A1 *in vitro*. Pri kombinaciji ciprofloksacina ali močnega *zaviralca CYP1A2* (npr. fluvoksamina) z erlotinibom je potrebna previdnost. V primeru pojava neželenih učinkov, povezanih z erlotinibom, lahko odmerek erlotiniba zmanjšamo. Predhodno ali sočasno zdravljenje z zdravilom Tarceva ni spremenilo očistka prototipov *substratov CYP3A4*, midazolama in eritromicina. Inhibicija glukoronidacije lahko povzroči interakcije z zdravili, ki so *substrati UGT1A1* in se izločajo samo po tej poti. Močni *zaviralci aktivnosti CYP3A4* zmanjšajo presnovo erlotiniba in zvečajo koncentracije erlotiniba v plazmi. Pri sočasnem jemanju erlotiniba in močnih zaviralcev CYP3A4 je zato potrebna previdnost. Če je treba, odmerek erlotiniba zmanjšamo, še posebno pri pojavu toksičnosti. Močni *spodbujevalci aktivnosti CYP3A4* zvečajo presnovo erlotiniba in pomembno zmanjšajo plazemske koncentracije erlotiniba. Sočasnemu dajanju zdravila Tarceva in induktorjev CYP3A4 se je treba izogibati. Pri bolnikih, ki potrebujejo sočasno zdravljenje z zdravilom Tarceva in močnim induktorjem CYP3A4, je treba premisliti o povečanju odmerka do 300 mg ob skrbnem spremljanju njihove varnosti. Zmanjšana izpostavljenost se lahko pojavi tudi z drugimi induktorji, kot so fenitoin, karbamazepin, barbiturati ali šentjanževka. Če te zdravilne učinkovine kombiniramo z erlotinibom, je potrebna previdnost. Kadar je mogoče, je treba razmisliti o drugih načinih zdravljenja, ki ne vključujejo močnega spodbujanja aktivnosti CYP3A4. Bolnikom, ki jemljejo *kumarinske antikoagulante*, je treba redno kontrolirati protrombinski čas ali INR. Sočasno zdravljenje z zdravilom Tarceva in *statinom* lahko poveča tveganje za miopatijo, povzročeno s statini, vključno z rabdomiolizo; to so opazili redko. Sočasna uporaba *zaviralcev P-glikoproteina*, kot sta ciklosporin in verapamil, lahko vodi v spremenjeno porazdelitev in/ali spremenjeno izločanje erlotiniba. Za erlotinib je značilno zmanjšanje topnosti pri pH nad 5. *Zdravila, ki spremenijo pH v zgornjem delu prebavil*, lahko spremenijo topnost erlotiniba in posledično njegovo biološko uporabnost. Učinka antacidov na absorpcijo erlotiniba niso proučevali, vendar je ta lahko zmanjšana, kar vodi v nižje plazemske koncentracije. Kombinaciji erlotiniba in zaviralca protonske črpalke se je treba izogibati. Če menimo, da je uporaba antacidov med zdravljenjem z zdravilom Tarceva potrebna, jih je treba jemati najmanj 4 ure pred ali 2 uri po dnevnem odmerku zdravila Tarceva. Če razmišljamo o uporabi ranitidina, moramo zdravili jemati ločeno: zdravilo Tarceva je treba vzeti najmanj 2 uri pred ali 10 ur po odmerku ranitidina. V študiji faze Ib ni bilo pomembnih učinkov *gemcitabina* na farmakokinetiko erlotiniba, prav tako ni bilo pomembnih učinkov erlotiniba na farmakokinetiko gemcitabina. Erlotinib poveča koncentracijo platine. Pomembnih učinkov *karboplatina* ali paklitaksela na farmakokinetiko erlotiniba ni bilo. *Kapecitabin* lahko poveča koncentracijo erlotiniba. Pomembnih učinkov erlotiniba na farmakokinetiko kapecitabina ni bilo.

Neželeni učinki: *Zelo pogosti neželeni učinki* so kožni izpuščaj in driska, kot tudi utrujenost, anoreksija, dispneja, kašelj, okužba, navzea, bruhanje, stomatitis, bolečina v trebuhu, pruritus, suha koža, suhi keratokonjunktivitis, konjunktivitis, zmanjšanje telesne mase, depresija, glavobol, nevropatija, dispepsija, flatulenca, alopecija, okorelost, pireksija, nenormalnosti testov jetrne funkcije. *Pogosti neželeni učinki* so krvavitve v prebavilih, epistaksa, keratitis, paronihija, folikulitis, akne/akneiformni dermatitis, fisure na koži. *Občasno* so poročali o perforacijah v prebavilih, hirzutizmu, spremembah obrvi, krhkih nohtih, odstopanju nohtov od kože, blagih reakcijah na koži (npr. hiperpigmentacija), spremembah trepalnic, hudi intersticijski bolezni pljuč (vključno s smrtnimi primeri). *Redko* pa so poročali o jetrni odpovedi. *Zelo redko* so poročali o Stevens-Johnsonovem sindromu/toksični epidermalni nekrolizi ter o ulceracijah in perforacijah roženice.

Režim izdaje zdravila: H/Rp. **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija. **Verzija:** 1.0/12. **Informacija pripravljena:** Januar 2013.

002-13-TAR

DODATNE INFORMACIJE SO NA VOLJO PRI:
Roche farmacevtska družba d.o.o., Vodovodna cesta 109, 1000 Ljubljana.
Povzetek glavnih značilnosti zdravila je dosegljiv na
www.roche.si ali www.onkologija.si.

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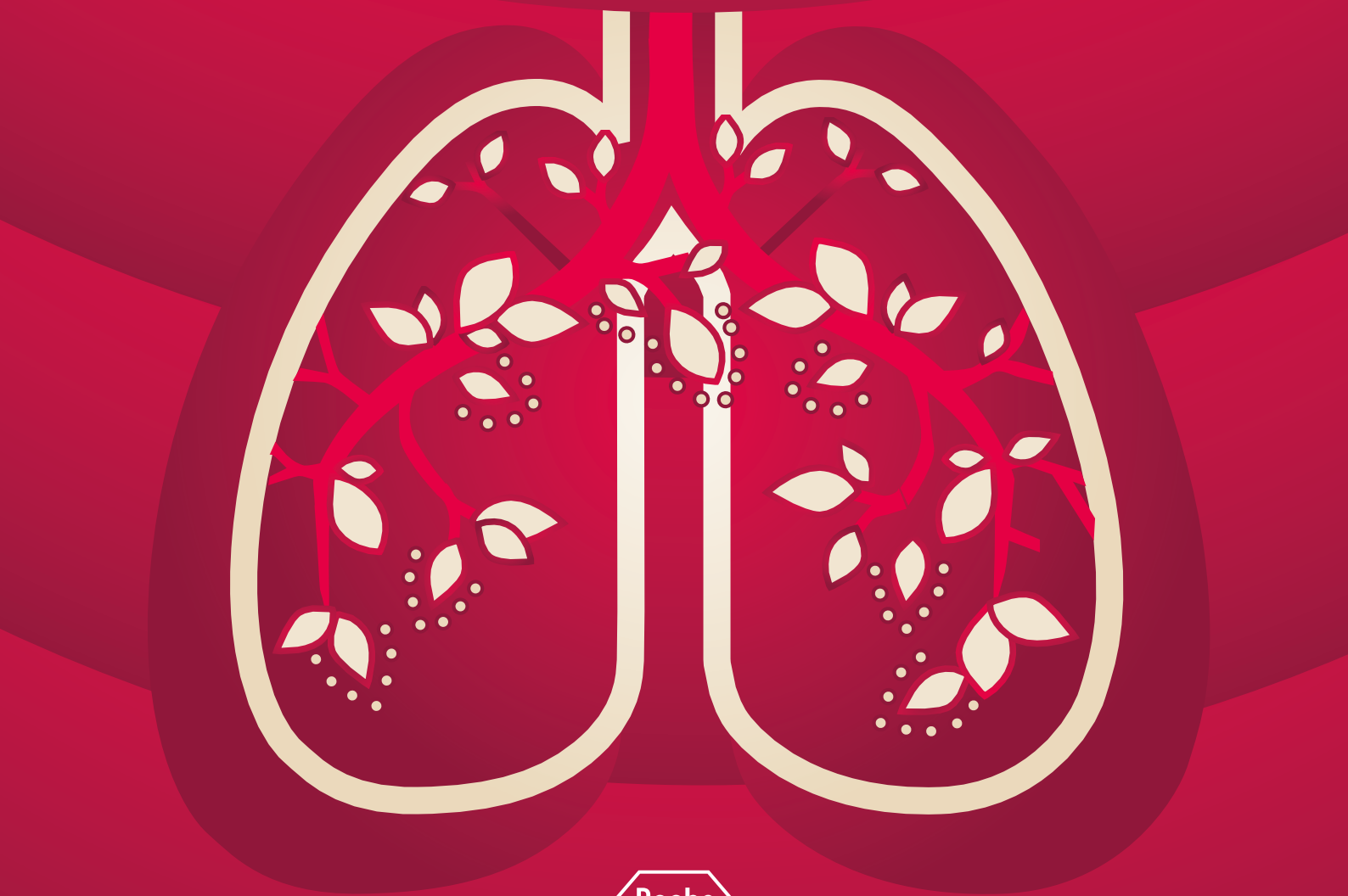
DOKAZANO PODALJŠA PREŽIVETJE PRI BOLNIKIH:

- z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč[1]
- z metastatskim rakom trebušne slinavke[1]

[1] Povzetek glavnih značilnosti zdravila Tarceva® 21.6.2012.
Dosegljiv na www.ema.europa.eu; dostopano: januar 2013.



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Preparation of manuscripts

Radiology and Oncology will consider manuscripts prepared according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals by International Committee of Medical Journal Editors (www.icmje.org). The manuscript should be typed double-spaced with a 3-cm margin at the top and left-hand side of the sheet. The manuscript should be written in grammatically and stylistically correct language. Abbreviations should be avoided. If their use is neccessary, they should be explained at the first time mentioned. The technical data should conform to the SI system. The manuscript, including the references, must not exceed 15 typewritten pages, and the number of figures and tables is limited to 8. If appropriate, organize the text so that it includes: Introduction, Materials and methods, Results and Discussion. Exceptionally, the results and discussion can be combined in a single section. Start each section on a new page, and number each page consecutively with Arabic numerals.

The Title page should include a concise and informative title, followed by the full name(s) of the author(s); the institutional affiliation of each author; the name and address of the corresponding author (including telephone, fax and E-mail), and an abbreviated title. This should be followed by the abstract page, summarizing in less than 250 words the reasons for the study, experimental approach, the major findings (with specific data if possible), and the principal conclusions, and providing 3-6 key words for indexing purposes. Structured abstracts are preferred. Slovene authors are requested to provide title and the abstract in Slovene language in a separate file. The text of the research article should then proceed as follows:

Introduction should summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Materials and methods should provide enough information to enable experiments to be repeated. New methods should be described in detail.

Results should be presented clearly and concisely without repeating the data in the figures and tables. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should discuss the results of the study in the light of previously published work.

Charts, Illustrations, Photographs and Tables must be numbered and referred to in the text, with the appropriate location indicated. Charts, illustrations and photographs, provided electronically, should be of appropriate quality for good reproduction. Illustrations and charts must be vector image, created in CMYK colour space, used font familie are encouraged "Century Gothic" and saved as .AI, .EPS or .PDF format. Color charts, illustrations and photographs are encouraged. Picture (image) size must be 2.000 pixels on the longer side and saved as .JPG (maximum quality) format. In photographs, mask the identities of the patients. Tables should be typed double-spaced, with a descriptive title and, if appropriate, units of numerical measurements included in the column heading. The files with the figures can be uploaded as separate files.

References must be numbered in the order in which they appear in the text and their corresponding numbers quoted in the text. Authors are responsible for the accuracy of their references. References to the Abstracts and Letters to the Editor must be identified as such. Citation of papers in preparation or submitted for publication, unpublished observations, and personal communications should not be included in the reference list. If essential, such material may be incorporated in the appropriate place in the text. References follow the style of Index Medicus. 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.

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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When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the Helsinki Declaration. Patients have the right to privacy; therefore the identifying information (patient's names, hospital unit numbers) should not be published unless it is essential. In such cases the patient's informed consent for publication is needed, and should appear as an appropriate statement in the article.

The research using animal subjects should be conducted according to the EU Directive 2010/63/EU and following the Guidelines for the welfare and use of animals in cancer research (*Br J Cancer 2010;* **102:** 1555 – 77). Authors must identify the committee approving the experiments, and must confirm that all experiments were performed in accordance with relevant regulations.

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ODPIRANJE NOVIH MOŽNOSTI

Zdravljenje metastatskega karcinoma ledvičnih celic (mRCC), gastrointestinalnega stromalnega tumorja (GIST) in nevroendokrinih tumorjev trebušne slinavke (pNET)

BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

SUTENT 12,5 mg, 25 mg, 37,5 mg, 50 mg trde kapsule
Sestava in oblika zdravila: Ena kapsula vsebuje 12,5 mg, 25 mg, 37,5 mg ali 50 mg sunitiniba (v obliki sunitinibijevega malata). **Indikacije:** Zdravljenje neizrezljivega in/ali metastatskega malignega gastrointestinalnega stromalnega tumorja (GIST) pri odraslih, če zdravljenje z imatinibom zaradi odpornosti ali neprenašanja ni bilo uspešno. Zdravljenje napredovalega in/ali metastatskega karcinoma ledvičnih celic (MRCC) pri odraslih. Zdravljenje neizrezljivih ali metastatskih, dobro diferenciranih nevroendokrinih tumorjev trebušne slinavke (pNET), kadar gre za napredovanje bolezni pri odraslih (izkušnje z zdravilom Sutent kot zdravilom prve izbire so omejene). **Odmerjanje in način uporabe:** Terapijo mora uvesti zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje rakavih bolezni. *GIST in MRCC:* Priporočeni odmerek je 50 mg peroralno enkrat na dan, 4 tedne zapored; temu sledi 2-tedenski premor (Shema 4/2), tako da celotni ciklus traja 6 tednov. *pNET*: Priporočeni odmerek je 37,5 mg peroralno enkrat na dan, brez načrtovanega premora. *Prilagajanje odmerka*: Odmerek je mogoče prilagajati v povečanjih po 12,5 mg, upoštevaje individualno varnost in prenašanje. Pri GIST in MRCC dnevni odmerek ne sme preseči 75 mg in ne sme biti manjši od 25 mg; pri pNET je največji odmerek 50 mg na dan, z možnimi prekinitvami zdravljenja. Pri sočasni uporabi z močnimi zaviralci ali induktorji CYP3A4 je treba odmerek ustrezno prilagoditi. *Pediatrična populacija:* Uporaba sunitiniba ni priporočljiva. *Starejši bolniki (≥ 65 let):* Med starejšimi in mlajšimi bolniki niso opazili pomembnih razlik v varnosti in učinkovitosti. *Okvara jeter:* Pri bolnikih z jetrno okvaro razreda A in B po Child-Pughu prilagoditev odmerka ni potrebna; pri bolnikih z okvaro razreda C sunitinib ni bil preizkušen, zato njegova uporaba ni priporočljiva. *Okvara ledvic:* Prilagajanje začetnega odmerka ni potrebno, nadaljnje prilagajanje odmerka naj temelji na varnosti in prenašanju pri posameznem bolniku. *Način uporabe:* Zdravilo Sutent se uporablja peroralno, bolnik ga lahko vzame s hrano ali brez nje. Če pozabi vzeti odmerek, ne sme dobiti dodatnega, temveč naj vzame običajni predpisani odmerek naslednji dan. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** *Bolezni kože in tkiv:* obarvanje kože, bolečine/draženje v ustih. Redko so poročali o hudih kožnih reakcijah (multiforni eritem (EM), Stevens-Johnsonov sindrom (SJS) in toksična epidermalna nekroliza (TEN)). Če so prisotni znaki EM, SJS ali TEN, je treba zdravljenje prekiniti. *Krvavitve* v prebavilih, dihalih, sečilih, možganih; najpogosteje epistaksa; krvavitve tumorja, včasih s smrtnim izidom. Pri bolnikih, ki se sočasno zdravijo z antikoagulanti, se lahko redno spremlja celotna krvna slika (trombociti), koagulacijski faktorji (PT / INR) in opravi telesni pregled. *Bolezni prebavil:* poleg navzeje, diareje, stomatitisa, dispepsije, bruhanja in ezofagitisa tudi resni zapleti (včasih s smrtnim izidom), vključno s perforacijo prebavil. *Hipertenzija,* povezana z zdravljenjem; pri bolnikih s hudo hipertenzijo, ki je ni mogoče urediti z zdravili, je priporočljivo začasno prenehanje zdravljenja. *Hematološke bolezni:* zmanjšanje števila nevtrofilcev, trombocitov, anemija. *Bolezni srca in ožilja:* srčno-žilni dogodki, vključno s srčnim popuščanjem, kardiomiopatijo in motnjami v delovanju miokarda, v nekaterih primerih s smrtnim izidom. Sunitinib povečuje tveganje za pojav kardiomiopatije. *Podaljšanje intervala QT*: previdna uporaba pri bolnikih z znano anamnezo podaljšanja intervala QT, tistih, ki jemljejo antiaritmike, in tistih z relevantno, že obstoječo srčno boleznijo, bradikardijo ali elektrolitskimi motnjami. *Venski in arterijski trombembolični dogodki*; arterijski včasih s smrtnim izidom. *Dogodki na dihalih:* dispneja, plevralni izliv, pljučna embolija ali pljučni edem; redki primeri s smrtnim izidom. *Moteno delovanje ščitnice:* bolnike je treba med zdravljenjem rutinsko spremljati glede delovanja ščitnice vsake 3 mesece. *Pankreatitis*, tudi resni primeri s smrtnim izidom. *Hepatotoksičnost*, nekateri primeri s smrtnim izidom. *Delovanje ledvic:* primeri zmanjšanega delovanja ledvic, odpovedi ledvic in/ali akutne odpovedi ledvic, v nekaterih primerih s smrtnim izidom. *Fistula:* če nastane fistula, je treba zdravljenje s sunitinibom prekiniti. *Oteženo celjenje ran:* pri bolnikih, pri katerih naj bi bil opravljen večji kirurški poseg, je priporočljiva začasna prekinitev zdravljenja s sunitinibom. *Osteonekroza čeljustnic:* pri sočasnem ali zaporednem dajanju zdravila Sutent in intravenskih difosfonatov je potrebna previdnost; invazivni zobozdravstveni posegi predstavljajo dodatni dejavnik tveganja. *Preobčutljivost/angioedem*. *Motnje okušanja*. *Konvulzije*. *Sindrom lize tumorja*, v nekaterih primerih s smrtnim izidom. *Okužbe:* hude okužbe z ali brez nevtropenije (okužbe dihal, sečil, kože in sepsa), vključno z nekaterimi s smrtnim izidom. **Medsebojno delovanje z drugimi zdravili:** (Študije so izvedli le pri odraslih.) Zdravila, ki lahko zvišajo koncentracijo sunitiniba v plazmi (ketokonazol, ritonavir, itrakonazol, eritromicin, klaritromicin ali sok grenivke). Zdravila, ki lahko znižajo koncentracijo sunitiniba v plazmi (deksametazon, fenitoin, karbamazepin, rifampin, fenobarbital, *Hypericum perforatum* oz. šentjanževka). **Plodnost, nosečnost in dojenje:** Zdravila Sutent ne smemo uporabljati med nosečnostjo in tudi ne pri ženskah, ki ne uporabljajo ustrezne kontracepcije, razen če možna korist odtehta možno tveganje za plod. Ženske v rodni dobi naj med zdravljenjem z zdravilom Sutent ne zanosijo. Ženske, ki jemljejo zdravilo Sutent, ne smejo dojiti. Neklinični izsledki kažejo, da lahko zdravljenje s sunitinibom poslabša plodnost samcev in samic. **Vpliv na sposobnost vožnje in upravljanja s stroji:** Sutent lahko povzroči omotico. **Neželeni učinki:** Najbolj resni neželeni učinki so odpoved ledvic, srčno popuščanje, pljučna embolija, predrtje črevesja in krvavitve (npr. dihal, prebavil, krvavitve tumorja). Najpogostejši neželeni učinki so: zmanjšan apetit, motnje okusa, hipertenzija, utrujenost, prebavne motnje (npr. driska, slabost, stomatitis, dispepsija in bruhanje), sprememba barve kože/motnje pigmentacije in sindrom palmarno-plantarne eritrodisestezije. Med najbolj pogostimi neželenimi učinki so hematološke motnje (nevtropenija, trombocitopenija in anemija). Ostali zelo pogosti neželeni učinki so: glavobol, epistaksa, bolečina v trebuhu/napihnjenost, zaprtje, glosodinija, izpuščaj, spremembe barve las, suha koža, bolečine v udih, vnetje sluznice, edemi. **Način in režim izdaje:** Predpisovanje in zdaja 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 Limited, Ramsgate Road, Sandwich, Kent, CT13 9NJ, Velika Britanija. **Datum zadnje revizije besedila:** 12.12.2012
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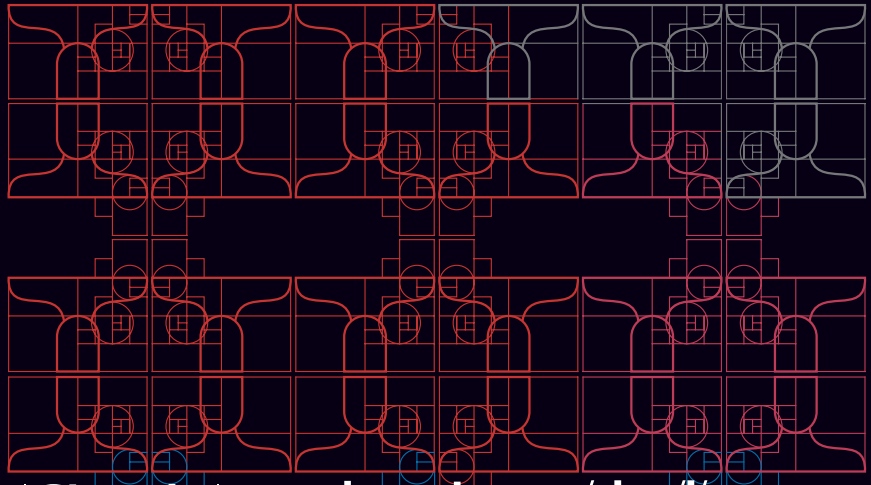

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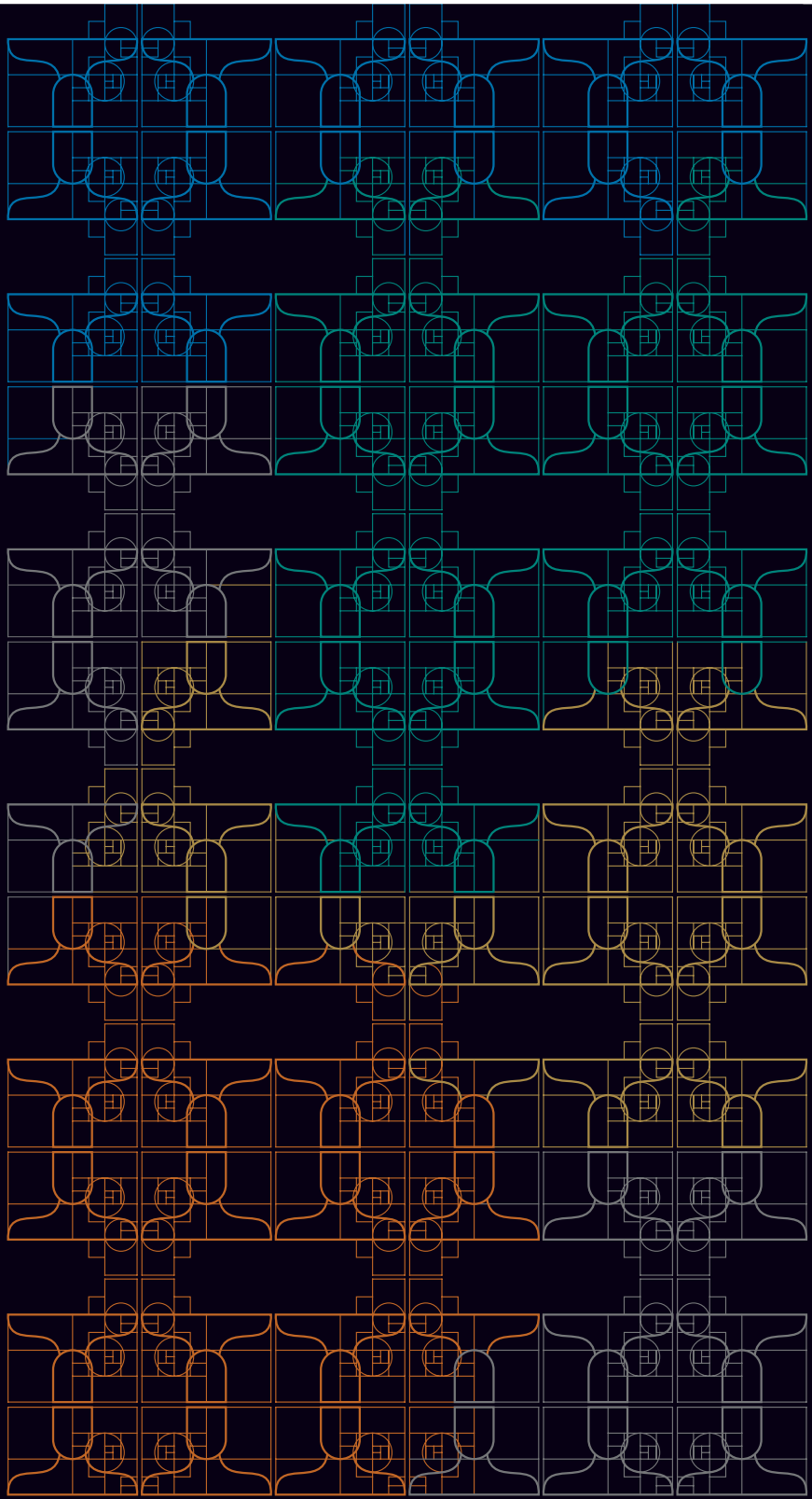

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