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NOVO

Cilja na 2 procesa nastanka CINV* v 1 odmerku
Zagotavlja učinkovito 5-dnevno preprečevanje CINV [1-5]

En odmerek
Dvojno delovanje
5-dnevno preprečevanje[1-5]

Akynzeo®
netupitant/palonosetron

* CINV: Chemotherapy-induced nausea and vomiting
[Slabost in bruhanje povzročena s kemoterapijo]

1. Aapro M et al. Ann Oncol. 2014 Jul;25(7):1328-33.
2. Hesketh et al. Ann Oncol. 2014 Jul;25(7):1340-46.
3. Gralla RJ et al, Ann Oncol. 2014 Jul;25(7):1333-39.
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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

▼Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

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TERAPEVTSKE INDIKACIJE Pri odraslih za preprečevanje akutne in zakasnjene navzee in bruhanja, povezanih z zelo emetogeno kemoterapijo na osnovi cisplatina za zdravljenje raka ter z zmerno emetogeno kemoterapijo za zdravljenje raka. **ODMERJANJE IN NAČIN UPORABE** Eno 300 mg/0,5 mg kapsulo je treba dati približno eno uro pred začetkom vsakega cikla kemoterapije. Trdo kapsulo je treba pogoltniti celo. Kapsulo je mogoče vzeti s hrano ali brez nje. Priporočeni peroralni odmerek deksametazona je treba ob sočasni uporabi z Akynzeom zmanjšati za približno 50 %. Prilagoditev odmerka pri starejših bolnikih ni potrebna. Pri uporabi tega zdravila pri bolnikih, starejših od 75 let, je potrebna previdnost zaradi dolgega razpolovnega časa zdravilnih učinkovin in omejenih izkušenj s to populacijo. Varnost in učinkovitost Akynzea pri pediatrični populaciji nista bili dokazani. Prilagoditev odmerka pri bolnikih z blago do hudo okvaro ledvic predvidoma ni potrebna. Potrebno se je izogibati uporabi Akynzea pri bolnikih s končnim stadijem bolezni ledvic, ki potrebujejo hemodializo. Pri bolnikih z blago ali zmerno okvaro jeter (stopnje 5-8 po lestvici Child-Pugh) prilagoditev odmerka ni potrebna. Pri bolnikih s hudo okvaro jeter (stopnja ≥9 po lestvici Child-Pugh) je treba Akynzeo uporabljati previdno. **KONTRAINDIKACIJE** Preobčutljivost na zdravilni učinkovini ali katero koli pomožno snov, nosečnost. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI** Ker lahko palonosetron podaljša čas prehoda skozi debelo črevo, je treba bolnike z anamnezo zaprtja ali znaki subakutne zapore črevesa po dajanju zdravila spremljati. Pri uporabi antagonistov 5-HT3 samih ali v kombinaciji z drugimi serotonergičnimi zdravili (vključno s selektivnimi zaviralci ponovnega privzema serotonina (SSRI) in zaviralci ponovnega privzema serotonina in noradrenalina (SNRI)) so poročali o serotoninskem sindromu. Priporočamo ustrezno opazovanje bolnikov glede simptomov, podobnih kot pri serotoninskem sindromu. Ker Akynzeo vsebuje antagonist receptorjev 5-HT3, je potrebna previdnost pri sočasni uporabi z zdravili, ki podaljšujejo interval QT, ali pri bolnikih, ki so razvili podaljšan interval QT, oziroma je verjetno, da ga bodo. Tega zdravila ne smemo uporabljati za preprečevanje navzee in bruhanja v dneh po kemoterapiji, razen v povezavi z dajanjem naslednjega cikla kemoterapije. Ne smemo ga uporabljati za zdravljenje navzee in bruhanja po kemoterapiji. Pri bolnikih s hudo okvaro jeter je potrebna previdnost, saj je za te bolnike na voljo malo podatkov. To zdravilo je treba uporabljati previdno pri bolnikih, ki sočasno peroralno prejemajo zdravilne učinkovine, ki se primarno presnavljajo prek CYP3A4 in imajo ozko terapevtsko območje. Netupitant je zmeren zaviralec CYP3A4 in lahko poveča izpostavljenost kemoterapevtskim zdravilom, ki so substrati za CYP3A4, npr. docetakselu. Zaradi tega je treba bolnike spremljati glede povečane toksičnosti kemoterapevtskih zdravil, ki so substrati za CYP3A4, vključno z irinotekanom. Poleg tega lahko netupitant vpliva tudi na učinkovitost kemoterapevtskih zdravil, pri katerih je potrebna aktivacija prek presnove s CYP3A4. Akynzeo vsebuje sorbitol in saharozo. Bolniki z redko dedno intoleranco za fruktozo, malabsorpcijo glukoze/galaktoze ali pomanjkanjem saharoza-izomaltaze ne smejo jemati tega zdravila. Poleg tega lahko vsebuje tudi sledi lecitina, pridobljenega iz soje. Zaradi tega je treba bolnike z znano preobčutljivostjo na arašide ali sojo skrbno spremljati glede znakov alergijske reakcije. Ženske v rodni dobi ne smejo biti noseče ali zanositi med zdravljenjem z Akynzeom. Pred začetkom zdravljenja je treba opraviti test nosečnosti pri vseh ženskah, ki še niso imele menopavze. Ženske v rodni dobi morajo uporabljati učinkovito kontracepcijo med zdravljenjem in še do en mesec po njem. Akynzeo je kontraindiciran med nosečnostjo. Med zdravljenjem z Akynzeom in še 1 mesec po zadnjem odmerku je treba prenehati z dojenjem. **INTERAKCIJE** Ob sočasni uporabi Akynzea z drugim zaviralcem CYP3A4 lahko pride do zvišanja plazemskih koncentracij netupitanta. Pri sočasni uporabi Akynzea in zdravil, ki spodbujajo delovanje CYP3A4, lahko pride do znižanja plazemskih koncentracij netupitanta, kar lahko privede do zmanjšane učinkovitosti. Akynzeo lahko zviša plazemske koncentracije sočasno uporabljenih zdravil, ki se presnavljajo prek CYP3A4. Ob sočasnem dajanju deksametazona z Akynzeom je treba peroralni odmerek deksametazona zmanjšati za približno 50 %. Ob sočasnem dajanju z Akynzeom se je izpostavljenost docetakselu in etopozidu povečala za 37 % oziroma 21 %. Pri ciklofosfamidu po sočasnem dajanju netupitanta niso opazili konsistentnih učinkov. Pri eritromicinu, midazolamu ali drugih benzodiazepinih, ki se presnavljajo prek CYP3A4 (alprazolam, triazolam), je treba ob sočasnem dajanju Akynzea upoštevati možne učinke njihovih zvišanih plazemskih koncentracij. Pri sočasnem dajanju Akynzea z močnimi zaviralci CYP3A4 (npr. ketokonazol) je potrebna previdnost, sočasnemu dajanju z močnimi spodbujevalci CYP3A4 (npr. rifampicin) pa se je treba izogibati. Priporočamo previdnost pri uporabi netupitanta v kombinaciji s peroralnim substratom encima UGT2B7 (npr. zidovudin, valprojska kislina, morfin), ker *in vitro* podatki kažejo, da netupitant zavira UGT2B7. Priporočamo previdnost pri kombiniranju netupitanta z digoksinom ali drugimi substrati P-gp, kot sta dabigatran ali kolhicin, ker podatki *in vitro* kažejo, da je netupitant zaviralec P-gp. **NEŽELENI UČINKI** *Pogosti (≥1/100 do <1/10):* glavobol, zaprtje, utrujenost. *Občasni (≥1/1.000 do <1/100):* nevtropenija, levkocitoza, zmanjšan apetit, nespečnost, omotica, vrtoglavica, atrioventrikularni blok prve stopnje, kardiomiopatija, motnja prevajanja, hipertenzija, kolcanje, bolečina v trebuhu, driska, dispepsija, napenjanje, navzea, alopecija, urtikarija, astenija, zvišane jetrne transaminaze, zvišana alkalna fosfataza v krvi, zvišan kreatinin v krvi, podaljšanje QT na elektrokardiogramu. *Redki (≥1/10.000 do <1/1.000):* cistitis, levkopenija, limfocitoza, hipokaliemija, akutna psihoza, sprememba razpoloženja, motnja spanja, hipestezija, konjuktivitis, zamegljen vid, aritmija, atrioventrikularni blok druge stopnje, kračni blok, popuščanje mitralne zaklopke, miokardna ishemija, ventrikularne ekstrasistole, hipotenzija, disfagija, obložen jezik, bolečina v hrbtu, občutek vročine, nekardialna bolečina v prsnem košu, nenormalen okus zdravila, zvišan bilirubin v krvi, zvišana kreatin fosfokinaza MB v krvi, depresija segmenta ST na elektrokardiogramu, nenormalen segment ST-T na elektrokardiogramu, zvišan troponin. **Vrsta ovojnine in vsebina:** Škatla z eno kapsulo v pretisnem omotu iz aluminija. **Režim izdaje:** Rp **Imetnik dovoljenja za promet:** Helsinn Birex Pharmaceuticals Ltd, Damastown, Mulhuddart, Dublin 15, Irska
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contents

review

Dynamic contrast-enhanced ultrasound of the bowel wall with quantitative assessment of Crohn's disease activity in childhood

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Background. Contrast-enhanced ultrasound (CEUS) has become an established non-invasive, patient-friendly imaging technique which improves the characterization of lesions. In addition, dynamic contrast-enhanced ultrasound (DCE-US) provides valuable information concerning perfusion of examined organs. This review addresses current applications of CEUS in children, focused on DCE-US of the bowel wall in patients with Crohn disease, which enables real-time assessment of the bowel wall vascularity with semi-quantitative and quantitative assessment of disease activity and response to medical treatment.

Conclusions. Crohn's disease is a chronic inflammatory relapsing disease. Frequent imaging re-evaluation is necessary. Therefore, imaging should be as little invasive as possible, children friendly with high diagnostic accuracy. US with wide varieties of techniques, including CEUS/DCE-US, can provide an important contribution for diagnosing and monitoring a disease activity. Even if the use of US contrast agent is off-label in children, it is welcome and widely accepted for intravesical use, and a little less for intravenous use, manly in evaluation of parenchymal lesions. To our knowledge this is the first time that the use of DCE-US in the evaluation of activity of small bowel Crohn disease with quantitative assessment of kinetic parameters is being described in children. Even if the results of the value and accuracy of different quantitative kinetic parameters in published studies in adult population often contradict one another there is a great potential of DCE-US to become a part of the entire sonographic evaluation not only in adults, but also in children. Further control studies should be performed.

Key words: contrast-enhanced ultrasound (CEUS); dynamic contrast-enhanced ultrasound (DCE-US); Crohn disease; quantification; children

Introduction

Contrast-enhanced ultrasound (CEUS) has been established as a valuable tool in many clinical applications. CEUS has improved the detection and characterization of different lesions in comparison to conventional ultrasound (US). The increasing role of CEUS is based on widespread availability of US equipment with available commercial contrast specific technique on the market, and commercially available safe ultrasound contrast agent (UCA) with no serious adverse effects. The use of CEUS is increasing, particularly in the abdominal US in adults in the diagnosis, differential diagnosis and follow-up in patients with focal liver lesions. The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) has published guidelines and recommendations for the clinical practice of CEUS on hepatic and also on non-hepatic applications in adults.[1,2] UCA is licensed only for

cardiac, liver, breast and vascular applications, but this is not an obstacle for a wider use in many other clinical applications.[2,3] Dynamic contrast-enhanced ultrasound (DCE-US) is a step forward in the quantification of tissue/lesion enhancement and tissue/lesion perfusion.

The second-generation UCA has not been registered for individuals younger than 18 years of age which makes the clinical application of UCA even harder.[4] The possibility of a fast, accurate and safe diagnosis of many diseases is thus significantly reduced even if safety considerations of UCA are most promising compared to iodine or paramagnetic contrast agent.[5-8] "Pediatric CEUS Data Registry" has been established on the EFSUMB website, which allows data input in a prospective manner and records any adverse events.[9] The main advantages of CEUS in childhood are that this is a radiation-free method, that there is no need for anaesthesia or sedation in small children and that it is easily performed in children because of a smaller body size and more favourable tissue composition compared to adults.[4] UCA is widely used in children for intravesical application to perform echo-enhanced voiding urosonography, an established method for vesicoureteric reflux evaluation.[10,11] Some papers have been published describing CEUS examination with intravenous application in the paediatric population after blunt injuries of the abdominal cavity (liver and spleen injuries) and for assessment, characterization, and monitoring of parenchymal lesions in childhood.[12-16] Meta-analysis of literature connected with CEUS performed in children was done.[4,17] To our knowledge, so far no report of CEUS/DCE-US examinations of the bowel wall in children with Crohn's disease has been published and no study has been done or published study that would evaluate DCE-US of the bowel wall with quantitative assessment of Crohn's disease activity or monitor medical therapy in children. However, DCE-US presents a huge step forward in combining morphologic and functional information regarding the disease activity and response to therapy.

Evaluation of inflammatory activity in Crohn's disease

Crohn's disease is a chronic inflammatory disease of the gastrointestinal tract. An important characteristic of this condition is that the episodes of inflammation alternate with the periods of remission. Therefore, frequent re-evaluation of the inflammatory activity to plan a proper therapy is necessary in many patients. There are some established methods for the evaluation of paediatrics Crohn's disease activity in children. A gold standard in the assessment of the degree of the Crohn's disease is upper and lower endoscopy (endoscopic severity score) with biopsy, but it is considered an invasive method, which also requires anaesthesia or deep sedation in paediatric patients. There are several surrogate markers for the disease activity that clinicians have traditionally used, including the clinical paediatric Crohn's disease activity index (PCDAI) and laboratory markers of inflammation C-reactive protein (CRP), and more recently faecal markers such as faecal calprotectin. All of these markers, however, have limitations.[18] Various imaging methods like US with colour and pulsed Doppler, CT and MR enterography play a significant role in the evaluation of the disease activity. No significant differences in diagnostic accuracy among the imaging techniques were observed.[19,20] In every day clinical praxis the determination of the activity depends on the results given by the various complementary markers and exams depending on local abilities.

In general, the main goal in paediatric patients with Crohn's disease is to find an accurate non-invasive method for the assessment of the disease activity, which is simple, quickly performed, widely available, and well tolerated by patients. High-resolution bowel US is an important imaging technique for the diagnosis and the follow-up of children with Crohn's disease. It can evaluate the localization and the length of the affected intestinal segments, and identify intra-abdominal complications. Doppler techniques can visualize and semi-quantify the bowel wall vascularization.[21-24] However, DCE-US of the bowel wall with the intravenous administration of the second-generation UCA has all the potentials to become the new technique because it allows real-time examination of the bowel wall perfusion and microvascularization, and enables an objective quantitative measurement of the enhancement by analysing the parameters of the time-intensity curve (TIC).[25-28]

Dynamic contrast-enhanced ultrasound of the bowel wall with quantitative assessment of Crohn's disease activity

Diagnostic value of CEUS and DCE-US of the bowel wall: review of the published articles

CEUS of the bowel wall was introduced to evaluate the intestinal wall hyperaemia and the bowel

wall microvascularization. An early pathological change in patients with active Crohn's disease is neovascularization of the bowel wall, characterized by the development of new capillary vessels in the *lamina propria* and *submucosa*. Low velocity flows in small vessels can be identified now due to advanced techniques using low-mechanical-index real-time harmonic sonography associated with the second-generation UCAs, which are more stable and remain for a longer period in the intestinal wall microcirculation. Due to the small size microbubbles (1-7 μm) the use of UCA allows the assessment of the microcirculation in micro-vessels and capillaries, where the vessels have very small diameters, as small as 40 microns. The spatial resolution of CEUS is 0.2–2 mm.[29] CEUS thus allows an accurate mapping of the bowel vasculature and perfusion, and it was proved to be superior to conventional colour or power Doppler imaging.[30]

The first studies for the evaluation of the bowel wall perfusion with CEUS were performed in the early 2000s by the first-generation US contrast agent Levovist (Schering, Berlin, Germany).[31] For the qualitative evaluation of the inflammatory activity in Crohn's disease four different types of perfusion enhancement patterns of the thickened bowel wall are described, including low or absent enhancement (in comparison with the adjacent mesentery), a prevalent submucosal enhancement sparing the *muscularis propria*, and complete transmural enhancement, either outward (centrifugal), starting from mucosa, or inward (centripetal), starting form perivisceral vessels. Serra *et al.* found that patients with complete enhancement of the bowel wall or enhancement of the inner layers had high sensitivity and specificity 81% and 63% in distinguishing active and inactive disease according to the Crohn disease activity index (CDAI).[32] The same study performed by Migaleddu *et al.* has shown even better results (sensitivity and specificity of 93.5% and 93.7%, respectively).[33] On the other hand, enhancement patterns are not useful in differentiating responders from non-responders to medical treatment among patients with Crohn's disease.[34,35] In fact, the degree of contrast-enhancement is dependent on several confounding factors, such as contrast agent and equipment used, depth of lesion and shadowing from air, or arteries filled with contrast. Inter-observer variability needs to be taken into account.

The next step of CEUS is DCE-US which enables the quantification of the disease activity by quantitative assessment of the bowel wall enhancement. Katzer *et al.* introduced the quantification of the bowel wall enhancement by using wide band harmonic imaging US and the HDI-Lab software, but there were no correlations between the enhancement and clinical and/or laboratory indices of the disease activity.[36] At the moment, different softwares for enhancement quantification are available on the market. One is the quantitative analysis of the brightness in regions of the interest localized in the most echogenic zone of the intestinal wall. The software automatically obtained a brightness-time curve, which correlated well with the disease activity found on endoscopy and MRI of the small bowel.[37,38] Other softwares are used to draw a TIC, which displays the average intensity of UCA in a region of interest as a function of time, reflecting its transit. The analysis of the TIC of the bowel wall offered various kinetic parameters.[39] The time-to-peak (the time from zero intensity to maximum intensity enhancement of the bowel wall), peak intensity (maximum value of intensity in arbitrary units), the slope of the first ascending tract of the curve (which correlates with time-to-peak enhancement and enhancement velocity), and the area under the TIC are the most promising in the evaluation of the disease activity. However, the results of published studies evaluating the capabilities of DCE-US, using different parameters of quantitative analysis of TIC to classify Crohn's disease activity and response to medical treatment compared to clinical activity index, laboratory inflammation markers, histopathologic analysis, endoscopy, contrast-enhance CT or MR enterography, often contradict one another. Girlich *et al.* found that a shorter time-to-peak in patients with higher peak enhancement corresponds well with the higher disease activity.[40] Romanini *et al.* compared various parameters obtained from TIC analysis during DCE-US to vascular density in biopsy specimens obtained during colonoscopy of the same bowel segment: a higher peak enhancement, a shorter time-to-peak enhancement, a high regional blood flow and a regional blood volume correlate well with a high vascular density in biopsy specimens.[41] De Franco *et al.* compared DCE-US parameters to the clinical and endoscopic score for Crohn's disease, and found sensitivity for detecting active Crohn's disease of 97% for maximum peak intensity and 86% for wash-in slope coefficient.[42] Białecki *et al.* compared parameters of TIC with a low-dose CT enterography and CDAI and found a good correlation between Crohn's disease activity in DCE-US and low-dose CT enterography.[43] According to Giangregorio *et al.* vascular activity cannot be simply correlated with clinical

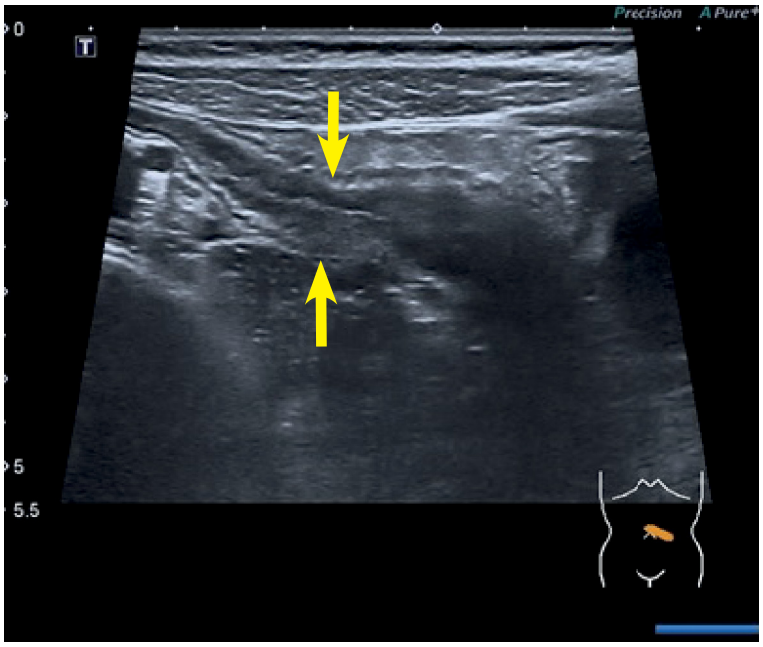


FIGURE 1. Ultrasound (US) of a jejunal segment with a thickened bowel wall.

activity, but its measurement is useful in clinical practice.[35] It was revealed that in the subgroup of patients with clinically inactive disease and with wall vascularization typical of inflammation, clinically relevant recurrence is present in near future. On the other hand, Wong *et al*. showed no significant association of TIC analysis (area under curve, time-to-peak peak intensity) with the endoscopic activity.[44] Girlich *et al.* performed a quantitative assessment of bowel wall vascularization in healthy volunteers and patients with acute exacerbation of proven Crohn's disease.[45] Patients had a significantly higher peak enhancement values and regional blood volume than volunteers, but the time-to-peak was significantly shorter in volunteers due to their low enhancement peak. The quantitative parameters should therefore be evaluated together, not individually.

The evaluation of the response to medical treatment with DCE-US provides even more contradictory results. The slope of the first ascending tract of the curve and the area under the TIC were significantly lower while the time-to-peak enhancement was significantly higher after the treatment with a significant correlation with CDAI score.[34] The last study by the same author contradicts the first one at some point and shows the area under the TIC as the only parameter to distinguish responders from non-responders.[46] Both of these studies have an important flaw; they were performed with video data that are a log-compressed version of the actual ultrasound intensities, which is not mathematically correct. Studies suggest that log-compression distorts the data and that only linear data of the actual ultrasound intensities (so-called "raw data") should be used for analysis.[47] Saevik *et al.* found a significant difference between groups for peak contrast enhancement, rate of wash-in and wash-out and the area under the TIC in wash-in phase at the examination 1 month after the start of the treatment, but there was no significant difference at any point in the time-related variables between the responders and non-responders groups.[48]

The discordant results of the studies may be due to the different methods used to collect the images (from raw data or from DICOM images) and different softwares available on the market to calculate the parameters of TIC. Even the definitions of some parameters are different using different software packages: *i.e.* TTP is defined as the time from *i.v.* administration of contrast medium to maximum enhancement of bowel wall, or time from first appearance of contrast medium in the bowel wall to its maximum enhancement. Therefore, there is no accepted agree about the normal value rangers of TIC parameters. Standardization of software programs should be obtained and further controlled comparable studies should be conducted to determine the real accuracy of the quantitative parameters.

DCE-US protocol

DCE-US protocol for the assessment of bowel wall vascularization is relatively simple. The adaption of US machine with contrast specific software and the software program for DCE-US quantification is necessary. In addition, software for the TIC evaluation is needed for the qualitative/quantitative evaluation of the bowel wall enhancement on workstation.[39] There is no need for patient preparation, only fasting for at least 6 hours before examination to ensure standard conditions during investigation. First, accurate baseline US and Doppler examination of the intestine are performed. One or more affected intestinal segments with thickened wall (≥ 3 mm) are chosen.[49] A high frequency transducer (7.5 to 12 MHz) is used and it should be kept as still as possible on the selected image of the intestinal segment. Contrast specific software with low mechanical index (MI < 0.10) is switched on. The gain setting is adapted to obtain an anechoic bowel wall. The patient is asked to breathe shallow or to hold the breath during examination. The examination of each examined bowel segment is recorded, starting at the time

of the intravenous administration in a dose of the second-generation UCA SonoVue (Bracco, Italy), followed by 10 ml of normal saline solution (0.9% NaCl). The dose of UCA for SonoVue varies from 1.2 ml in case of low-frequency transducers to 2.4 to 4.8 ml in case of high-frequency transducers per cycle with maximal doze of 10 ml in adults, and it depends also on the sensitivity of the equipment used.[28] The dose of UCA in children can be calculated according to the formula: dose (ml) = child age (years) / 10, but not less than 0.1 ml per single application.[49] Continuous imaging is performed for 60 – 90 seconds. The examination is stored in the scanner as a raw data video clip. One or more regions of interest (ROI) are drawn encompassing the thickened anterior wall, excluding the lumen and perivisceral tissues. In postprocesing the raw data is sent to the workstation by one of the software packages, either to draw a TIC and automatically calculate various kinetic quantitative parameters as described above, or to draw a brightness time curve using quantitative analysis of the brightness in ROI, and to calculate the percentage of the increase in the wall brightness.

Clinical indications, advantages and limitation of CEUS/DCE-US of the bowel wall

According to EFSUMB guidelines and recommendations on the clinical practice of CEUS/DCE-US considering the gastrointestinal tract CEUS/DCE-US is recommended when estimating the disease activity in inflammatory bowel disease, when discerning between fibrous and inflammatory strictures in Crohn's disease, when characterizing the suspected abscesses, and when confirming and following the route of fistulas.[4] Even if the studies contradict one another to a certain degree, as described above the DCE-US with the assessment of the quantitative results might be able to differentiate between responders and non-responders to the treatment in patients with Crohn's disease, and thus to improve therapy planning and monitoring of the efficacy of the treatment, together with other non-invasive inflammatory markers and imaging examinations. To determine the most valuable parameters of TIC, further controlled and comparable studies should be conducted. However, we should be aware that there are no absolute numbers and that quantitative parameters should be analysed as a whole. A comparison of the parameters is mandatory in follow-up studies monitoring the therapy.

The main advantage of CEUS/DCE-US examination of the bowel wall particularly in children is that the examination is non-invasive, radiation-free, easy repeatable, patient friendly, very well tolerated and accepted, and it does not require any specific preparation. An additional advantage in children is that CEUS/DCE-US is easily performed because of the smaller body size and more favourable tissue composition compared to adults, and that there is no need for anaesthesia in smaller children. Motion artefacts produced by peristalsis or intestinal contents do not impair CEUS/DCE-US as is the case with colour Doppler or during MR enterography. Even if the ROI cannot be continuously placed over a defined area because of the motion of the intestinal wall, it is possible to analyse the contrast agent enhancement from individual images.[25] In addition, UCA is considered the safest contrast agent with the lowest rate of adverse effects.[5-7]

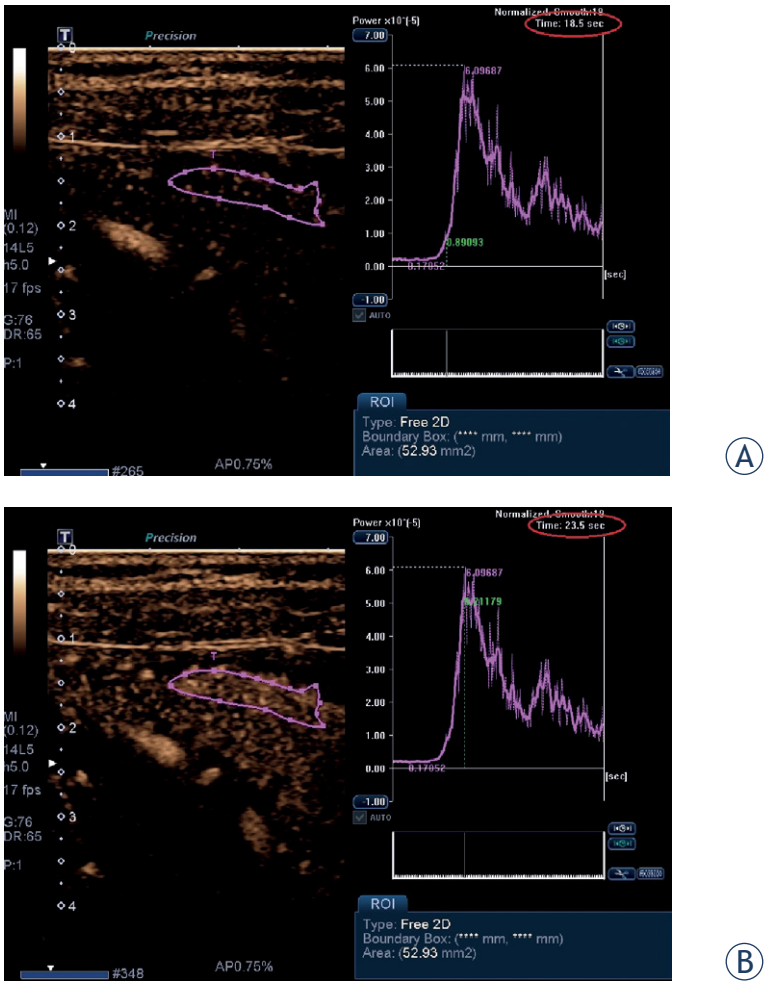
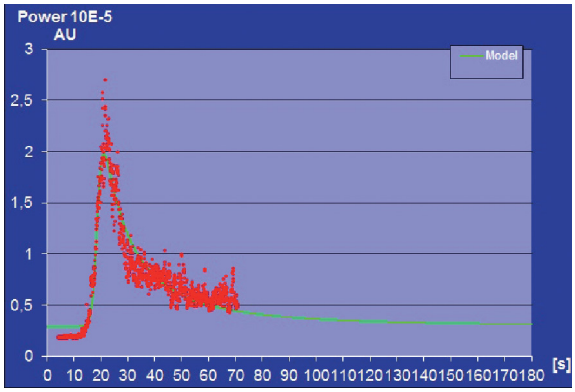


FIGURE 2. Contrast-enhanced ultrasound: the region of interest (ROI) is determined **(A)** when the contrast medium first appears in the affected bowel wall corresponding to starting point at time-intensity curve, **(B)** when the maximum enhancement was seen corresponding to peak at time-intensity curve. The calculated time-to-peak is 5 seconds (18.5 sec to 23.5).



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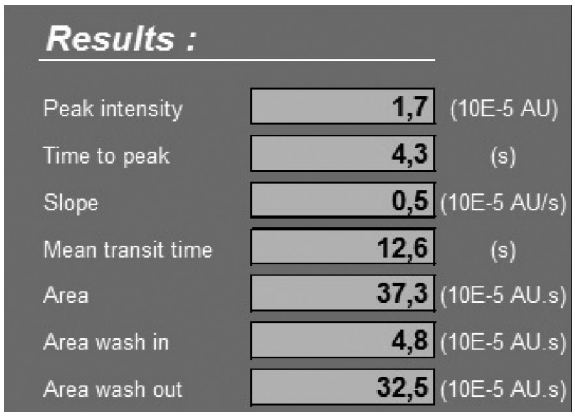

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FIGURE 3. Postprocesing results obtained from raw data imported in workstation with dedicated quantification software (Workstation UltraExtend FX with IGR protocol): **(A)** Time-intensity curve (TIC), and **(B)** the table of results of the calculated quantitative kinetic parameters from TIC.

The main limitations are the off-label use of UCA in childhood and the evaluation of the limited number of the effected bowel segments seen by the use of on basic examination. It is not possible to evaluate the enhancement if the bowel wall is not clearly identified, or it is too thin (it should be ≥ 3 mm). Each bowel segment needs an injection of UCA. DCE-US needs specific software installed on US machine to be able to quantify the enhancement. Postprocesing software packages of different kinds are on the market and a comparison of these programs has not been obtained yet. The selection of the ROI to create TIC also depends on the radiologist and it can be a potential cause of interobserver variability.

In our case a 13-year-old boy with known Crohn's disease was admitted to the hospital because of the acute exacerbation of the disease, presented by vomiting, severe abdominal cramps, weight loss and inappetence. He had been diagnosed with isolated small bowel Crohn's disease two years ago. At the time of the diagnosis remission had been induced by exclusive enteral nutrition and it was well maintained by azathioprine (2.5 mg/kg daily) for 2 years. At the time of the exacerbation, he had elevated laboratory markers (erythrocyte sedimentation rate [ESR] 43, CRP 25) and faecal calprotectin (310 mg/kg, normal value < 50 mg/kg of stool). The estimated PCDAI was 37. The abdominal US showed aperistaltic segments of the small intestine with a thickened wall surrounded by echogenic mesentery and enlarged lymph nodes. The terminal ileum and colon were normal on US. MR enterography confirmed irregular thickened segments of the small bowel wall with entero-enteric fistulas and intense enhancement of the effected bowel wall. Upper endoscopy and ileocolonoscopy showed normal mucosa of the upper gastrointestinal tract, ileum and colon. Anti-TNFα (infliximab) biological treatment was introduced. Control US and control MR enterography showed only a slightly improvement of the inflammation. Laboratory findings were normal, except for the increased calprotectin. Anti-TNFα treatment was continued and the patient was in clinical remission. Three months later he complained of fatigue, inappetence and some weight lost. At that time laboratory markers were normal (normal ESR and CRP), only calprotectin was elevated (495 mg/kg). Estimated PDCAI was 40.The boy refused to perform control MR enterography for the disease activity evaluation because he felt sick after drinking the contrast medium and had nausea after the application of glucagon. Therefore, DCE-US of the bowel wall was suggested to evaluate its inflammatory activity and a written informed consent by him and his parents was obtained (Figures 1 and 2). The boy was very cooperative during the examination. He tolerated it very well, without any side effects compared to previous MR enterography. The quantitative analyses of TIC suggest according to some literature and our own experiences that there is still a quite active inflammation of jejunum: time-to-peak enhancement was short, less than 5 seconds, the intensity of the enhancement was high (Figure 3). Time-to-peak peak intensity (TTP) less than 7 seconds and intense enhancement of the bowel wall are sings for active inflammation of the bowel wall.[40] According to the clinical condition and the results of DCE-US, anti-TNFα treatment was optimized: the application interval was shortened from 8 to 4 weeks. Five months later control US and the blood inflammatory parameters were normal, PCDAI was below 10 and calprotectin decreased to 56 mg/kg. The boy is doing clinically well now, shows no fatigue and he is gaining weight and height.

To our knowledge, no study evaluating DCE-US of the bowel wall with quantitative assessment of Crohn's disease activity in children has yet been published. Our case shows the importance of DCE-US as a non-invasive method for children with Crohn's disease, especially during follow-up of the disease activity. In this case the results of DCE-US combined with clinical signs and elevated calprotectin presented the main indicator, which influenced the therapy regime.

Conclusions

DCE-US is a dynamic real-time examination of the bowel wall perfusion, which can be performed in a short period of time, is inexpensive, easy available compared to MR enterography, and radiation free compared to CT enterography. It is well tolerated by patients, which is very important in chronic patients and children, in whom frequent examinations are necessary. At the moment DCE-US cannot replace other cross-sectional imaging for global bowel assessment, but it has a great potential to become an important part in algorithms for the diagnosis and monitoring of the disease activity in patients with Crohn's disease. However, controlled studies with comparable softwares of TIC evaluation should be performed to establish accuracy of the method and its value in children.

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review

Granulomatosis after autologous stem cell transplantation in nonHodgkin lymphoma - experience of single institution and a review of literature

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Background. Sarcoidosis before and after treatment of malignancy is an important differential diagnosis that has to be distinguished from lymphoma.

Patients and methods. Hodgkin lymphoma, diffuse large B-cell lymphoma and aggressive follicular lymphoma are being staged and treatment effect is evaluated with PET-CT. We report three cases of aggressive lymphoma after high dose therapy and autologous stem cell transplantation with positive lymph nodes on PET-CT, which were histologically diagnosed as sarcoidosis/granulomatosis. In the literature, we found that false positive lymph nodes were more common after allogeneic than after autologous transplantation.

Conclusions. Post-treatment PET-CT positive lymph nodes should always be examined histologically prior to any further treatment decision to avoid unnecessary toxic procedures.

Key words: granulomatosis; nonHodgkin lymphoma; PET-CT; differential diagnosis; tissue biopsy

Introduction

Sarcoidosis is a multisystemic disease of so far unknown cause. It is characterised by CD4+ T cell alveolitis, followed by formation of noncaseating immune granulomas in involved organs, mainly the lungs and the lymphatic system. Mortality is higher in patients with sarcoidosis than in general population, pulmonary fibrosis being the main cause of death. Systemic corticosteroids are the main treatment.[1] Sarcoidosis has been linked to malignancy in various aspects. Usually it precedes malignancy, less frequently is it diagnosed after the initial treatment of cancer. Pathogenesis of sarcoidosis after treatment of malignancy is still unknown but it can be a misleading finding especially in lymphoma patients.[2] Positron emission tomographic (PET) scanning using 18F-fluorodeoxyglucose (18F-FDG) combined with the computer tomography (CT) is used for initial diagnosis of lymphoma and for later follow-up examinations, especially in Hodgkin lymphoma, diffuse large B-cell lymphoma and aggressive follicular lymphoma.[3,4] Still, positive post-treatment scans should be interpreted with caution. Positive lymph nodes in this setting are namely highly suspicious of a lymphoma relapse.[5-8] Nonetheless, further treatment without biopsy of the suspicious sites could have detrimental consequences for the patient, starting with unnecessary conventional chemotherapy and proceeding to even more toxic allogeneic stem cell transplantation.

PET-CT imaging and evaluation

Numerous studies have documented that PET scanning detects actively metabolizing tumor cells in residual masses of FDG-avid lymphomas following chemotherapy. It was shown that the persistent abnormal uptake is a predictor of early relapse or poor survival.[9,10] After completion of the treatment, the CT scan of patients with nonHodgkin lymphomas (NHL) may show evident residual mass which can be either fibrosis or non-viable tumour. Yet, the PET scanning of these sites or combined PET-CT scanning appears to differentiate quite well between the relapse and the remission in most cases.[5,11] Even though, post-therapy PET positivity requires tissue biopsy or further evaluation[5-8], same as the mid-therapy PET positivity.[8] An important supplementation of the PET scan with the CT imaging is to identify the site for optimal sampling of the tissue therefore defining the most appropriate invasive procedure - in all our cases the bronchoscopy with biopsy - or to define the morphology of the extranodal tissue which was positive on PET-CT.[8]

In 2014, the new consensus of the international conference on malignant lymphoma imaging work group was published.[3] It includes recommendations on the use of PET-CT scanning using 18F-FDG for the assessment of treatment of Hodgkin lymphoma, diffuse large B-cell lymphoma and aggressive follicular lymphoma. For primary mediastinal large B-cell lymphoma the routine usage of PET-CT scan is not validated and further prospective studies are warranted.[3] Still, the primary mediastinal lymphomas are like the diffuse large B cell lymphomas aggressive lymphomas with high proliferation rate and the PET-CT should supposedly be an adequate diagnostic procedure both for staging and response evaluation purposes.

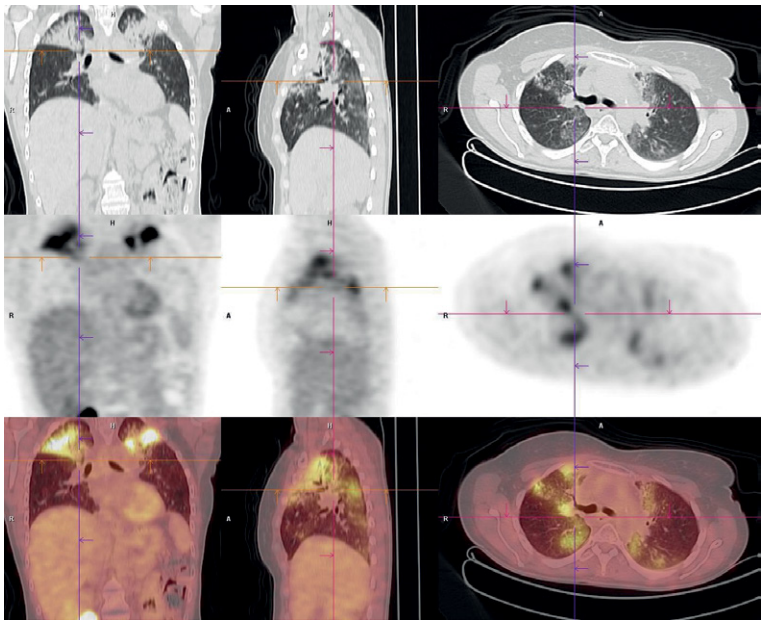

FIGURE 1. PET-CT patient1: Suspicious relapse in pulmonal tissue.

Our patients after autologous stem cell transplantation

In our observation through past few years, we found three different cases of pulmonary granulomatosis and sarcoidosis in lymphoma patients. All three patients were young females, aged between 33 and 40 years. Two of them had the primary mediastinal large B cell lymphoma and one had the diffuse large B-cell lymphoma. All three received standard first line immune-chemotherapy, relapsed and then following conventional salvage treatment underwent high dose treatment and autologous stem cell transplantation (AuSCT). All three had the control PET-CT scan 4-6 months following AuSCT (Figure 1, 2 and 3). Patient in Figure 1 had suspicious infiltrates in the lungs, paramediastinally and in the right pulmonary hilus. The thoracic X-ray was normal. The transbronchial biopsy showed granulomatosis and with bronchoalveolar lavage lymphocytic alveolitis with abnormal CD4/CD8 ratio was discovered. Therefore, the diagnosis of sarcoidosis was set and the patient initiated the corticosteroid therapy. Patient in Figure 2 had suspicious infiltrates on PET-CT paratracheally, below the carina, close to the aortic arcus, between the liver and thoracic wall, under the diaphragm, in the spleen, some lesions in the liver and in the mesenterium, nodular lesions in the lungs and in the left and right pulmonary hili. The X-ray revealed enlarged mediastinum due to the lymph nodes. The transbronchial biopsy showed granulomatosis and the bronchoalveolar lavage discovered lymphocytic alveolitis with abnormal CD4/CD8 ratio. A normal serum level of calcium and angiotensin converting enzyme was found in the blood. The corticosteroid therapy was initiated. Patient in Figure 3 had an enlarged mediastinum on the X-ray of the thorax and suspicious infiltrates on PET-CT in the neck region, paratracheally, behind the aortic arc, in the right and left pulmonary hili, subcarinally, between the pancreas and the stomach and beside the hepatic artery and inferior vena cava. The transbronchial biopsy showed granulomatose changes, yet alveolitis was not present and

the serum concentrations of calcium and angiotensin converting enzyme were normal. The patient was observed and the corticosteroid therapy has not been initiated. At present, all three patients are under regular control and none of them relapsed with lymphoma for the second time between 6 and 46 months following AuSCT. None of our patients complained of any symptoms of systemic sarcoidosis like the two cases described below.[12,13]

All three patients were immunocompromised following the stem cells transplantation. Lymphadenopathy in immunocompromised patients may be observed with acute viral infections (Cytomegalovirus, Ebstein-Barr virus), sarcoidosis, and infections due to mycobacteria, Cryptococcus spp., and with some drug reactions (e.g. trimethoprim-sulfamethoxazole).[14]

Sarcoidosis and malignancies

In the 1960s, a higher incidence of cancer was observed in sarcoidosis patients. Lung cancer occurred 3 times and malignant lymphoma 11.5 times more frequently than in control population.[15] In 1986, Brincker introduced a new condition called the sarcoidosis-lymphoma-syndrome.[16] Most of the patients with this syndrome had chronic, active sarcoidosis and later developed the lymphoproliferative malignancy. The most frequently observed was Hodgkin lymphoma, yet NHL, chronic lymphocytic leukaemia and paraproteinaemia were also being noticed. The latest onset of malignancy was 35 years after sarcoidosis had been confirmed.[16]

Cohen and Kurzrock define malignancy and sarcoidosis in three settings: sarcoidosis followed by a hematologic malignancy, sarcoidosis followed by a solid tumor and sarcoidosis as a paraneoplastic reaction due to malignancy. They also described sarcoid reaction (i.e. the development of noncaseating granulomas in patients who do not fulfil the criteria for systemic sarcoidosis)[17], which is restricted to regional lymph nodes or the visceral organ of tumor origin, but rarely appears in the skin.[18] Several studies evaluated a number of sarcoid reactions on a PET-CT scan before or after treatment of a malignancy.[17,19-22]

A few authors report malignancy first and then followed by the histologically proven sarcoidosis.[12,13,23-25] Suen *et al*. reported of a reverse condition than Bricker, they called it a "syndrome of sarcoidosis following malignancy with or without chemotherapy".[12] The malignancies reported were lymphoproliferative diseases[13], breast[12,17] and ovarian cancer[12,25], Hodgkin lymphoma[24], MALT lymphoma[26], nonseminomatous malignant germ cell tumor, osteosarcoma and oral floor carcinoma.[17] We found two separate articles describing two patients who underwent autologous stem cell transplantation and were later diagnosed with sarcoidosis, which was not present at the time of treatment of lymphoma.[12,13] One was a 41-year-old male with diffuse large B-cell lymphoma, who un-

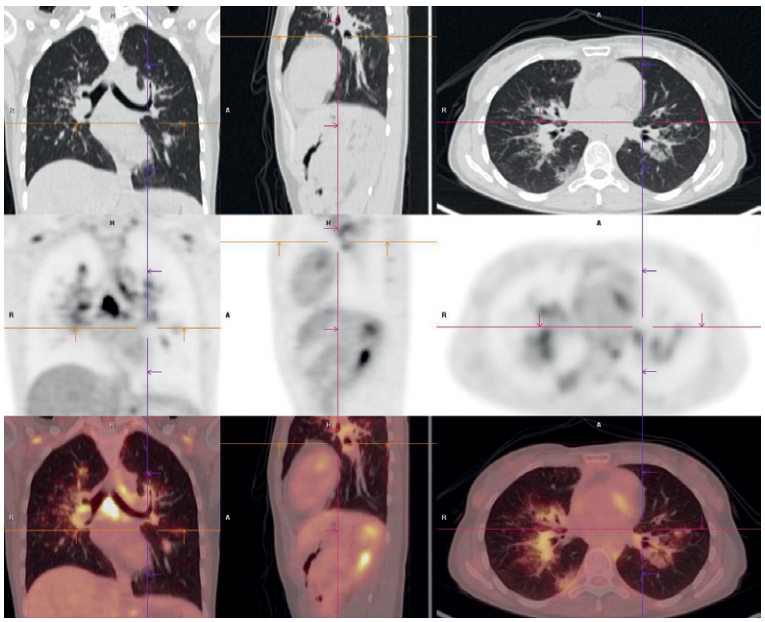

FIGURE 2. PET-CT patient 2: Suspicious relapse in hilar lymph nodes.

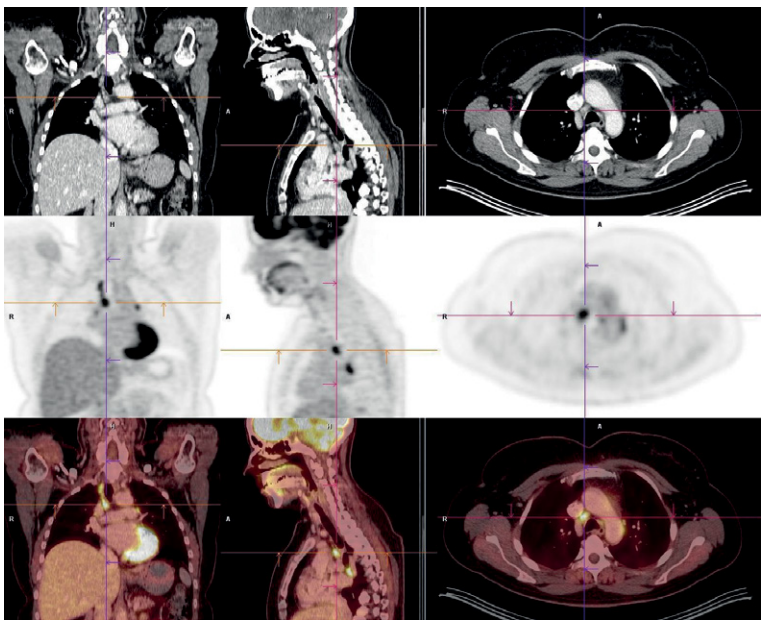

FIGURE 3. PET-CT patient 3: Suspicious relapse in mediastinal lymph nodes.

derwent chemotherapy and AuSCT and after few months complained of arthralgias with undulating fever and erythematous lesions of the skin, which were attributed to sarcoidosis.[13] The other case was a 28-year-old male with T-cell lymphoma who underwent chemotherapy and allogeneic stem cell transplantation (AlSCT). After 15 months he complained of weight loss, cough and dyspnea. He was treated with steroids, yet developed severe pulmonary fibrosis and died 28 months after the onset of sarcoidosis.[12]

The largest study by Ulaner *et al.* involved 251 patients after autologous or allogeneic stem cell transplantation treatment for lymphoma. In AlSCT group, 50 out of 107 patients had FDG-avid lesion after transplantation, 21 of which were histologically identified as benign – there were no specific data provided by the authors about the biopsies, benign changes were attributed to subclinical inflammation due to graft-versus-host-disease or graft-versus-lymphoma process. In AuSCT group, 65 out of 144 patients had FDG-lesion after transplantation on a PET-CT scan, 91% of them were demonstrated to be lymphoma by biopsy or follow-up imaging.[2] Considering their subdivision of groups, our three patients would fit in the 9% of non-malignant changes after AuSCT, which are by their criteria a rare entity.

The pathogenesis of sarcoidosis after treatment of malignancy is still unknown and different theories and hypotheses were proposed. Merchant *et al.* suggested that sarcoidosis develops as a direct immunosuppressive effect of chemotherapy or due to the influence of a specific chemotherapy agent, bleomycin, which is known to have a relatively high lymph node, skin and lung tissue concentrations.[24] Brincker, on the other hand, proposed that the formation of epithelioid-cell granulomas is driven by the antigenic factors derived from tumor cells, eliciting an immunological hypersensitivity reaction which results in granuloma formation.[22] And finally, Kornacker *et al*. suggested that the development of sarcoidosis is a consequence of an underlying immunologic disturbance associated with the primary malignancy.[13]

Conclusions

The PET-CT is the recommended diagnostic tool for post-treatment assessment in some of the NHL – namely the FDG-avid ones. It gives us valuable information about the residual disease but it is not one hundred percent accurate. Our cases clearly demonstrate that the confirmation by biopsy of a residual mass or lymph node is needed as there are other pathologies that could mimic relapse and would lead us into the wrong direction of treatment. Sarcoidosis prior to malignancy is a known condition, though not very frequently observed. Sarcoidosis after treatment of malignancy is even less frequent but still possible. Biopsy is crucial.

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research

Prognosis estimation under the light of metabolic tumor parameters on initial FDG-PET/CT in patients with primary extranodal lymphoma

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Background. Non-Hodgkin's lymphomas arising from the tissues other than primary lymphatic organs are named primary extranodal lymphoma. Most of the studies evaluated metabolic tumor parameters in different organs and histopathologic variants of this disease generally for treatment response. We aimed to evaluate the prognostic value of metabolic tumor parameters derived from initial FDG-PET/CT in patients with a medley of primary extranodal lymphoma in this study.

Patients and methods. There were 67 patients with primary extranodal lymphoma for whom FDG-PET/CT was requested for primary staging. Quantitative PET/CT parameters: maximum standardized uptake value (SUVmax), average standardized uptake value (SUVmean), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) were used to estimate disease-free survival and overall survival.

Results. SUVmean, MTV and TLG were found statistically significant after multivariate analysis. SUVmean remained significant after ROC curve analysis. Sensitivity and specificity were calculated as 88% and 64%, respectively, when the cut-off value of SUVmean was chosen as 5.15. After the investigation of primary presentation sites and histopathological variants according to recurrence, there is no difference amongst the variants. Primary site of extranodal lymphomas however, is statistically important ($p = 0.014$). Testis and central nervous system lymphomas have higher recurrence rate (62.5%, 73%, respectively).

Conclusions. High SUVmean, MTV and TLG values obtained from primary staging FDG-PET/CT are potential risk factors for both disease-free survival and overall survival in primary extranodal lymphoma. SUVmean is the most significant one amongst them for estimating recurrence/metastasis.

Key words: 18-fluorodeoxyglucose positron emission tomography/computed tomography; metabolic tumor parameters; primary extranodal lymphoma

Introduction

Non-Hodgkin's lymphomas (NHLs) arising from the tissues other than primary lymphatic organs (lymph nodes, bone marrow, spleen, thymus and Waldeyer's ring of pharyngeal lymphatics) are named primary extranodal lymphoma (PEL).[1,2] Although PEL can arise in almost every organ, gas-

trointestinal tract is the most frequently involved localization. Its incidence accounts for 30–40% of all extranodal cases in hospital and population-based series published so far. The most common locations in gastrointestinal system (GIS) are stomach (50–60%) and the small intestine (approximately 30%).[3] PEL usually presents at stage I-II in up to 74% of the patients.[4] Disseminated nodal disease involving an extranodal site is different from PEL. Extranodal involvement is seen in approximately 25–40% of lymphomas and less common in Hodgkin's lymphoma (HL).[5] On the other hand, involvement of an extranodal organ as the predominant site with a few minor draining lymph nodes (LNs) only, can be categorized as PEL.

PELs have different etiopathogenesis, genetic origin, biologic features, clinical characteristics and outcome. It has been claimed in previous studies that extranodal lymphomas should be regarded as separate nosological entities.[6] Computed tomography (CT) is the most frequently used imaging modality in the management of patients with PEL. CT, 18-fluorodeoxyglucose positron emission tomography (FDG-PET) and FDG-PET/CT are used to stage PEL. FDG-PET is a superior imaging technique which proved its utility especially in oncologic field. It is able to show functional alterations that precede the anatomical changes. Integration of CT to FDG-PET combines anatomical detail with functional information and yields excellent anatomic and functional information, increasing accuracy and detection capability. All these advantages of FDG-PET/CT potentially makes it a superior imaging modality for primary staging, evaluation of treatment response and restaging in PEL just like in other types of HL and many of NHL lymphomas.

FDG-PET/CT also has a high prognostic value with respect to overall survival (OS) and disease-free survival (DFS). The semi-quantitative measurement of standardized uptake value (SUV) is an easy-to-calculate and noninvasive index reflecting FDG metabolic rate. Its assessment has additional prognostic value in early response to treatment and long-term outcome in lymphoma patients and improves the prognostic value of the test manifestly according to visual analysis.[7] A great majority of the studies pertaining to PEL in literature evaluated metabolic tumor parameters in different primary sites (organs) and histopathologic variants generally for treatment response. We aimed to evaluate the prognostic value of metabolic tumor indices over quantitative parameters derived from initial FDG-PET/CT in patients with a medley of PEL in this study.

Patients and methods

There were 67 patients of NHL with PEL histopathologically proven by biopsy in our retrospective cohort study. The study was conducted at Nuclear Medicine Department of a training and research hospital of a medical school between 2004 and 2015. FDG-PET/CT was requested for primary staging. These patients were treated and followed up by Medical Oncology Department of our hospital. CD20-positive cases were treated by R-CHOP protocol (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone), CD20-negative cases by CHOP protocol. Radiotherapy (RT) was applied in selective cases for curative purpose or consolidation.

The patients were followed by clinical history, physical examination, LDH and sedimentation rate measurement, haemogram, liver function tests, CT and/or FDG-PET/CT. Information and data were obtained from clinic follow-up files, radiation therapy records, physician records of other departments at our hospital or personal contact with the patients by telephone. Extranodal disease with LN involvement, cutaneous T-cell lymphomas or cases originating from LN, spleen, thymus, bone marrow and Waldeyer's ring were excluded from the study. Patients who didn't have primary staging FDG-PET/CT and inadequate follow-up were also omitted. Patients having a primary extranodal site with a minor regional LN, primary head and neck lymphomas not originating from the lymphatic tissues of this region were included. Primary orbital extranodal lymphomas were accepted as CNS lymphoma, primary natural killer (NK)/T-cell lymphomas of nose and paranasal sinuses as head and neck lymphoma.

Staging with PET/CT is usually reserved for highly metabolically active (high-grade) PEL and it is not an appropriate method for MALT lymphomas because of potential false negative results.[8] But this is not a definite rule for primary staging of PEL of MALT type. The histopathological diagnosis was MALT lymphoma in our 12 patients and PET/CT results might be false negative necessitating the exclusion of these cases from the study. However, all these cases with MALT lymphoma had no other metastasis detected by primary staging FDG-PET at initial diagnosis (no false negative results were seen). This was proven by CT component, other imaging modalities (USG, MR), laboratory tests and clinical staging. Besides, no recurrence/metastasis was seen during their follow-ups. According to our study design, primary site (organ) and variants of PEL (DLBC, MALT, T cell, Burkitt, man-

tle cell) were accepted as predefined risk factors. Also, they belong to an organ (some of the orbital lymphomas and many of gastric lymphomas were MALT type). Although these patients had MALT lymphomas, we included them in the study due to the above mentioned reasons.

FDG-PET/CT imaging protocol

Patients fasted for 6 hours and their blood glucose level had to be under 150 mg/dl before the injection of an activity of 370–555 MBq of 18F-FDG according to patient's weight. Image acquisitions were performed 1 hour later with an integrated PET/CT scanner (Discovery 690-GE Healthcare). Unenhanced low dose CT and PET emission data were acquired from mid-thigh to the vertex of the skull in supine position with the arms raised over head. CT data were obtained by automated dose modulation of 120 kVp (maximal 100 mA), collimation of 64 × 0.625 mm, measured field of view (FOV) of 50 cm, noise index of 20% and reconstructed to images of 0.625 mm transverse pixel size and 3.75 mm slice thickness. PET data were acquired in 3D mode with scan duration of 2 min per bed position and an axial FOV of 153 mm. The emission data were corrected in a standardized way (random, scatter and attenuation) and iteratively reconstructed (matrix size 256 × 256, Fourier rebinning, VUE Point FX [3D] with 3 iterations, 18 subsets).

Visual and quantitative interpretation

Quantitative PET/CT parameters used in the study were maximum standardized uptake value (SUVmax), average standardized uptake value (SUVmean), metabolic tumor volume (MTV) and total lesion glycolysis (TLG). They were calculated according to a standard protocol on a dedicated workstation (Volumetrix for PET-CT and AW volume share 4.5, GE Healthcare, Waukesha, WI, USA). SUV max and SUV mean corrected for body weight were computed by standard methods from the activity at the most intense voxel in three-dimensional tumor region from the transaxial whole body images on attenuation-corrected PET/CT images. MTV (cm^3) was measured with semiautomatic PET analysis software using an automatic isocontour threshold method based on a theory of being greater than 42% of the SUVmax value within the tumor. TLG values were calculated by multiplying MTV and SUVmean.

We retrospectively examined demography, clinic, histology, clinical stage, response to treatment and outcome of the patients. OS was defined as the time from diagnosis to death of any cause (including ones other than the disease itself too) or to the last follow-up. DFS was defined as the time from diagnosis to detection of relapse or to the last follow-up. Ann-Arbor staging system and definitions were used in this study.

Statistical analysis

The whole data were analyzed using IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY:IBM Corp. Number and percentage values were used for the description of categorical data; mean, median, standard deviation (SD), minimum (min) and maximum (max) values were used for the description of continuous data. Univariate and multivariate Cox regression models were performed to determine related factors with disease free survival time. The variables having a value of $p < 0.20$ were included in multivariate analysis. Backward LR (logistic regression) elimination method was used to refine regression model. ROC (receiver operating characteristic) curve was drawn to evaluate the diagnostic value of SUVmean, MTV and TLG. SUVmean was dichotomized by splitting two groups according to ROC curve. Kaplan-Meier method with log-rank test was used to compare disease free survival times of SUVmean groups. One way ANOVA test was used for the comparison of histopathological variants of PEL according to metabolic tumor parameters. Chi-square test was used for the comparison of primary site and histopathologic variant of PEL according to recurrens/metastasis (rec/met). Informed consent was deemed as a retrospective study using records, documents and data of patients referred to our clinic for the test. The study was approved by Our Institutional Review Board Committee.

Results

Mean age of the patients at diagnosis was 52 ± 17 years (2–87). 30% of the patients were female (n: 20), 70% (n: 47) male (male/female ratio: 2.35). 42/67 (63%) of the patients had DLBC, 12/67 (18%) MALT, 5/67 (7.5%) T cell, 4/67 (6%) Burkitt and 4/67 (6%) mantle cell (MC) lymphoma. 25/67 (37%) of the cases in our study group was GIS lymphoma, 8/67 (12%) testis lymphoma, 11/67 (16.5%) central nervous system (CNS) lymphoma, 13/67 (19.5%) bone lymphoma, 7/67 (10.5%) head and

TABLE 1. Patient characteristics and demography; clinicopathologic features and follow-up data

Patient no	Age	Gender	Histology	Organ	Presentation site	Rec/Met	Ex	SUV max	SUV mean	MTV	TLG	DFS	OS
1	52	M	DLBC	GIS	Colon	-	-	7.6	4.5	12.5	56.3	44	44
2	75	M	DLBC	GIS	Stomach	-	-	26.9	15.2	1212	18422	40	40
3	45	M	DLBC	GIS	Pancreas	+	+	20	13.1	113	1483	10	18
4	65	M	DLBC	GIS	Jejunum	-	-	10	4.5	7	31.3	67	67
5	52	F	DLBC	GIS	Colon	+	-	8.6	5.8	37.3	216.4	23	143
6	72	F	DLBC	GIS	Stomach	+	+	27.4	18.1	29.9	541.3	8	26
7	69	M	MC	GIS	Stomach	-	+	10	5.2	70.1	367.6	35	35
8	64	F	MALT	GIS	Stomach	-	-	5.1	2.6	57	148.2	63	63
9	52	F	DLBC	GIS	Rectum	+	-	14.8	7.1	13.7	97.7	4	39
10	82	M	DLBC	GIS	Stomach	-	+	15	8.8	90.1	792.8	48	48
11	50	M	DLBC	GIS	Ileum	-	-	6.1	4.4	35.2	154.8	27	27
12	25	M	MALT	GIS	Duodenum	-	-	6.6	4.1	30.8	126.3	122	122
13	47	M	DLBC	GIS	Stomach	-	-	9.9	5.7	96	547.2	111	111
14	65	F	MALT	GIS	Stomach	-	-	10.1	6	135.2	811.2	88	88
15	62	M	MC	GIS	Jejunum	-	-	5.2	3.1	29	89.9	40	40
16	80	F	DLBC	GIS	Stomach	-	-	5.2	2.85	10	28.5	59	59
17	35	M	DLBC	GIS	Stomach	-	-	39.9	21.1	144	3037	17	17
18	87	M	T cell	GIS	Colon	-	-	7.2	4	14.3	57.2	5	5
19	33	M	MALT	GIS	Stomach	-	-	3.2	2	17.9	36.5	34	34
20	57	M	MALT	GIS	Ileum	-	-	7.6	4	18.2	72.8	61	61
21	61	F	DLBC	GIS	Stomach	-	+	20.1	11.15	32.1	358.2	70	70
22	56	M	DLBC	GIS	Stomach	-	+	15.1	8.3	50.5	419.1	123	123
23	49	M	MALT	GIS	Stomach	-	-	3.45	2.8	8.5	23.75	57	57
24	77	M	MALT	GIS	Stomach	-	-	2.9	2.7	7.9	21.25	160	160
25	21	M	Burkitt	GIS	Colon	+	-	10.6	5.2	468	2423	7	32
26	60	M	DLBC	Testis	L;R testicle	+	-	14.8	8.1	98	793.8	21	34
27	53	M	DLBC	Testis	L testicle	-	-	6.5	4	124	496	50	50
28	66	M	DLBC	Testis	L testicle	-	-	7.2	3.8	45	171	101	101
29	68	M	DLBC	Testis	R testicle	+	+	6.9	4.5	143	643.5	16	26
30	67	M	DLBC	Testis	L testicle	+	+	7.8	4.3	112.5	483.7	24	88
31	2	M	Burkitt	Testis	R testicle	-	-	7.5	3.8	33	125.4	42	42
32	21	M	DLBC	Testis	L testicle	+	+	8.6	5.7	128	729.6	9	12
33	57	M	DLBC	Testis	L testicle	+	+	9.5	6.2	77	477.4	35	47
34	56	F	DLBC	CNS	Corpus callosum	+	+	19.2	10.4	43.9	456.4	8	58
35	31	M	DLBC	CNS	Occipital lobe	+	+	9.8	6.5	36.3	236	6	27
36	52	F	MALT	CNS	R orbit	-	-	3.1	2	5.6	11.2	119	119
37	49	M	DLBC	CNS	Frontoparietal lobe;cerebellum	+	+	16.2	8.9	183	1628.7	6	9
38	66	F	DLBC	CNS	Parietooccipital lobe	+	-	9.8	7.2	30.2	217.2	9	30
39	64	M	MC	CNS	R orbit	+	-	3.7	2.9	2.6	7.45	19	38
40	40	F	DLBC	CNS	Cerebellum	+	-	17.5	10.5	10	105	3	7
41	66	M	MALT	CNS	R orbit	-	-	5.8	3.8	1.95	7.4	33	33
42	45	M	DLBC	CNS	Occipital lobe; cerebellum	+	+	22.3	12.4	63.3	782.2	3	3

Patient no	Age	Gender	Histology	Organ	Presentation site	Rec/Met	Ex	SUV max	SUV mean	MTV	TLG	DFS	OS
43	60	M	MALT	CNS	L orbit	-	-	7.1	4.5	2.6	11.8	36	36
44	34	F	DLBC	CNS	Cerebellum; lateral ventricle	+	+	15.6	8.2	21.8	180.1	9	11
45	50	F	DLBC	Bone	Sacrum	-	-	29.1	13.3	218	2896	34	34
46	45	F	DLBC	Bone	Maxilla	+	+	12.2	6.9	29	200.1	18	24
47	85	M	T cell	Bone	Maxilla	+	-	10.5	6.2	14	88	43	95
48	53	F	DLBC	Bone	Mandible	-	-	6.4	3.5	8.2	28.4	64	64
49	69	M	DLBC	Bone	Ethmoid bone	-	-	7.2	5.1	23	117.3	76	76
50	66	M	DLBC	Bone	Distal femur	-	+	8.5	6.4	13	83.2	43	43
51	69	F	DLBC	Bone	Sacrum	-	+	13.6	7.9	190	1501	93	93
52	49	M	DLBC	Bone	Sphenoid bone	-	-	5.7	3.2	61.4	196.5	129	129
53	43	F	DLBC	Bone	T11 vertebrae	-	-	7.2	4.8	8.2	39.4	59	59
54	49	M	DLBC	Bone	Mandible	-	-	13.2	7.5	17.2	129	119	119
55	15	F	DLBC	Bone	Ilium;sacrum	-	-	6.8	4.3	39.2	168.6	114	114
56	27	M	DLBC	Bone	Sphenoid bone	+	-	9.1	5.6	22	123.2	36	123
57	23	M	DLBC	Bone	Iliac bone	-	-	9.7	6.1	43.5	265.4	31	31
58	23	M	T cell	HN	Nose	+	+	9.1	5.7	38.2	217.7	13	35
59	42	M	DLBC	HN	Nasopharynx	-	-	7.1	3.9	9	35.1	100	100
60	39	F	DLBC	HN	Velum (palatum molle)	-	-	18.6	10.2	20	203	41	41
61	41	M	Burkitt	HN	Gum	+	+	16.6	8.5	18.6	158.1	16	23
62	54	M	MC	HN	Nasopharynx	-	-	7.9	4.4	5	22	127	127
63	75	M	T cell	HN	Parotid gland	+	+	10.2	6.6	27.6	182.2	13	29
64	53	M	T cell	HN	Nose	-	-	11.2	6.4	8.3	53.1	133	133
65	41	F	Burkitt	Breast	R breast	+	-	42	14.5	108	1555	10	63
66	36	M	MALT	Lung	L lung	-	-	3.8	2.3	7.75	17.7	50	50
67	48	M	MALT	Lung	R lung	-	-	6.4	3.95	109.9	431.8	53	53

CNS = Central Nervous System; DLBC = Diffuse Large B Cell; GIS = Gastrointestinal System; HN = Head and Neck; F = Female; M = Male; MALT = Mucosa-associated Lymphoid Tissue; MC = Mantle Cell; L = Left; R = Right; Rec = Recurrence

neck lymphoma, 2/67 (3%) pulmonary lymphoma and 1/67 (1.5%) breast lymphoma. 62/67 (92.5%) of our patients were at stage I, 5/67 (7.5%) at stage II. Mean SUVmax value was 11.5 ± 7.8 (2.9–42), average SUVmean 6.5±3.8 (2–21.1), mean MTV 73.75 cm^3 (1.95–1212, median: 30.8), mean TLG 696 (7.4–18422, median: 180). Mean OS was 59 ± 39 months (3–160). Mean DFS was 49 ± 40 months (3–160). 21 patients (31%) died, 25 patients (37%) developed recurrence and/or metastasis during the follow-up. Patient characteristics and demography, clinicopathologic features and follow-up data were detailed in Table 1. 6 patients died of causes other than the disease (cardiovascular events, aging, etc). 15 patients died of the disease itself (widespread metastasis and its complications). OS at 5th year was 75%, at 10th year 70%. Recurrence rate was 37.5%. Average period untill recurrence or metastasis was 14.5 months (3–43). DFS was 81% at first year, 67% at second year, 58% at fifth year.

Univariate cox regression was performed for all potential risk factors (sex, age, pathology, primary site, SUVmax, SUVmean, MTV, TLG) impacting recurrence/metastasis development. Factors with $p < 0.2$ values after univariate analysis (SUVmax, SUVmean, MTV, TLG and age) were processed with multivariate model. SUVmean, MTV and TLG were found statistically significant after multivariate analysis. The results of univariate and multivariate Cox regression analyses are shown in Table 2,3. ROC curve drawn to evaluate the diagnostic value of SUVmean, MTV and TLG is shown in Figure 1. SUVmean remained significant after ROC curve analysis. One unit increment of SUVmean amplifies recurrence rate 1.4 times. Sensitivity and specificity were calculated as 88%

and 64%, respectively, when the cut-off value of SUVmean was set at 5.15. Cut-off values, sensitivity and specificity of SUVmean, MTV and TLG are shown in Table 4. SUVmean was dichotomized by splitting two groups according to ROC curve. Kaplan-Meier method with log-rank test was used to compare DFS of SUVmean groups. Kaplan-Meier curve drawn for SUVmean with a cut-off value of 5.15 is shown in Figure 2. When we analyze metabolic tumor parameters for histopathological subtypes, SUVmax and SUVmean prove meaningful (p = 0.003 and p = 0.005, respectively). After the investigation of primary presentation sites and histopathological variants according to recurrence, there is no difference amongst the variants. Primary site (organ) of extranodal lymphomas however, appears to be statistically important (p = 0.014). Testis and CNS lymphomas have higher recurrence rate (62.5%, 73%, respectively). Risk of recurrence/metastasis development increases 3.5 times in testis lymphomas and 216 times in CNS lymphomas with comparison to GIS lymphomas.

TABLE 2. Univariate Cox regression analysis

Factors	Significance (p value)	Hazard Ratio	95% CI for Hazard Ratio	
			Lower	Upper
Sex*	0.363	0.495	0.108	2.254
Age	0.080	0.971	0.939	1,004
DLBC**	0.265		Reference	
Mantle Cell	0.672	0.550	0.034	8.783
T Cell	0.038	10.535	1.135	97.758
Burkitt	0.720	1.535	0.147	15.982
MALT	0.962	0.000	0.000	-
GIS***	0.000		Reference	
Testis	0.163	3.503	0,602	20.378
CNS	0.000	216.611	20.786	2257.305
Bone	0.898	1.135	0.165	7.818
Head and neck	0.916	0.879	0.080	9.709
Lungs	0.999	3.422	0.000	-
SUVmax	0.032	0.680	0.478	0.968
SUVmean	0.000	3.630	1.791	7.355
MTV	0.001	1,035	1.015	1.056
TLG	0.011	0.996	0.993	0.999

Reference groups: *male sex, **DLBC = Diffuse Large B Cell ,***GIS = Gastrointestinal System

TABLE 3. Multivariate Cox regression analysis

Factors	Significance (p value)	Hazard Ratio	95% CI for Hazard Ratio	
			Lower	Upper
SUVmean	0.000	1.418	1.226	1.640
MTV	0.000	1.020	1.009	1.031
TLG	0.002	0.998	0.996	0.999

MTV = metabolic tumor volume; TLG = total lesion glycolysis

TABLE 4. Cut-off values, sensitivity, specificity of SUVmean, MTV and TLG

Factors	Cut-off Value	Sensitivity (%)	Specificity (%)
SUVmean	5.15	88	64
MTV (cm3)	18.4	84	45
TLG	175.55	76	64

MTV = metabolic tumor volume; TLG = total lesion glycolysis

Discussion

FDG-PET/CT was performed for 435 patients with NHL during this study in our department. The incidence of PEL in our study group is 15% (67/435) and apparently under the literature average. Because our patients formed a highly selective population after a meticulous exclusion according to the study criteria. The peak incidence is in the 6th-7th decade with a male predominance.[9] Average age of our study group is 52 years with male preponderance and younger according to literature. Firstly, we want to give descriptive information about our patients with a medley of PEL.

The most frequent form of PEL is constituted by GIS lymphomas. Stomach is the most common site of primary GIS lymphoma and MALT lymphoma is the most common variety.[10] Small intestine fills the second ranking. A heterogeneous group of lymphomas including MALT, DLBC, MC, Burkitt and T cell affect the small bowel. Primary colon lymphoma has features similar to small bowel disease with wall thickening without obstruction.[11] DLBC, Burkitt and T cell lymphomas are strongly FDG-avid. 25/67 (37%) of our patients had primary GIS lymphoma. 14/25 (56%) of them were primary gastric lymphoma, 5/25 (20%) primary intestinal lymphoma and 5/25 (20%) primary colon lymphoma. 5/14 (36%) of gastric lymphomas were MALT type, while 8/14 (57%) DLBC variant (Figure 3). DLBC variants exhibited usually high FDG accumulation. MALT types had variable (usually moderate) uptake. Our incidence of gastric DLBC outnumbered gastric MALToma. This is an interesting result contrary to the literature. Other findings are nearly the same as in previous studies.

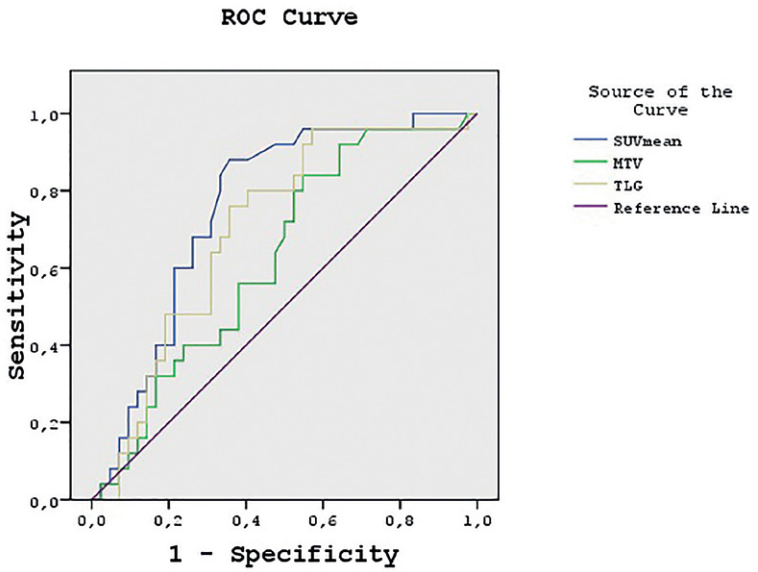

FIGURE 1. ROC curve for SUVmean, metaboloc tumor volume (MTV) and total lesion glycolysis (TLG).

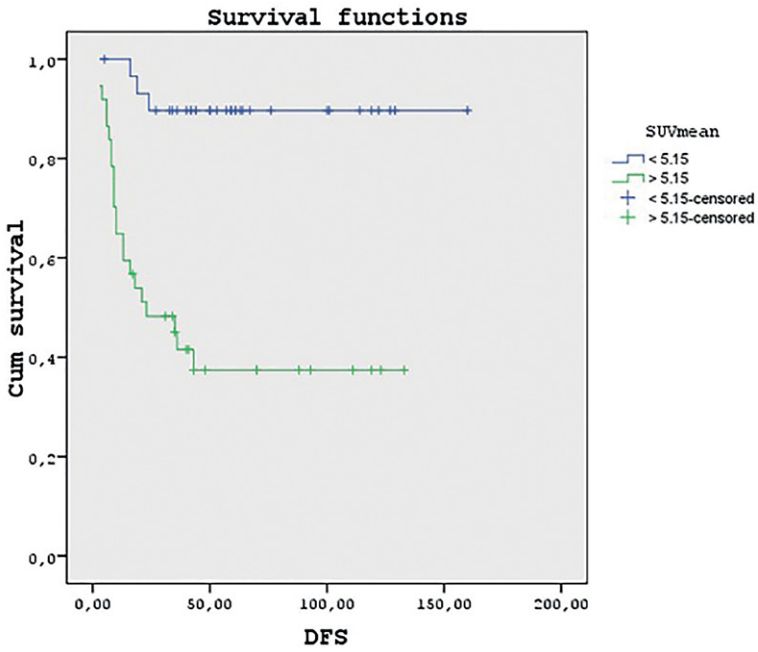

FIGURE 2. Kaplan-Meier curve of SUVmean with a cut-off value of 5.15.

Primary testicular lymphoma is mostly DLBC and accounts for up to 5% of testicular masses presenting with painless swelling. It is usually aggressive with spread into the nervous system.[12] Asymmetrical intense FDG uptake is usually seen. Over half of our patients either recurred or metastasized mainly into the nervous system. The disease showed its wicked face during its fatal cruise. Primary CNS lymphomas account for approximately 6.6–15.4% of CNS neoplasms and are usually of DLBC type.[13] Although MRI is the choice of imaging modality due to the fact that presence of high physiologic FDG activity in cerebral cortex may hinder the visualization of lesions, FDG-PET/CT is now well established in the evaluation of CNS lymphomas with a pattern of intense FDG uptake. All our cases of primary CNS lymphoma were DLBC type with high FDG accumulations. The disease was very aggressive and fatal (Figure 4). All the cases recurred and 5/7 (71%) of the patients died during the follow-up. Orbital lymphomas constitute approximately 8% of extranodal disease. Marginal zone (MALT) lymphoma is the most frequent variant, DLBC is the second most common type.[14] They are invariably FDG-avid ranging from moderate to high uptake.[14] 4/11 (36%) of our CNS lymphomas were primary orbital lymphoma and mostly MALT showing mild to moderate uptake. Their prognosis was indisputably very well contrary to the intracranial DLBC subtype.

Primary extranodal head and neck lymphomas are usually DLBC variant showing marked and asymmetrical FDG-avidity with the enlargement of organs and corresponding changes in the anatomical contours. A particular variant affecting the nose and paranasal sinuses is the NK/T cell variant. It is a locally aggressive form of lymphoma involving the nasal cavity, septum, paranasal sinuses and hard palate with the erosion of underlying bone unlike DLBC.[2] These lesions are also intensely FDG-avid. Our patients had DLBC and T cell variants showing intense FDG-avidity too. Primary bone lymphoma is most usually a DLBC type and shows intense uptake.[15] Our patents are fully in agreement with the literature. Primarily lung lymphoma is more common with HD than with NHL.[16] Lung involvement is usually associated with mediastinal nodal disease in HD, as NHL presents with lung disease alone.[16] The most common histologic variant of primary lung lymphoma is MALT arising from the bronchus.[17] Lung MALToma has variable FDG uptake. There were two patients with lung MALToma having mild to moderate uptake in our study group concordant with the literature. Primary breast lymphomas constitute 0.1–0.5% of all breast neoplasms.[18] Involvement is by mostly DLBC with intense FDG-avidity. Our single case of primary breast lymphoma was a Burkitt which is an extremely rare variant in the breast.

There is a correlation between FDG uptake and histologic grade of lymphoma. Although low-grade NHLs such as follicular lymphoma and MC lymphoma do not demonstrate FDG-avidity to the same degree that high-grade lymphomas do, they

are still FDG-avid enough to be determined.[19] MC lymphoma is a subtype of NHL. It accounts for approximately 5% of all cases of lymphoma.[20] The majority of patients present with advanced-stage disease and often have extranodal sites of involvement. These patients have a poor prognosis with a median survival of 3 to 4 years.[20] MC lymphomas in the study took up mild FDG and had good prognosis. However, it must be taken into consideration that our patients were at stage I. MALT lymphoma is the third most common NHL following only DLBC and follicular lymphoma in incidence and it comprises approximately 8% of all NHL.[21] Most studies report that MALT lymphomas show moderate to high FDG accumulation.[21,22] But a few studies with limited numbers of patients claim that FDG-PET imaging is unreliable for primary extranodal MALT lymphomas.[19,21,22] We found usually moderate uptake and 50% decreased recurrence risk according to DLBC in our cases of MALT lymphoma with a favorable prognosis.

DLBC lymphoma is the most common histologic subtype of NHL accounting for approximately 25% of NHL cases.[23] 42/67 (63%) of our patients were DLBC with high FDG uptake. Burkitt lymphoma is a highly aggressive B-cell NHL. It is the most frequent NHL in childhood (30–40%), presenting almost always as a rapidly growing tumoral mass in the abdomen (60–80%, typically in the ileocecal region).[24] Our patients with Burkitt lymphoma had high FDG uptake and their prognosis was bad. T cell lymphomas (PTCL) are a heterogeneous group of generally aggressive neoplasms that constitute less than 15% of all NHLs in adults.[25] Our cases had bad prognosis with intense FDG-avidity. We found 10.5 times increased recurrence risk in T cell lymphomas in comparison to DLBC.

Fifteen (22%) patients died of the disease itself (widespread metastasis) and its complications. 5/15 (33%) of them had CNS, 4/15 (27%) testis, 3/15 (20%) head and neck, 2 GIS, one bone lymphoma. Of these, 12/15 (80%) were DLBC, 2 T cell and 1 Burkitt lymphoma. We observed complete remission in 42 patients and DFS was 54% at the end of the study (at 160th month). Mean follow-up time of this group was 72 months (13–160). OS at 5th year was 75%, 70% at 10th year. These results are in line with the other studies in literature.

FDG-PET/CT is being widely used in many cancers and lymphoma patients. Some quantitative metabolic parameters derived from initial staging PET/CT (SUVmax, SUVmean, MTV, TLG) have also been used in prognosis estimation and evaluation of treatment response for many cancers and lymphomas. They consume glucose at a higher metabolic rate reflected by the abnormal FDG uptake. This event is measured by SUV and correlates with cellular metabolism.[26] SUVmax is the first used one and represents the highest FDG uptake within the tumor. SUVmean is the average activity in a tumor volume. More lately increasing recognition of volume-based metabolic parameters (MTV and TLG) emerged for this purpose.[27]

Esfahani *et al.* researched TLG and other parameters in DLBC for DFS estimation on initial and interim PET.[28] They found TLG the most signifi-

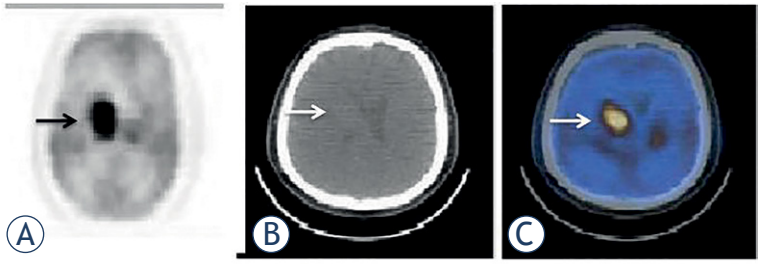

FIGURE 3. There was a mass in antrum of stomach on transaxial CT **(A)**, PET **(B)**, fusion **(C)** and maximum intensity projection (MIP) images **(D)** (arrows) of a 60-year old female patient with primary gastric lymphoma of diffuse large B cell (DLBC) type. She had metabolic tumor parameters of SUVmax: 11, SUVmean: 5, matabolov tumor volume (MTV): 34 cm^3, total lesion glycolysis (TLG): 150. Her outcome was excellent with a disease free survival (DFS) and overall survival (OS) of 111 months.

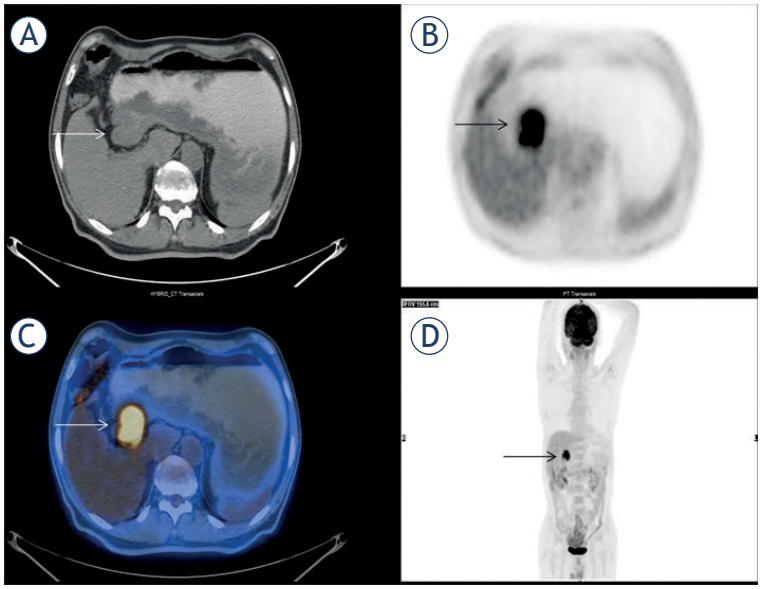

FIGURE 4. 61-year old female patient was diagnosed with primary central nervous system (CNS) lymphoma of diffuse large B cell (DLBC) type. There was a mass in right periventricular region adjacent to right thalamus on transaxial PET **(A)**, CT **(B)** and fusion **(C)** images (arrows). She was in serious risk because of her high metabolic tumor parameters (SUVmax: 35, SUVmean: 25, metabolic tumor volume (MTV): 425 cm^3, total lesion glycolysis (TLG): 2543) and died of the disease 11 months after the diagnosis.

cant parameter with regard to recurrence and their recurrence rate was 30%.[28] Gallicchio *et al.* in their study of 52 patients found these quantitative parameters helpful in the management of DLBC lymphoma.[29] Especially TLG proved its utility in this area and came out as a striking predictor in many cancers and lymphomas. As it combines the assessment of tumor volume and metabolism, it can stratify patients or predict the effectiveness of therapy regimens. Ceriani *et al.* in their cohort study of 103 patients with DLBC showed that TLG is the most powerful predictor on baseline PET/CT.[30] However, no study is available researching the use of these parameters in a mixed group of PEL patients with different subtypes currently. Most of the studies investigated them for separate organs and unique variants with limited numbers of patients or compared different treatment approaches. To the best of our knowledge, our study is the first one in which the prognosis of a mixed group of PEL was predicted with these metabolic indicators. The results of previous studies on PEL are controversial with respect to the use of metabolic tumor parameters for prediction of their prognosis in the literature. After evaluation of all potential risk factors affecting metastasis/recurrence development with univariate cox regression analysis and multivariate model; SUVmean, MTV and TLG were found to have statistically significant correlation with DFS time in our study. The most meaningful of them was SUVmean. The first used metabolic index, SUVmax is not as effective in our study as compared with the previous ones claiming that it is the most useful in many of the studies. SUVmax can be a misleading metabolic parameter for some tumors in which cells are in different phases of mitotic cycle, causing nonuniform FDG distribution. SUVmean may reflect tumoral activity more correctly in these cases. When we evaluated the diagnostic value of SUVmean over ROC curve, we observed a sensitivity of 88% and a specificity of 64% with a cut-off value of 5.15. First impressions show that metabolic tumor parameters, especially SUVmean may be used in the management of PEL. However, our results should be supported with studies of larger number of subjects in more specific subgroups with regard to primary site (organ) with unique variants.

Conclusions

High SUVmean, MTV and TLG values obtained from primary staging FDG-PET/CT are potential risk factors (predictors) for both disease-free survival and overall survival in patients with PEL. SUVmean is the most significant one amongst them for estimating the risk of recurrence/metastasis development.

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research

Anatomic variations of the pancreatic duct and their relevance with the Cambridge classification system: MRCP findings of 1158 consecutive patients

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Background. The study was conducted to evaluate the frequencies of the anatomic variations and the gender distributions of these variations of the pancreatic duct and their relevance with the Cambridge classification system as morphological sign of chronic pancreatitis using magnetic resonance cholangiopancreatography (MRCP).

Patients and methods. We retrospectively reviewed 1312 consecutive patients who referred to our department for MRCP between January 2013 and August 2015. We excluded 154 patients from the study because of less than optimal results due to imaging limitations or a history of surgery on pancreas. Finally a total of 1158 patients were included in the study.

Results. Among the 1158 patients included in the study, 54 (4.6%) patients showed *pancreas divisum*, 13 patients (1.2%) were defined as *ansa pancreatica*. When we evaluated the course of the pancreatic duct, we found the prevalence 62.5% for descending, 30% for sigmoid, 5.5% for vertical and 2% for loop. The most commonly observed pancreatic duct configuration was Type 3 in 528 patients (45.6%) where 521 patients (45%) had Type 1 configuration.

Conclusions. Vertical course ($p = 0.004$) and Type 2 ($p = 0.03$) configuration of pancreatic duct were more frequent in females than males. There were no statistically significant differences between the gender for the other pancreatic duct variations such as *pancreas divisium*, *ansa pancreatica* and course types other than vertical course ($p > 0.05$ for all). Variants of *pancreas divisum* and normal pancreatic duct variants were not associated with morphologic findings of chronic pancreatitis by using the Cambridge classification system. The *ansa pancreatica* is a rare type of anatomical variation of the pancreatic duct, which might be considered as a predisposing factor to the onset of idiopathic pancreatitis.

Key words: pancreas divisum; pancreatic duct variants; magnetic resonance imaging; magnetic resonance cholangiopancreatography

Introduction

Pancreatitis can be fatal and remains a serious disease. It occurs in two forms as acute and chronic with different clinic, morphological and histological features.[1] Chronic pancreatitis is characterized by progressive inflammation, fibrosis of pancreas leading to irreversible structural changes.[1] Excessive alcohol consumption, cigarette smoking, autoimmunity, high protein diet, heredity factor and also several morphological anomalies of the pancreaticobiliary ductal system like abnormal

pancreaticobiliary junction and *pancreas divisum* are thought as causes of chronic pancreatitis.[1,2] Except *pancreas divisum*, pancreatic duct variations are not well evaluated.[3] To define risk factors for the appearance of chronic pancreatitis and to understand the pathophysiology including further underlying causes of chronic pancreatitis are important.

Normal variants and congenital anomalies of the pancreas and the pancreatic duct may not be detected in asymptomatic patients until maturity and even when they are detected, it is often incidental.[4,5] The variations and anomalies of the pancreatic and the biliary ductal system are commonly experienced during radiologic examinations.[6] Endoscopic retrograde cholangiopancreatography (ERCP) is considered as the gold standard for the evaluation of pancreatic ductal system and chronic pancreatitis because of its superior spatial resolution and its ability to show main duct and side branch abnormalities with severity assessed using the Cambridge classification. The Cambridge classification divides chronic pancreatitis to five severity groups according to morphologic changes of the main pancreatic duct and its side branches from normal or equivocal to mild, moderate, severe.[7] Magnetic resonance cholangiopancreatography (MRCP) is becoming more commonly used in the noninvasive evaluation of the pancreatic and the biliary ducts.[1-8] MRCP detects pancreatic ductal system and also these variations with similar accuracy as the invasive technique of endoscopic retrograde cholangiopancreatography (ERCP).[3,9] In MRCP, heavily T2 weighted sequences are used to image fluid filled structures without using contrast agent. MRCP has certain advantages over ERCP, it is safer (no exposure to ionizing radiation, no using contrast agent, no premedication), it can be used for staging malignancy and it does not carry the risk of developing complications, can be applied during acute attacks of pancreatitis and cholangitis, gives the chance to view the extraductal structures by using the conventional T1–T2-weighted images.[10] Also, the Cambridge classification has been modified for the MRCP technique.[11-13] The using of MRCP in adult patients with persistent and unexplained signs and symptoms such as abdominal pain, nausea and vomiting resulting from chronic pancreatitis or gastric outlet obstruction gives an option to look for a developmental anomaly of pancreas and pancreatic duct. It is important to recognize of these anomalies because they may be a surgically correcTable cause of recurrent pancreatitis or the cause of gastric outlet obstruction. Awareness of these anomalies may provide useful information in surgical planning and prevent of inadvertent ductal injury.

Pancreas divisum is the most frequent anatomical variation of pancreatic ductal patterns.[2-6] While the frequency of classical *pancreas divisum* has been evaluated to be between 5 and 10% in large series[2], the frequency of other anatomic variations of pancreatic duct, sub-type of *pancreas divisum* and the gender distributions of these variations of the pancreatic duct and their relevance with the Cambridge classification system are unknown. This study was conducted in order to evaluate anatomic variations and developmental anomalies of pancreatic duct; including the variations of the course and the configuration of the pancreatic duct, anomalous pancreaticobiliary ductal junction, subtypes of *pancreas divisum* and the gender distributions of these variations of the pancreatic duct. We also aimed to show their relevance with the Cambridge classification system which we used for scoring ductal changes as morphological sign of chronic pancreatitis with the largest sample size in the literature best of our knowledge. This sample consists of a group of patients who underwent MRCP for various reasons in one center during a certain time interval.

Patients and methods

Patient population

We retrospectively reviewed MRCPs obtained at our radiology department between January 1, 2013 and August 30, 2015 after obtaining the approval of the ethical board. A total of 1312 cases were examined, 154 cases with less than optimal results due to imaging limitations, failure to visualize the main pancreatic duct and with a history of surgery on pancreas, were excluded from the study. A total of 1158 cases were included in the study. The study was performed according the Helsinki Declaration and the Institutional Review Board Committee was approved it.

Imaging

We performed the MRI examinations of the patients in our radiology department using a 1.5 T MR device (Philips Achiva, Philips Medical System, the Netherlands). Patients were informed about the MRCP imaging and following a 6-hour fasting period and after any metal items or objects on the patients which may produce artifacts were removed. Oral or intravenous contrast material was not used during the investigations. But

TABLE 1. MRI sequence parameters

	TR	TE	MATRIX	N OF SLICE	SLICE THICKNESS	FOV	NSA	TSE-TFE FACTOR	SLAB THICK.
T2 W Images (Ax and Coronal)	962ms	100 ms	256x256	24	6 mm	350-400 mm	2	158	-
GRE Balanced TFE (Ax and Coronal)	4 ms	1.24 ms	156x213	24	7 mm	300-400 mm	2	219	-
3D-TSE T2 W respiratory-triggered	1466 ms	650 ms	256x256	1	0.8 mm	250-300 mm	1	105	40 mm

Ax = axial; ETL = echo train length; GRE = gradient-recalled echo; N = number; NSA = number of signal acquired; TE = echo time; TFE = turbo field echo; TR = repetition time; TSE = turbo spin echo; W = weighted

in some cases, we also performed abdominal MRI with intravenous contrast material at the time of MRCP or sometimes after MRCP when we found some lesions which need additional information.

In all patients, MR examinations were made including coronal and axial T2- weighted turbo spin echo (TSE) images (repetition time [TR]: 962 ms, echo time [TE]:100 ms, Matrix: 256 x 256, number of slice: 24, slice thickness: 6 mm, field of view [FOV]: 350–400 mm, SENSE factor: 4, number of signal acquired [NSA]: 2), coronal and axial gradient-recalled echo (GRE) balanced turbo field echo (TFE) images (TR: 4 ms, TE: 1.24 ms, Matrix: 156 x 213, number of slice: 24, slice thickness: 7 mm, FOV: 300–400 mm, Flip angle: 80, NSA: 2). The choledochus was located in the images in the axial-coronal plane, then respiratory-triggered high-resolution with SENSE 3D-TSE T2-weighted (TR: 1466 ms, TE: 650 ms, echo train length [ETL]: 128, matrix: 256 × 256, NSA: 1, slice thickness: 0.8 mm, FOV: 250–300 mm); para-coronal MRCP source and maximum intensity projection (MIP) reformatted images were obtained. MRCP is performed with heavily T2-weighted sequences with a torso phased-array coil (Table 1).

Evaluation of the images

Two radiologists with experience in abdominal imaging of 15 years (Z.H.A.) and 1 year (M.A.) reviewed the MRCP images retrieved from Picture Archiving and Communication System (PACS) of our hospital. Discordant interpretations were subsequently resolved by consensus of the 2 radiologists.

The course of the pancreatic duct was evaluated as descending (Figure 1), sigmoid (Figure 2), vertical (Figure 3), and loop (Figure 4) shaped courses.[5,14]

The ductal configuration was evaluated as Type 1–Type 5 (Figure 5). At Type 1, there was a bifid configuration with dominant duct of Wirsung, at Type 2, there was a dominant duct of Santorini without *divisum*, at Type 3, Wirsung duct was seen

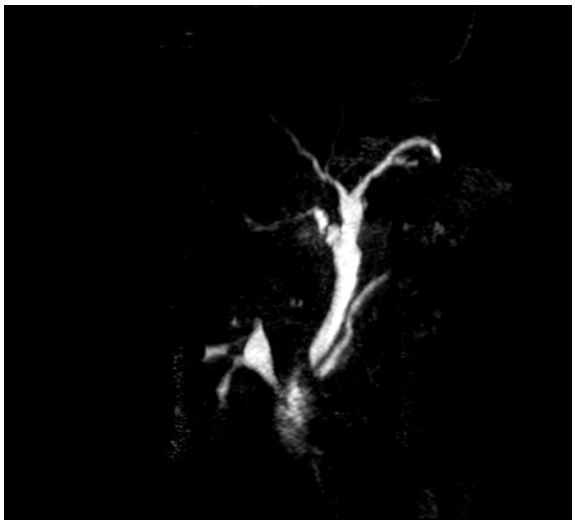

FIGURE 1. Descending course of pancreatic duct in 60 year of woman who had cholecystectomy. There is a Type 3b variation (the right posterior duct drained into the main hepatic duct) at the level of bifurcation of the biliary ducts and mild forms of renal pelvis dilatation.

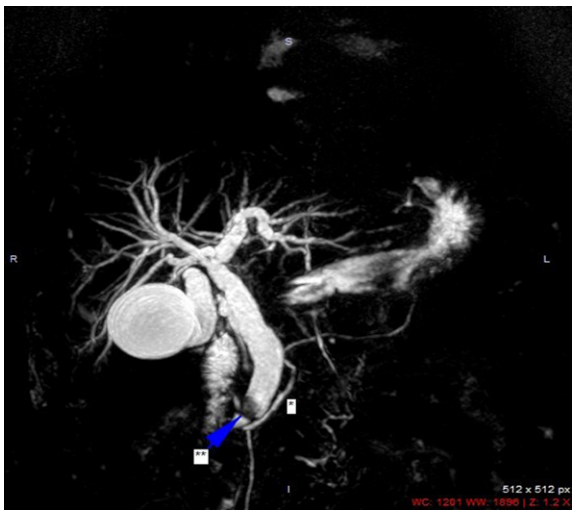

FIGURE 2. Sigmoid course of pancreatic duct in 49 year- old woman with and trifurcation at the level of bifurcation of the biliary ducts (*): Sigmoid course of the pancreatic duct, (**): choledocholithiasis.

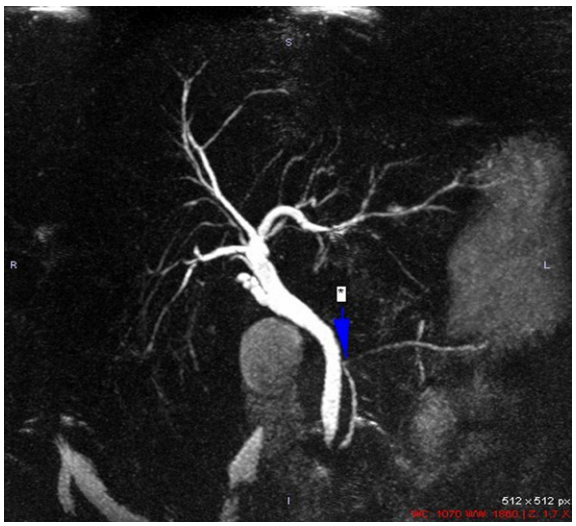

FIGURE 3. Vertical course (*) of pancreatic duct in 55 year-old woman who had cholecystectomy.

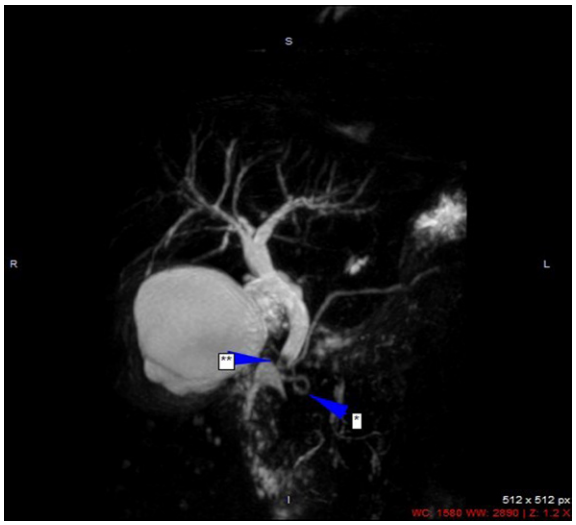

FIGURE 4. Loop course (*) of pancreatic duct in in 38 year-old woman with choledocholithiasis (**).

with absent duct of Santorini. We mentioned *pancreas divisum* as Type 4 and 'ansa pancreatica' as Type 5, where the duct of Santorini formed an inferior loop and connected with a side branch of the duct of Wirsung in the *uncinate* process.[5,6,14]

Three variants of *pancreas divisum* were evaluated as; in subtype 1 or classical *divisum*, there was total failure of fusion; in subtype 2, there was only dominant dorsal drainage with the absence of the duct of Wirsung; in subtype 3 or incomplete *divisum*, a small communicating branch was present.[5]

Anomalous pancreaticobiliary ductal junction was described the abnormal junction of the common biliary duct (CBD) and the pancreatic duct outside the duodenal wall forming a long common channel (> 15 mm).[5,6]

We used the Cambridge classification system which has been modified for the MRCP technique: Cambridge 1 (normal pancreas): pancreatic ducts are normal; Cambridge 2 (*equivocal pancreas*): 1–2 side branches and main duct 2–4 mm, Cambridge 3 (mild disease): ≥ 3 side branches and main duct 2–4 mm; Cambridge 4 (moderate disease): ≥ 3 side branches and main duct > 4 mm; Cambridge 5 (marked disease): additional feature include a large cavity, obstruction, a filling defect, severe dilatation or irregularity[11-13] MRCP was called normal when main duct calibers at the pancreatic head, body, and tail were less than 3 mm, 2.5 mm, and 1.5 mm, respectively, and when no pancreatic duct side branch ectasia was identified. We could not differentiate Cambridge 2 and 3 from each other where main pancreatic duct was normal by using MRCP; we evaluated Cambridge 2 and 3 together.

Statistical analysis

Fisher's exact test and χ square test were used as statistical methods in the study. When samples were small and the assumptions for the χ square were violated, the Fisher's exact test was used. For example, in a 2 x 2 Table when expected cell counts were less than 5, or any were less than 1 even, Yates correction does not work and Fisher exact test was used instead of χ square test. Statistical significance was

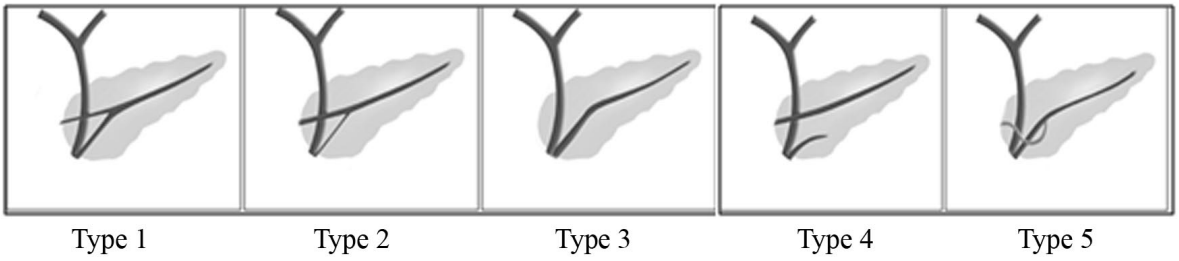

FIGURE 5. Variations in the configuration of the pancreas duct. Type 1: Bifid configuration with dominant duct of Wirsung, Type 2: Bifid configuration with dominant duct of Santorini without divisum, Type 3: Rudimentary non-draining duct of Santorini, Type 4: *Pancreas divisum*, Type 5: *Ansa pancreatica*.

TABLE 2. The distribution of the course types of main pancreatic duct

	Number of variations n (%)	Male n (%)	Female n (%)
Descending type	724 (62.5)	321 (65.5)	403 (60)
Sigmoid type	343 (30)	134 (27.5)	199 (30)
Vertical type	68 (5.5)	17 (5.4)	51 (7.8)
Loop type	23 (2)	8 (1.6)	15 (2.2)
Total	**1158**	**490**	**668**

TABLE 3. The distribution of ductal configuration types of main pancreatic duct

Variation in configuration	Number of Variations, n (%)	Male, n (%)	Female, n (%)
Type 1	521 (45)	233 (47.5)	288 (43.1)
Type 2	42 (3.6)	11 (2.3)	31 (4.6)
Type 3	528 (45.6)	220 (44.9)	308 (46.1)
Type 4	54 (4.6)	19 (3.9)	35 (5.3)
Type 5	13 (1.2)	7 (1.4)	6 (0.9)
Total	**1158**	**490**	**668**

Type 1 = a bifid configuration with a dominant duct of Wirsung; Type 2 = a bifid configuration with dominant duct of Santorini without *divisum*; Type 3 = Wirsung duct is seen with absent duct of Santorini or rudiment duct of Santorini without communication with Wirsung; Type 4 = *pancreas divisum*; Type 5 = '*ansa pancreatica*', where the duct of Santorini forms an inferior loop and connects with a side branch of the duct of Wirsung

TABLE 4. The distribution of *pancreas divisum* subtypes

Pancreas divisum subtypes	Number of variations	Male, n (%)	Female, n (%)
P. divisum subtype 1	24 (44.4)	8 (40)	16 (47)
P. divisum subtype 2	20 (37)	7 (35)	13 (38.2)
P. divisum subtype 3	10 (18.6)	5 (25)	5 (14.8)
Total	**54**	**20**	**34**

Pancreas divisum were evaluated as; in subtype 1 or classical *divisum,* there was total failure of fusion; in subtype 2, there was only dominant dorsal drainage with the absence of the duct of Wirsung; in subtype 3 or incomplete *divisum*, a small communicating branch was present.

assumed at a P-value of < 0.05.Data documentation and statistical analyses were performed using Excel (v.2007, Microsoft Corporation, Redmond, WA, USA) and SPSS v.14 (SPSS Inc, Chicago, IL, USA).

Results

We retrospectively evaluated the results of 1312 patients who underwent MRCP at our radiology department prior to liver resection surgery or due to suspicion of pancreatobiliary disease. One-hundred fifty four cases were excluded from the study for low MRCP image quality, pancreatic head mass making it impossible to analyze the ductal system, and with history of pancreatic surgery. Finally 1158 patients were included in the study, 668 were female (57.69%) and 490 were male (42.31%). The mean age was 60.8 ± 13.4 and the ages were between 16 and 102. The mean age of female patients was 60.5; the mean age of male patients was 61.1.

When we evaluated the course of pancreatic duct, descending type was the most commonly observed (62.5%), and the second most common course type was sigmoid type (30%). The distribution of the pancreatic course types was summarized in Table 2.

The most commonly observed pancreatic duct configuration was Type 3 in 528 patients (45.6%) where 521 patients (45%) had Type 1 configuration. The distribution of the ductal configuration of pancreas is shown in Table 3.

Pancreas divisum was seen in 54 (4.6%) patients. The distribution of *pancreas divisum* subtypes was shown in Table 4. The anatomic variations between the genders are also shown Table 2–4.

There were only two female patients (0.17%) who had pancreatobiliary junction anomaly.

The relationship between Cambridge types and the distribution of the course types, ductal configuration types of main pancreatic duct, *pancreas divisum* subtypes were shown at Table 5.

Besides the anatomical variations of pancreatic duct, we did not observe any *annular pancreas*, pancreatic agenesis, hypoplasia of dorsal pancreas, accessory lobe; we did not demonstrate ectopic pancreas.

Discussion

The pancreas and the pancreatic ductal embryology is moderately complicated, a number of congenital ductal variations have been described such as complete or incomplete *pancreas divisum*, functional *pancreas divisum*, *ansa pancreatica*, *annular pancreas*, anomalous pancreaticobiliary junction.[5,6] We used MRCP to determine the frequency of anatomic variations of the main pancreatic duct and the prevalence of *pancreas divisum* and its subtype.

Our study has the largest sample size in the literature to the best of our knowledge, was to evaluate the frequency of anatomic variations of pancreatic duct by using MRCP during a certain time interval. When we evaluated the course of pancreatic ducts of our study population, descending type was the most common type (62.59%) where sigmoid type

TABLE 5. The relationship between Cambridge classification and the distribution of the course types, ductal configuration types of main pancreatic duct, *pancreas divisum* subtypes

	Cambridge 1; n - (%)	Cambridge 2 and 3; n - (%)	Cambridge 4; n - (%)	Cambridge 5; n - (%)	TOTAL n - (%)
Descending type	612 (85)	82 (11)	25 (3)	5 (1)	724 (62.5)
Sigmoid type	292 (85)	34 (10)	13 (4)	4 (1)	343 (30)
Vertical type	57 (84)	7 (10)	3 (4)	1 (2)	68 (5.5)
Loop type	17 (74)	4 (17)	2 (9)	0 (0)	23 (2)
TOTAL	**978 (85)**	**127 (11)**	**43 (4)**	**10 (1)**	**1158 (100)**
Type 1	445 (85)	54(10)	18 (4)	4 (1)	521 (45)
Type 2	34 (81)	6 (14)	2 (5)	0 (0)	42 (3.6)
Type 3	447 (84)	57 (11)	19 (4)	5 (1)	528 (45.6)
Type 4	44 (81)	8 (15)	2 (4)	0 (0)	54 (4.6)
Type 5	8 (62)	2 (15)	2 (15)	1 (8)	13 (1.2)
TOTAL	**978 (85)**	**127 (11)**	**43 (4)**	**10 (1)**	**1158 (100)**
PD subtype 1	19 (79)	4 (17)	1 (4)	0 (0)	24 (44.4)
PD subtype 2	16 (80)	3 (15)	1 (5)	0 (0)	20 (37)
PD. subtype 3	9 (90)	1 (10)	0 (0)	0 (0)	10 (18.6)
TOTAL FOR PD	**44 (81)**	**8 (15)**	**2 (4)**	**0 (0)**	**54 (100)**

PD = *pancreas divisum*

was the second one as seen in Table 2. In literature, the course of the pancreatic duct varies greatly and the most common one is a descending course with 50%.[5,14] Itoh *et al.*[14] evaluated pancreatic duct of 77 patients by using multi-slice computed tomography (MSCT) in 2003. Their study population was very small comparing to ours and also they used different imaging modality which was not specific for pancreatic ducts. Shu *et al.*[15] evaluated MRCP investigations of 300 patients, they found that the pancreatic duct courses included descending (66.0%, 192/300), sigmoid (16.0%, 48/300), vertical (10.7%, 32/300), and loop configurations (9.3 %, 28/300) in Chinese population. Gonoi *et al.*[16] evaluated that 2.2% (11/504) of subjects had loop and reverse-Z type pancreatic course in Japanese. In our study group, descending course was found in about 62.5%, sigmoid course 30% and vertical course 5.5%, loop course 2%, so the prevalence of vertical and sigmoid course were marked different from their value. The different prevalence may be because of ethnicity.

In our study, the most commonly observed pancreatic duct configuration was Type 3 in 528 patients (45.6%) where 521 patients (45%) had Type 1 configuration. These two Wirsung dominant configurations had a rate of 90.6% totally. In literature, the bifid configuration with dominant duct of Wirsung drainage is most common (60%), a rudimentary, non-draining duct of Santorini (30%), or dominant duct of Santorini without *divisum* (1%) may be present.[5,6,17] The prevalence rates of types of configuration of the pancreatic ducts are different in our study group and the different prevalence may be because of ethnicity, too. *Ansa pancreatica* is a rare variant where the duct of Santorini takes a curved or looped course before its fusion with the duct of Wirsung. We defined *ansa pancreatica* (Type 5) in 13 patients (1.2%).We could not find an exact prevalence rate of *ansa pancreatica* in literature.

Anomalous pancreaticobiliary ductal junction was found in 2 female patients (0.17%). In this condition, pancreatobiliary reflux occurs into the ducts because of the failure of the sphincter of Oddi.[18] In literature, complications or associated conditions with anomalous pancreaticobiliary ductal junction are cholodochal cyst, recurrent cholangitis, bile duct, choledocholithiasis, gallbladder cancer and peritonitis caused by spontaneous perforation.[18] Our two patients had solo anomalous pancreaticobiliary ductal junction, they did not have its complications or associated conditions. We follow up these patients.

During embryonic development, *pancreas divisum* occurs due to the failure of fusion of the ven-

tral and dorsal pancreatic buds. *Pancreas divisum* is a common finding with a reported frequency of 3–13% in autopsy, MRCP and ERCP studies.[3,6,19-21] In clinical imaging studies using MRCP, *pancreas divisum* was detected in approximately 12% of cases, typically as incidental findings.[9] Onder *at al.*[22] revealed only one patient with *pancreas divisum* among the 590 patients included in the study. Shu *et al.*[15] found the prevalence of the side branch, the Santorini duct and *pancreas divisum* as 4.67% (14/300), 44.3% (133/300) and 7.7% (23/300), respectively in Chinese population. Gonoi *et al.*[16] evaluated that the prevalence of *pancreas divisum* was 2.6 (13/504) in Japanese. We found a prevalence rate of *pancreas divisum* 4.6%. Our study population was mostly from western part of Turkey and our study population was larger than those studies. And we think that the different prevalence may be because of ethnicity, too.

In our study population, female to male ratio was 1.36. When we consider pancreatic duct anatomic variations, we found that female-to-male ratios of Type 2 (p = 0.03) configuration and vertical course (p = 0.0048) of pancreatic duct were statistically significant. The gender distributions between the other types of configurations of the pancreatic duct were not statistically significant (for Type 1, p = 0.35; for Type 3, p = 0.80; for Type 4, p = 0.29; for Type 5, p = 0.40) (Table 3). And also there were not statistically significant differences between the gender for the descending (p = 0.38), loop (p = 0.46) and sigmoid (p = 0.49) course of the pancreatic duct (Table 2). When we analyzed the distributions of subtypes of *pancreas divisum* between the gender, it wasn't any statistically significant difference (for subtype 1, p = 0.80; for subtype 2 p = 0.99; for subtype 3, p = 0.49) (Table 4).

It has been shown that MRCP has been sensitive and specific (85%–100 % for 1.5 Tesla systems) for evaluating pancreatic ductal system.[1,9,16] The ductal changes of main pancreatic duct are demonstrated on MRCP, however subtle side branch changes can be missed.[1] One of the major limitation of MRCP is the lack of functional information and inability to image the ductal system in distended condition. According to the previous MRCP-based studies, ductal alterations suggesting chronic pancreatitis were reported in approximately 16%.[23] Our study was concordant with the literature; we could only demonstrate dilated side branches of main pancreatic duct in approximately 15% of the cases (Table 5). This can be overcome by using secretin which is known as secretin stimulated MRCP (s-MRCP). Secretin injection during MRCP enhances the morphology of the main pancreatic duct and side branches and provides information on pancreatic outflow dynamics at the same time.[23-25] Secretin is a safe drug and can be administered without any serious side effect. In normal pancreas, the side branches are not visualized after secretin but in patient with early chronic pancreatitis the side branches can show dilatation which is not seen on conventional MRCP.[1,23-25] Thus s-MRCP has the capability to provide both the structural and functional information.

In our study population, dilatation of pancreatic side branches as a chronic pancreatitis feature was observed in 15% of the patients (Table 5) and 85% of the patients had normal caliber of main pancreatic duct and side branches. When we evaluated the distributions of Cambridge classification between course types and configuration types of the pancreatic duct, only Type 5 configuration (which was *ansa pancreatica*) had statistically different from Type 1, Type 3 configurations (p = 0.0059 for Type 1, P = 0.0129 for Type 3). The frequency of ductal alterations suggesting chronic pancreatitis was significantly higher in Type 5 configuration by using the Cambridge classification. The Type 4 configuration (which was pancreatic *divisum*) and *pancreas divisum* subtypes did not have any statistically significant difference from the others, our study showed no correlation between *pancreas divisum* and ductal alterations suggesting chronic pancreatitis.

There are some limitations of our study. One of them is that we do not have a reference standard such as ERCP or surgery because of ethical issues. Second is the retrospective nature of the study. And third limitation is that most of our patients underwent MRCP at our radiology department with suspected biliary or pancreatic disease, because of that our study population may not sample the whole population.

In conclusion, the results of our study indicated that variants of *pancreas divisum* and normal pancreatic duct variants were not associated with morphologic findings of chronic pancreatitis by using Cambridge classification system. But *ansa pancreatica* might be considered a relevant factor to the onset of chronic pancreatitis. We found that the drainage occurring through the major papilla via the duct of Wirsung had a rate of 90.6% and the prevalence of Type 4 (*pancreas divisum*) and vertical course as 4.6% and 5.5% respectively. The gender distributions of vertical course and Type 2 configurations of pancreatic duct were statistically significant.

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research

Endovascular treatment of unruptured aneurysms of cavernous and ophthalmic segment of internal carotid artery with flow diverter device Pipeline

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Background. Intra-arterial treatment of aneurysms by redirecting blood flow is a newer method. The redirection is based on a significantly more densely braided wire stent. The stent wall keeps the blood in the lumen of the stent and slows down the turbulent flow in the aneurysms. Stagnation of blood in the aneurysm sac leads to the formation of thrombus and subsequent exclusion of the aneurysm from the circulation. The aim of the study was to evaluate flow diverter device Pipeline for broad neck and giant aneurysm treatment.

Methods. Fifteen patients with discovered aneurysm of the internal carotid artery were treated between November 2010 and February 2014. The majority of aneurysms of the internal carotid artery were located intradural at the ophthalmic part of the artery. The patients were treated using a flow diverter device Pipeline, which was placed over the aneurysm neck. Treatment success was assessed clinically and angiographically using O'Kelly Marotta scale.

Results. Control angiography immediately after the release of the stent showed stagnation of the blood flow in the aneurysm sac. In none of the patients procedural and periprocedural complications were observed. 6 months after the procedure, control CT or MR angiography showed in almost all cases exclusion of the aneurysm from the circulation and normal blood flow in the treated artery. Neurological status six months after the procedure was normal in all patients.

Conclusions. Treatment of aneurysms with flow diverter Pipeline device is a safe and significantly less time consuming method in comparison with standard techniques. This new method is a promising approach in treatment of broad neck aneurysms.

Key words: flow diversion; Pipeline; aneurysm; ophthalmic segment

Introduction

Surgical craniotomy was the only possible treatment of brain vessels aneurysms in the past. First surgical procedures started in the early 1990s.

Intra-arterial embolization treatment of aneurysms with electrolytically detachable coils came into clinical use in 1994, when Guido Guglielmi introduced his invention.[1]

Within few years endovascular treatment became a popular method equivalent to surgery.

ISAT study was the first large-scale randomized comparative study of endovascular and surgical treatment of ruptured cerebral aneurysms.[2] Results of the study showed that endovascular therapy is safer and more effective in comparison with surgery. The study also showed that endovascular treatment improves survival. The consequence of the study was a re-thinking in the approach of the treatment of these cerebral vascular diseases.

The only disadvantage of the endovascular treatment was the appearance of the recanali-

zation of the aneurysm. Different studies have shown that this can occur in 10 to 15%, even 25% in some studies.[3,4] Recanalization rate was higher in the aneurysm with a wider neck and in complex aneurysms. With the introduction of stents and supporting balloons the percentage of recanalization of the wide neck aneurysms decreased significantly.[5,6]

Attempts were also made with a non-adhesive liquid embolic agent Onyx (ev3, Covidien, USA) and balloon remodeling. Onyx consists of a solution of 20% ethylene vinyl alcohol copolymer dissolved in dimethyl sulfoxide, to which tantalum powder is added. Tantalum powder ensures radiopaque appearance under the fluoroscopy.

There still remained a problem with the complex and gigantic aneurysms. Finding a solution for the treatment of such type of aneurysms has led to the development of a new generation of stents which are densely braided and which retain most of the blood flow within the lumen of the stent. This results in the diversion of the blood flow in the aneurysmatic sac and its slowing down which leads to the formation of a thrombus and subsequent scarification of the thrombus.[7] At the end of this process, the aneurysm is completely excluded from the circulation.[8,9] This method was used only in the cavernous sinus and the ophthalmic part of the internal carotid artery at first, but nowadays these stents are also used for the more narrow, distal A1 and M1 segments of the cerebral arteries. They are also useful for the treatment of small blister aneurysms, which are, due to their small size, unsuitable for the embolization with coils.[10]

Indications for the use of flow diverting device are still in the formation stage. Also the method itself is not yet fully defined.[11] Flow diverting device is most commonly used alone. It can be also used in combination with another flow diverting device or in combination with coils, which are inserted into the aneurysm prior to the deployment of the stent. These coils should foster the formation of the thrombus, as well as protect against a possible aneurysm rupture.

TABLE 1. Patients by age, size and position of an aneurysm, complications, and follow-up

No/Age (years)/ Gender	Clinical presentation	Location	Type	Size (mm)/ Neck (mm)	Dilatation of the stent with the PTA balloon	Late clinical complications	Type of control to confirm closure/ Status (month)	OKM Scale
1/57/ Female	Mass effect (vision loss)	Parophthalmic	S	12/5	-	-	CTA/Complete (3)	C3
2/72/ Female	Incidental (headache)	Parophthalmic	S	7/5	-	-	CTA/ Complete (14)	A2
3/55/ Female	Recurrent (coil) incidental (headache)	Parophthalmic	S	5/3	-	-	CTA/ Complete (5)	B3
4/47/ Female	Incidental (headache)	Petrous ICA	S	4/4	PTA	-	CTA/ Complete (6)	B3
5/62/ Female	Incidental (headache)	ICA – PCOM	S	6/6	-	-	CTA/ Complete (2)	D
6/57/ Female	Mass effect (vision loss)	Carotid-cavernous	S	7/4	-	-	CTA/ Complete (1)	D
7/56/ Female	Mass effect (diplopia)	Parophthalmic	S	12/7	PTA	-	CTA/ Complete (3)	C2
8/48/ Female	Mass effect (diplopia)	Parophthalmic	S	17/7	-	Stent shortening	MRA/ Complete (15)	A3
9/22/ Female	Mass effect (diplopia)	Parophthalmic	F	20/10	-	-	MRA/ Complete (6)	B2
10/52/ Female	Mass effect (diplopia)	ICA – PCOM	S	23/9	-	-	MRA / Complete (6)	B3
11/52/ Female	Mass effect (diplopia)	Parophthalmic	S	20/3	-	-	DSA/ Complete (3)	C2
12/55/ Female	Mass effect (diplopia)	Parophthalmic	S	22/5	PTA	-	MRA/Complete (6)	C1
13/47/ Female	Incidental (headache)	ICA – PCOM	S	4/3	-	-	DSA/Complete (3)	C3
14/54/ Male	Recurrent aneurysm (clip) (headache)	ICA – cisternal	F	16/14	-	-	MRA/Complete (6)	B2
15/28/ Male	Incidental (headache)	M1	S	6/5	-	-	MRA/Complete (6)	B3

CTA = computed tomography angiography; DSA = digital subtraction angiography; ICA = internal carotid artery; MRA = magnetic resonance *angiography; OKM* = O'Kelly-Marotta; PCOM = *posterior communicating artery,* PTA = percutaneous transluminal angioplasty

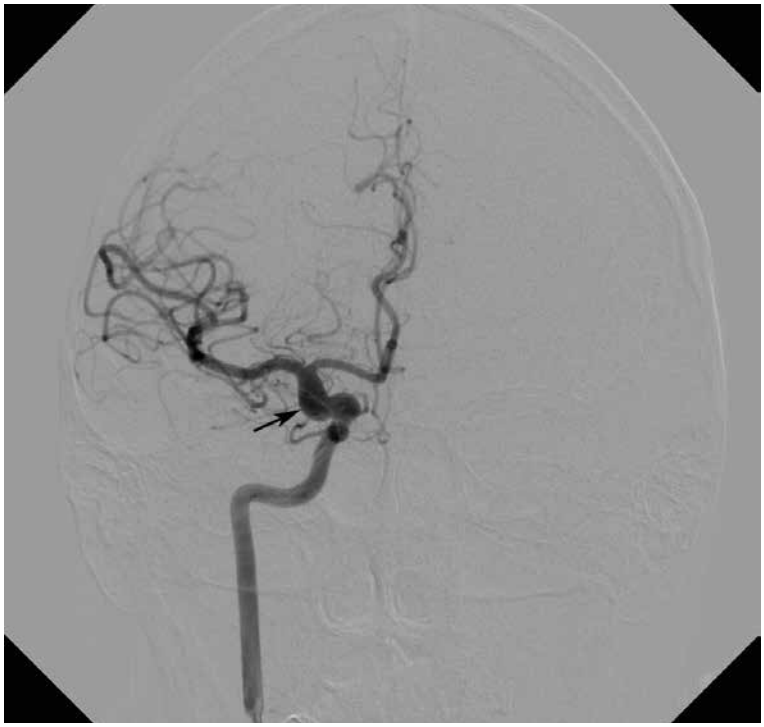

FIGURE 1. A fusiform aneurism of the internal carotid artery is indicated by the arrow.

The aim of the study was to evaluate flow diverter device Pipeline for broad neck and giant aneurysm treatment.

Patients and methods

Patients and study design

Fifteen patients were treated with a flow diverting device in our institution so far. Average age was 50.9 years (range 22–72). There were 13 female and 2 male patients. The procedures were carried out between November 2010 and February 2014. The procedures described in this study were in accordance with the Helsinki declaration. All patients gave their written informed consent before being included in the present study. This study was approved by the National Ethics Committee (No. 145/02/0).

All aneurysms were discovered accidentally or as part of the diagnostic process for headaches, visual disturbances or clinical symptoms of mass effect. None of the patient suffered subarachnoid hemorrhage.

Aneurysms were located predominantly intradural in the ophthalmic and precommunicant part of the internal carotid artery. Aneurysms were mostly of unknown etiology. Only in patient No. 9 the etiology was known. She underwent radiation therapy of the meningioma of the cavernous sinus (Table 1).

Anticoagulant protocols and drugs

The patients began with the preoperative preparation 5 days before hospitalization with 75 mg clopidogrel p.o. a day and 100 mg acetylsalicylic acid p.o. a day. Before the procedure the prothrombin time, using a standard method with a VerifyNow meter (Accumetrics, San Diego, USA) and a standardized lab test for acetylsalicylic acid and clopidogrel, was measured ensuring that the patient is not resistant to clopidogrel therapy.[12,13]

After the introduction of a guiding catheter an intravenous bolus of heparin, 5000 units, was administrated, also taking into account the weight of the patient. Heparinization continued for 24 hours after the procedure. Patients were hospitalized for one or two days and discharged with antithrombotic and antiplatelet protection with a daily dose of 75 mg clopidogrel for 6 months and 100 mg acetylsalicylic acid for a period of one year. Before hospitalization and before discharge, the neurological examination was performed. The neurological examination was also performed prior to the control MR angiography.

To prevent perioperative vasospasm 2 ml (0.2 mg/ml) nimodipine diluted in 10 ml physiological saline were administrated *i.a.* in all cases prior to the procedure. In patients with large aneurysms corticosteroids for the duration of 48 hours were prescribed for the prevention of the inflammatory response after the embolization.

Methods and the procedure

The placement of the flow diverting device is performed under general anesthesia, which ensures that the patient is perfectly calm during the procedure. In our institution we have a single-plain C-arm system Axiom Artis (Siemens, Germany).

We use a classical approach using the Seldinger technique. After the puncture of the femoral artery we introduce the angiographic catheter fist into the femoral artery and then via the aorta and the aortic arch into the common carotid artery. A detailed angiographic processing of the aneurysms with preoperative angiography in typical projections, rotating three-dimensional subtraction angiography with computerized data processing, construction and reconstruction of an aneurysm in the head-up display technique is performed (Figure 1). This al-

lows us detailed measurements of the aneurysm. It also helps us to select working projections and to assess the dimensions of the stent. Then, using an exchange wire 265 cm in length, a long vascular introducer sheath Destination (Terumo, USA) is placed into the common carotid artery. Through the introducer sheath a soft 6F guiding catheter Neuron 53 (Penumbra, USA) is introduced into the internal carotid artery at the level of the skull base. The Neuron 53 (Penumbra, USA) catheter enables us good maneuverability and is the new generation of guiding catheters, which can be introduced even through the cavernous part of the internal carotid artery all the way to its bifurcation.

5000 units of heparin bolus *i.a.* are administered followed by the passage of the neck of the aneurysm with Marxsmann microcatheter (ev3, Covidien, USA) with the help of the micro guide wire. The guide wire is than removed and the Pipeline stent with the radiopaque pusher wire (ev3, Covidien, USA) is introduced. Pusher wire, serving now as a guide wire, is introduced in the initial segment of the middle cerebral artery (M1), in order to achieve an adequate stability of the catheter. A control angiography through the guiding catheter is performed to control the position of the stent in relation to the neck of the aneurysm. Release of the stent is performed by extraction of the guiding catheter and the simultaneous introduction of the pusher wire. The stent must open completely and it must fit the vessel wall tightly (Figure 2). Then the pusher wire can be extracted and the final angiography is performed, to check the position of the stent, if the stent is completely opened and fits the vessel wall tightly. Particular attention should be paid to the possible occurrence of dissection at the start and the end of the stent. Care should be taken that the stent fits the vessel wall perfectly, otherwise clotting of blood between the stent and the vessel wall can occur, which may lead to thrombus formation, and the closure of the stent lumen (Figure 3).

After the procedure a CT examination was performed to exclude any intracranial bleeding due to vessel wall injury, which may occur because of the manipulation of the guiding wires.

Evaluation of procedure success

Closing of the aneurysm is a long process and therefore angiography after stent placement does not say much about the effectiveness of the intervention. The process of stagnation of blood and thrombosis of the aneurysms may persist for sev-

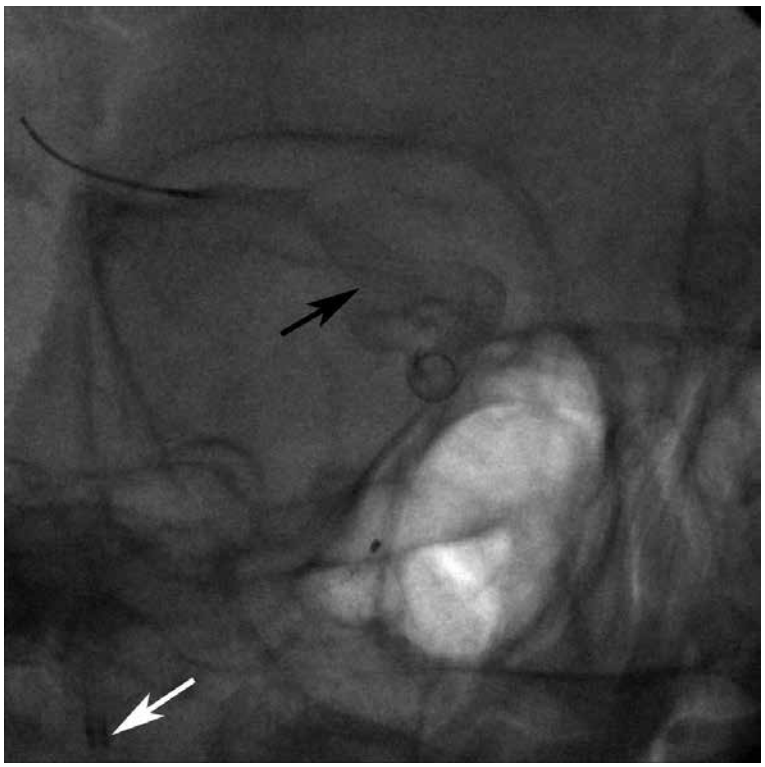

FIGURE 2. The position of the flow diverter stent on the native radiogram is indicated by the black arrow. The white arrow indicates the tip of the guiding catheter.

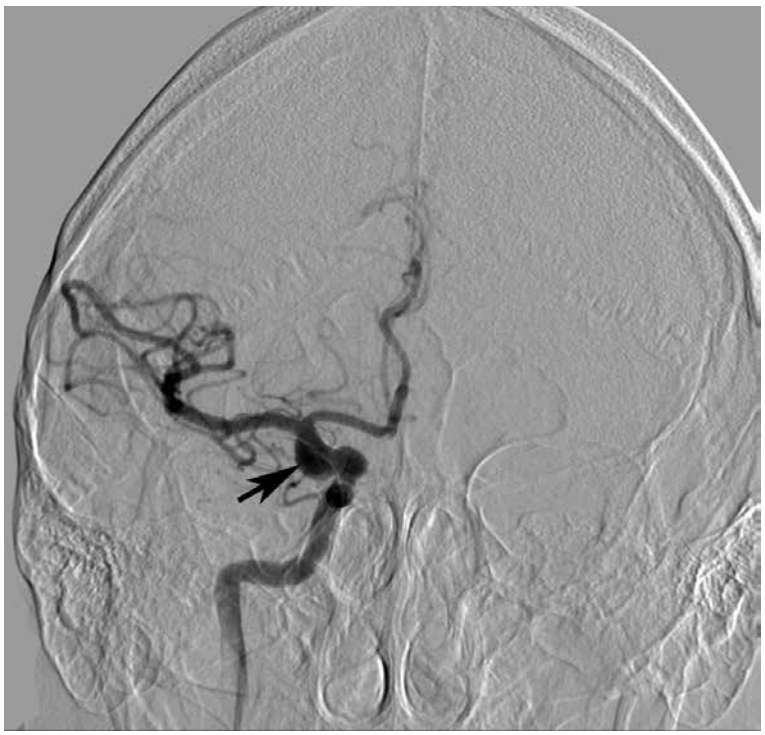

FIGURE 3. Control DSA direct after the positioning of the flow diverter stent shows some residual filling of the aneurism (arrow). The stent fits the vessel wall perfectly.

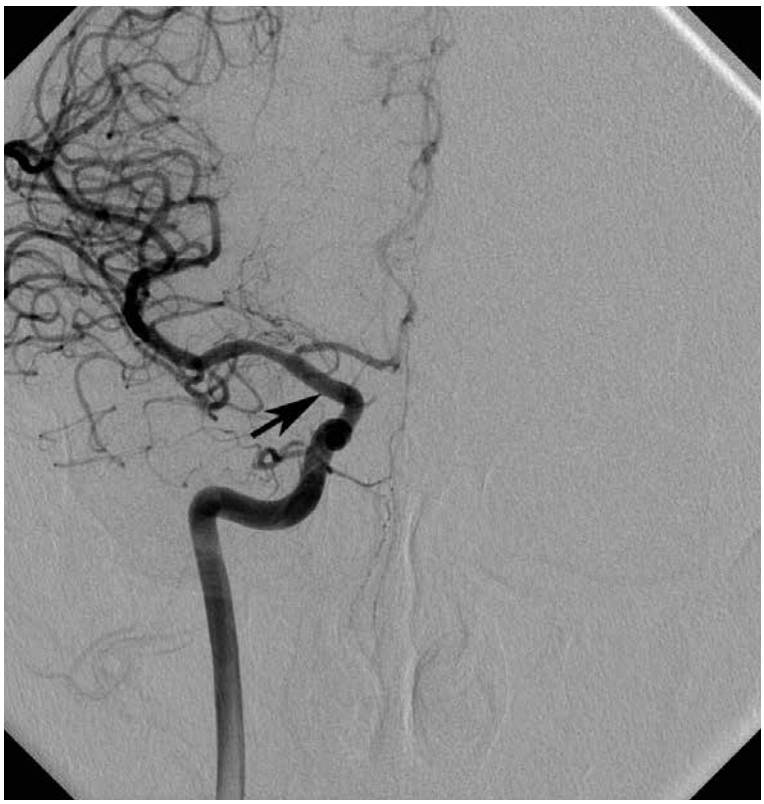

FIGURE 4. Control DSA after 12 months shows complete thrombosis of the aneurism (arrow) and good patency of the distal arteries.

eral weeks or months after placing the flow diverting stent. In the case of endovascular aneurysm closure with coils residual filling of the aneurysm, after Roy-Raymond scale, is a bad result. Residual filling of the aneurysm in the case of flow diverting stent placement however is acceptable and even desirable.[14]

Traditional evaluating scales, such as the Roy-Raymond scale, which are used for evaluating the success of the procedure in the case of endovascular and surgical aneurysm treatment, are unsuitable for evaluation of flow diverting stent procedure success. According to Roy-Raymond scale residual filling of the aneurysm after the procedure is undesirable. In the case of flow diverting stent placement however such a result, immediately after the procedure, is expected. Therefore, a new scale, named after O'Kelly and Marotta was made.[15] This scale consists of two parameters, which correspond to the mechanism of flow diverting stent action in protecting the aneurysm:

Reduced aneurysm filling (filling rate), which corresponds to the anatomical aspect.

Accelerated blood retention within the aneurysm (retention rate) in the arterial, parenchymal, and venous phase, which corresponds to a more dynamic and physiological parameter.

All of the patients have had MRA (and axial T2) between 6–12 month after the procedure to asses silent ischemic lesions and aneurysm exclusion.

To assess only stent patency and aneurysm exclusion or late reperfusion we performed either computed tomography angiography (CTA) or magnetic resonance angiography (MRA) first during 3–6 month period, second 6–12 month period and third 24 month period after the procedure.

Results

Between 2010 and 2014 we treated 15 patients with flow diverting device. In 14 patients we used one stent and in one patient it was necessary to use two stents, applying a telescope technique in which the second stent works like an extension of the first. There were no serious technical complications during the procedure in none of the patients. In three patients there was need of using a balloon for dilation of the stent to achieve tight fit to the arterial wall.

Control angiography also showed exclusion of the aneurysm from the circulation and a normal patency of the stent (Figure 4, Figure 5). In three patients with multiple aneurysms, which were covered with a Pipeline (ev3, Covidien, USA), all the aneurysms were excluded from the circulation.

All control examinations, except of two, were within normal limits. In case of one patient there were ischemic changes in the area of right nucleus lentiformis, however there were no clinically manifestations. In another patient some residual filling of the aneurysm on the 12 months MRI angiography control was observed, which was caused by flow diverter shrinkage.

The symptoms that patients had prior the procedure mostly disappeared. One patient had double images prior the procedure and was symptomless three days after the procedure. Later double vision reoccurred and lasted for 12 months. In patients in whom the stent covered the ophthalmic artery no deterioration of the vision was observed, probably due to the suction effect of the artery.[16] Patients who had headaches prior to the procedure were without symptoms after the procedure. (Figure 5)

Discussion

Large and giant aneurysms represent a challenge for both surgical and endovascular approaches.

Such aneurysms often present with mass effect and corresponding compression syndrome on the neighboring neural tissue. Therefore, the goal of treatment is not only to prevent rupture but also to reduce the pulsating mass and eliminate the associated mass effect. Unfortunately, this goal is difficult to achieve by conventional endovascular techniques.[17]

For the endovascular treatment of cerebral aneurysms, a number of options are available. We can use bare coils or coils covered with hydrogel which are 2- or 3D shaped. We can also use a balloon or a stent for remodeling the neck of the aneurysm in combination with coil embolization. The use of stents and coils can be successfully used in treatment of wide neck aneurysms. There is also a possibility of using a liquid polymer with a supporting balloon. Currently no drug eluting stents are available for treatment of aneurysms. The method of using stents and coil deployment after stent placing showed good short-term results, but the long-term results were not encouraging because of a high recanalization rate. This has been found in approximately 20%.[3-6]

With the development of technology and the development of new, much more flexible, stents with very densely braided mesh a new technique for treating these aneurysms emerged. Wider use of flow diverting stents occurred after 2010, both in Europe and in the USA. Definitive indications for the flow diverting stent usage are not yet established, but mainly it is used on randomly discovered, complex aneurysms of the internal carotid artery in both extracranial and intracranial part of the carotid artery and its branches. It is often used on abnormalities of the artery walls, which cover a longer segment of the vessel and not just saccular aneurysm as the simplest form of the lesion.[7,8]

Concerns that may arise with the use of flow diverting stents are the formation of thrombus within the aneurysm and its fibrous transformation. Review of literature and experience in placing the flow diverting stents in the treatment of the aneurysms of the abdominal aorta show that after the formation of the thrombus, especially in giant aneurysms, the thrombus can get infected causing swelling and headaches. The extent of these symptoms is often related to the size of the aneurysm. The focus of the inflammation is in the thrombus within the aneurysm.[18] In favor of this hypothesis are a number of published cases with extensive edema around a giant aneurysm before the treatment of the aneurysm. Inflammatory changes may also be present in the wall of small aneurysms,

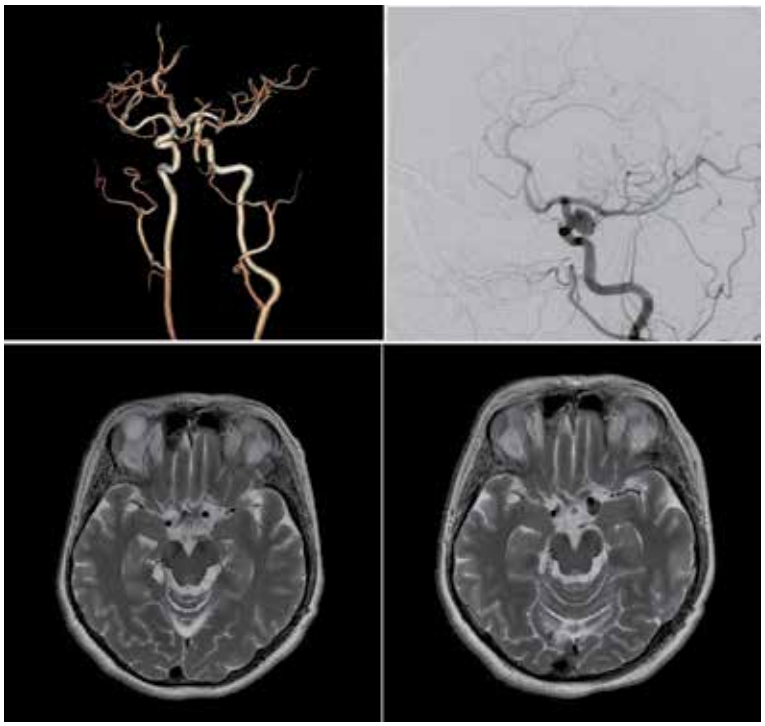

FIGURE 5. Control MR after 12 months shows complete thrombosis of the aneurism (arrow) and aneurysm collapse following flow diverter treatment.

but these changes usually do not cause complications and can heal by themselves or remain dormant. It appears that the physiology of large and giant aneurysms by itself cause inflammation. Histopathological studies have shown that the wall of the aneurysm, which is covered with a thick layer of thrombus, is thinner with less smooth muscle cells, less elastin and the increased number of inflammatory cells in comparison with the aneurysm with the thinner thrombus.[19,20]

Another problem that can occur, are the arteries that must be covered during the placement of the stent. The major problem is the ophthalmic artery. Here the placement of the stent can cause an occlusion. However, in all our patients, in which deceased filling of the ophthalmic artery was observed, additional anastomoses through external carotid artery appeared. Despite impeding flow to the ophthalmic artery using flowdiverter this did not result in clinical sequelae in the current study which correlates with Durst CR at all study.[21] The obstruction of the artery is prevented by the vacuum effect that can be best observed in the case of anterior cerebral artery covering, where after the stent placement no obstruction of blood flow is observed. The vacuum effect can be the main cause of prolonged thrombus formation.[22]

Technical barrier to successful procedure is a phenomenon of vasospasm. Therefore, initial bolus of nimodipine followed by nimodipine drip infusion during the procedure is obligatory.

Permanent stent patency does not represent a big problem. Although the treatment of these aneurysms is complicated, the stents are placed in the part of the artery, which shows no intraluminal atherosclerotic changes. At the same time, it is a relatively large artery.

There is also the question of reducing the size of the aneurysm due to thrombosis and subsequent fibrotic transformation of the thrombus after stent placement. Based on the clinical picture, we believe that this effect occurs, although indirectly (Figure 5).

In our study none of the procedural and periprocedural complications were observed. 6 months after the procedure, control CT or MR angiography showed in almost all cases exclusion of the aneurysm from the circulation and normal blood flow in the treated artery. Neurological status six months after the procedure was normal in all patients.

Conclusions

Initial clinical results of our single center series with flow diverter device Pipeline are encouraging. We had no technical difficulties so far. All aneurysms were excluded from circulation. For the full implementation of the method however, further studies are required. Special attention should be paid to the impact of the stent on the covered arteries.

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research

Somatic mutations of isocitrate dehydrogenases 1 and 2 are prognostic and follow-up markers in patients with acute myeloid leukaemia with normal karyotype

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Background. Mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) genes are frequent molecular lesions in acute myeloid leukaemia with normal karyotype (AML-NK). The effects of *IDH* mutations on clinical features and treatment outcome in AML-NK have been widely investigated, but only a few studies monitored these mutations during follow-up.

Patients and methods. In our study samples from 110 adult *de novo* AML-NK were studied for the presence of *IDH1* and *IDH2* mutations, their associations with other prognostic markers and disease outcome. We also analyzed the stability of these mutations during the course of the disease in complete remission (CR) and relapse.

Results. *IDH* mutations were found in 25 (23%) patients. IDH^+ patients tend to have lower CR rate compared to IDH^- patients (44% *vs* 62.2%, p = 0.152), and had slightly lower disease free survival (12 months *vs* 17 months; p = 0.091). On the other hand, the presence of *IDH* mutations had significant impact on overall survival (2 *vs* 7 months; p = 0.039). The stability of *IDH* mutations were studied sequentially in 19 IDH^+ patients. All of them lost the mutation in CR, and the same *IDH* mutations were detected in relapsed samples.

Conclusions. Our study shows that the presence of *IDH* mutations confer an adverse effect in AML-NK patients, which in combination with other molecular markers can lead to an improved risk stratification and better treatment. Also, *IDH* mutations are very stable during the course of the disease and can be potentially used as markers for minimal residual disease detection.

Key words: *IDH1* mutations; *IDH2* mutations; acute myeloid leukaemia; normal karyotype

Introduction

Patients with acute myeloid leukaemia with normal karyotype (AML-NK) comprise 40-50% of all AML patients.[1] They are characterized by high heterogeneity in terms of clinical features, biological characteristics and response to treatment. Nevertheless, all of the AML-NK patients are categorized into intermediate risk group. The need for more precise risk stratification of such cases led to the discovery of numerous new molecular markers. Some of them, such as mutations in fms-related

tyrosine kinase-3 (*FLT3*), nucleophosmin (*NPM1)* and CCAAT/enhancer binding protein alpha (*CEBPA*) genes have made an impact on prognosis of AML-NK patients. Those mutations have been already included in the revised version of World Health Organisation classification of leukaemia.[2] This new classification implies that all AML-NK patients with mutated *NPM1* without *FLT3-* (internal tandem duplication) *ITD* and mutated *CEBPA* have favourable genotype.

Mardis *et al.* have reported the entire genome sequence of leukemic cells from a single *de novo* AML-NK patient and compared it with the genome sequence from normal skin cells of the same patient.[3] After that, from the number of possible somatic mutations, only a handfull of genes were recurrently mutated in multiple AML genomes, including mutations in the genes for isocitrate dehydrogenase 1 (*IDH1*) and isocitrate dehydrogenase 2 (*IDH2*).

The *IDH1* and *IDH2* genes, located at chromosome bands 2q33.3 and 15q26.1 respectively, encode NADPH (reduced nicotinamide adenine dinucleotide phosphate) - dependent isocitrate dehydrogenase 1/2 enzymes, whose main role is to protect cells from oxidative stress.[4]

Heterozygous point mutations in *IDH1* and *IDH2* genes most likely affect the evolutionarily conserved arginine at position R132 in exon 4 of *IDH1 (IDH*^{R132}) and either the homologous position R172 (*IDH*^{R172}) or the second arginine R140 in the *IDH2* gene (*IDH*^{R140}).[5]

IDH1 and *IDH2* mutations occur in approximately 20% of AML-NK cases.[6-11] Clinical characteristics commonly found in these patients compared to those with wild-type *IDH* are older age, higher platelet counts and concurrent presence of *NPM1* mutations.[5,6,8,9,11,18] The relatively high incidence of *IDH* mutations and their association with the most commonly detected mutations in AML patients (*NPM1* mutations) indicates possible mutual interactions in the pathogenesis of the disease.[19,22]

Despite the results of numerous studies investigating the effect of the presence of *IDH* mutations on clinical outcome, the prognostic significance of these mutations remains controversial.[11] A number of studies showed that the presence of these mutations have no effect in response to therapy and survival[5,14-16], while there are others that suggest a negative prognostic effect.[8-10,17-20] Nevertheless, most studies agree with the fact that *IDH* mutations have adverse prognostic impact in the so called low-risk group of patients (*NPM1*$^{+}$/*FLT3-ITD*$^{-}$ AML-NK patients).[8-10,13,20,21]

Some studies investigated the potential of *IDH* mutations as a follow-up markers.[13,16,22-24] *IDH1* and *IDH2* mutations are relatively stable and show direct correlation with disease status. Thus, *IDH* mutations could be useful markers for monitoring disease, including treatment response, minimal residual disease (MRD), and early relapse.

The purpose of our study was to analyze the frequency of mutations in *IDH1/2* genes and their potential associations with other prognostic markers and outcome in 110 adult *de novo* AML-NK patients. We also analyzed the stability of these mutations during the course of the disease in complete remission (CR) and relapse.

Patients and methods

Patients

From 2009-2014, pre-treatment bone marrow (BM) samples from 110 consecutive consenting patients with *de novo* AML-NK were analysed at the Clinic for Hematology. This study was approved by the by the Ethics Committee of the Clinical Centre of Serbia, Belgrade, Serbia. Written informed consent was obtained for all patients. Diagnostic procedures comprised cytomorphology, cytogenetics, molecular genetics and immunophenotyping of BM. Morphologic diagnosis was made according to the French–American–British classification.[25] Conventional G-band karyotyping was employed for cytogenetic analysis.[26] Immunophenotyping by flow cytometry was performed using the direct multicolour immunofluorescent technique applied to whole BM specimens.[2] A WBC count ≥ $30x10^9$/L was considered as leukocytosis. Organ dysfunctions, as well as non-disease mortality risk were estimated by the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI).[27] Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) scale.[28] All patients < 60 years of age were treated with standard "3+7" induction chemotherapy, consisting of daunorubicin at a daily dose of 60 mg/m^2 on days 1–3, in combination with cytarabine at 200 mg/m^2 daily as a continuous intravenous infusion for 7 days. Patients > 60 years old were treated with reduced doses in the same regimen. Patients who achieved CR after induction chemotherapy received three cycles of consolidation chemotherapy: cytarabine 3 g/m^2 per q12h on days 1, 3 and 5 for those younger than 60 years and cytarabine 0.5-1g/m^{2}per q12h on days 1, 3 and 5 for those older than 60 years. Patients aged ≤55 years under-

went allogeneic stem cell transplantation (SCT), in total 15 (25.42%) patients. Definitions of CR, overall survival (OS), disease free survival (DFS) and early death (ED) were established by proposed criteria.[29]

Molecular analyses

BM samples collected at diagnosis, in CR (after induction therapy and after consolidation) and at relapse were analysed. Mononuclear cells were separated by Ficoll density gradient centrifugation and cryopreserved until mutational analyses. Genomic *DNA* was extracted from the mononuclear cells using a QIAamp Blood Mini Kit (Qiagene, Germany) according to the manufacturer's protocol. *DNA* fragments spanning exons 4 of the *IDH1* and *IDH2* genes were amplified by polymerase chain reaction (PCR) as described before.[24] PCR reaction products were further subjected to direct sequencing, and the resulting sequences compared to wild-type *IDH1* and *IDH2 cDNA* (GenBank Accession numbers NM_005896.2 and NM_002168.2, respectively). Mutational analyses of *FLT3* and *NPM1* gene mutations were performed as previously reported.[30-32] We investigated the impact of *IDH* mutations on OS in AML-NK patients in relation to three different risk groups defined by *FLT3* and *NPM1* mutation status (*favourable* risk-*NPM1*$^+$/*FLT3-ITD*$^-$; *intermediate-NPM1*$^-$/*FLT3*-ITD$^-$; *unfavorable-FLT3-ITD*$^+$), according to the recommendation of European Leukaemia Net.[1]

Statistical analysis

Differences in continuous variables were analysed using the Mann-Whitney *U* test for distribution between two groups. Frequencies were analysed using the Pearson χ^2 test for 2x2 tables or the Fisher exact test for larger tables. Survival probabilities were estimated by the Kaplan-Meier method, and differences in survival distributions were evaluated using the Log rank test. Patients undergoing allogeneic SCT were censored at the time of transplantation. Multivariate logistic regression model was applied to analyse factors related to the probability of CR failure. Cox's regression model was applied to determine the association of *NPM1* mutations with OS and DFS with adjustment for other factors. The statistical analyses were performed using SPSS computer software 15.0 (Chicago, IL, USA). For all analyses, the probability (p) values were 2-tailed and $p < 0.05$ was considered statistically significant.

TABLE 1. Type of *IDH1* and *IDH2* mutations identified in 110 AML-NK patients

Mutation	Nucleotide change	Amino acid change	No. of patients
IDH1			
	c.394C>T	R132C	4
	c.395G>A	R132H	2
	c.394C>G	R132G	1
	c.394C>A	R132S	1
IDH2			
	c.419G>A	R140Q	12
	c.418C>T	R140W	1
	c.419G>T	R140L	1
	c.418C>G	R140G	1
	c.515G>A	R172K	2

C = cysteine; G = glycine; H = histidine; K = lysine; L = leucine; S = serine. Q = glutamine

IDH1 and *IDH2* nucleotide numbering based upon the NCBI sequence NM_005896.2 and NM_002168.2, respectively.

Results

Frequency of IDH1 and IDH2 mutations in AML-NK patients

Among the 110 AML-NK patients, 25 (23%) harboured missense mutations in *IDH* genes. Eight (7%) patients had *IDH1* mutations, all of them *IDH*^{R132}. Seventeen (16%) patients had *IDH2* mutations: fifteen *IDH*^{R140} and two *IDH*^{R172} (Table 1). The wild-type allele was retained in all *IDH* positive samples, and no patient had both *IDH1* and *IDH2* mutations. As *IDH1* and *IDH2* mutations were mutually exclusive and appear to have the same functions, we examined the clinical significance these mutations as a collective group as previously reported.[11]

Association of IDH mutations with clinical characteristics and other molecular markers

Pre-treatment clinical characteristics of the patients are summarized in Table 2. Their mean age was 54 years (range 19–78), while 31 (31.8%) patients were ≥ 60 years of age. There were 62 (56.4%) men and 48 (43.6%) women. Distribution of *IDH*$^+$ patients across FAB groups was uneven, being most frequent in the M2 group - nine (29%) patients, followed by six (27.3%) in the M1 and five (21%) in the M4 group. *IDH*$^+$ patients had higher platelet counts (p = 0.024), as well as a higher percentage of pe-

TABLE 2. Clinical characteristics of patients with *de novo* AML-NK stratified by the presence or absence of *IDH* mutations

Parameter	Total (n = 110)	*IDH*$^+$ (n = 25)	*IDH*$^-$ (n = 85)	*p* value
Sex				**0.617**
Male (%)	62 (56.4)	13 (21)	49 (79)	
Female (%)	48 (43.6)	12 (25)	36 (75)	
Age, years, median (range)	53.5(19-78)	50(23-73)	54(19-78)	0.783
ECOG ≥2				0.081
Yes No	45(40.9) 65(59.1)	14(31.1) 11(16.9)	31 (68.9) 54 (83.1)	
HCT-CI ≥3				**0.300**
Yes No	8(7.3) 102(92.7)	3 (37.5) 22(21.6)	5 (62.5) 80(78.4)	
WBC count, x10^9/l (range)	16.8 (0.5-195)	6.9 (0.5-160)	17.4 (0.8-195)	0.373
Haemoglobin median, range	95.5 (6-178)	100 (57-178)	94 (6-140)	0.810
Platelets (x10^9/L) median, range	68 (1-420)	109(16-193)	56 (1-420)	0.024
LDH (U/L) median, range	917 (273-7180)	901 (315-5105)	922.5 (273-7180)	0.825
Peripheral blood blast (%)	26 (0-96)	60.5 (0-96)	21 (0-96)	0.031
Bone marrow blasts (%)	71 (23-97)	67 (33-97)	73 (23-97)	0.920
FAB (%)				0.139
M0	10	4 (40)	6 (60)	
M1	22	6 (27.3)	16(72.7)	
M2	31	9 (29)	22 (71)	
M4	24	5 (21)	19 (79)	
M5	22	1 (0.05)	21 (95.5)	
M6	1	0 (0.0)	1 (100.0)	
***FLT3*-ITD**				0.626
present (%)	26(23.6)	5 (19.2)	21 (80.8)	
absent (%)	84(76.4)	20 (23.8)	64 (76.2)	
***FLT3*-D835**				0.428
present (%)	9	3 (33.3)	6 (66.7)	
absent (%)	101	22 (21.8)	79 (78.2)	
NPM1				0.496
present (%)	42(38.2)	11(26.2)	31(73.8)	
absent (%)	68(61.8)	14(20.6)	54(79.4)	

ECOG = performance status of the Eastern Cooperative Oncology Group; FAB = French-American-British classification; *FLT3-ITD* = *FLT3* internal tandem duplication; HCT-CI = hematopoietic cell transplantation-comorbidity index; IDH = isocitrate dehydrogenase; LDH = lactate dehydrogenase; *NPM1* = nucleophosmin; WBC = white blood cell count

ripheral blood (PB) blasts (p = 0.031) compared to *IDH*$^-$ patients. There were no differences between *IDH*$^+$ and *IDH*$^-$ patients regarding age, sex, WBC count, BM blast percentage, haemoglobin and serum LDH level.

IDH mutations occurred evenly in *NPM1*$^+$ and *NPM1*$^-$ patients (26.2% *vs* 20.6%, p = 0.496). Moreover, *IDH* mutations were not associated with *FLT3-ITD* mutations: 19.2% *vs* 23.8% (p = 0.626).

Response to induction therapy and prognostic relevance of IDH mutations

Out of the 85 *IDH*$^-$ patients, 51 (62.2%) achieved CR, while 11/25 (44%) of the *IDH*$^+$ patients achieved CR. The difference was not statistically significant (p = 0.152). The presence of *IDH* mutations was not associated with ED (*IDH*$^+$-36% *vs* *IDH*$^-$- 24.7%; p = 0.310), too. Overall 36/110 (32.7%) participants

exhibited disease relapse, 6 (24%) *IDH*$^+$ and 30 (35.3%) *IDH*$^-$patients. The impact of *IDH* mutations on DFS failed to reach statistical significance (*IDH*$^+$- 12 months *vs* *IDH*$^-$- 17 months; p = 0.266). In contrast, OS was significantly impaired in the presence of *IDH* mutations (*IDH*$^+$-2 months vs *IDH*$^-$-7 months; p = 0.039) (Figure 1).

In the univariate analysis, leukocytosis (p = 0.016) was found to be significantly correlated with a poor rate of CR. The most important factor associated with poor CR rate in the multivariate analysis was leukocytosis (p = 0.015, RR 0.34, 95% CI 0,143-0,809). Univariate analysis showed that significant factors for poor DFS were *FLT3-ITD* positivity (p = 0.03) and *NPM1* positivity (p = 0.032). The most significant risk factor for DFS using the multivariate method was *FLT3-ITD* positivity (p = 0.030, RR = 2.465, 95% CI 1.089-5.579). Univariate COX proportional regression analysis indicated that the following tested features were significant predictors of poor OS: age ≥ 55 years (p = 0.023), leukocytosis (p = 0.001) and *IDH* positivity (p = 0.039). The multivariate COX proportional regression method pointed to leukocytosis (p = 0.001, RR = 1.768, 95% CI 1.084–2.883) as the most significant predictor of poor OS.

In our study, patients aged 55 years or less received conventional or reduced intensity allogeneic SCT. OS rate in *IDH*$^+$ patients not given allogeneic SCT was markedly lower than that in *IDH*$^+$ patients who received it (2 *vs* 15 months; p = 0.006) (Figure 2). Conversely, among patients who did receive allogeneic SCT, the difference in OS rates between those with or without *IDH* mutations was not significant (p = 0.07).

We found that the presence of *IDH*$^+$ had a negative impact on OS in the *intermediate* risk subgroup (5 *vs* 12 months; p = 0.050) (Figure 3). However, *IDH* mutations did not affect OS in the *favourable* and *unfavourable* subgroups (1 *vs* 3 months, p = 0.668; 1 *vs* 7 months, p = 0.114, respectively).

Sequential studies of IDH mutation

The *IDH* mutational status was serially studied in relapsed samples of *IDH*$^-$ patients and in follow-up and/or relapsed samples in *IDH*$^+$ patients. None of the available relapsed samples of *IDH*$^-$ patients acquired *IDH* mutations. Among the nineteen *IDH*$^+$ cases who were alive after induction, eleven (44%) achieved CR. Nine of them lost *IDH* mutations after induction therapy but two patients retained it. One of them achieved CR after the first induction therapy. He lost *FLT3-D835* and *NPM1* positive status, but remained *IDH2*$^+$ positive and died

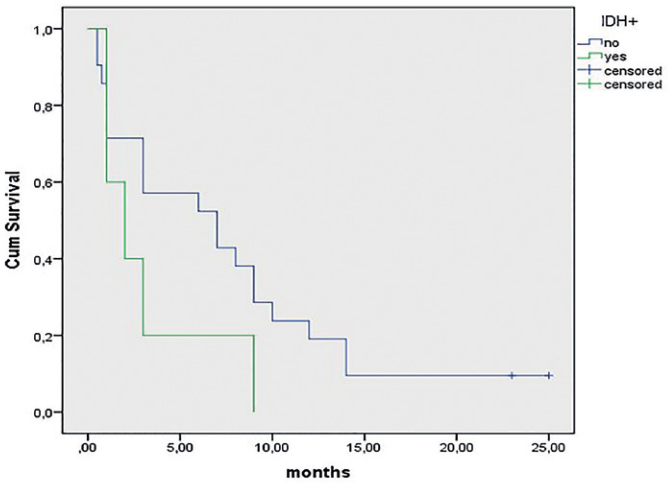

FIGURE 1. Impact of *IDH* mutation on overall survival (p = 0.039 by Kaplan-Meier method).

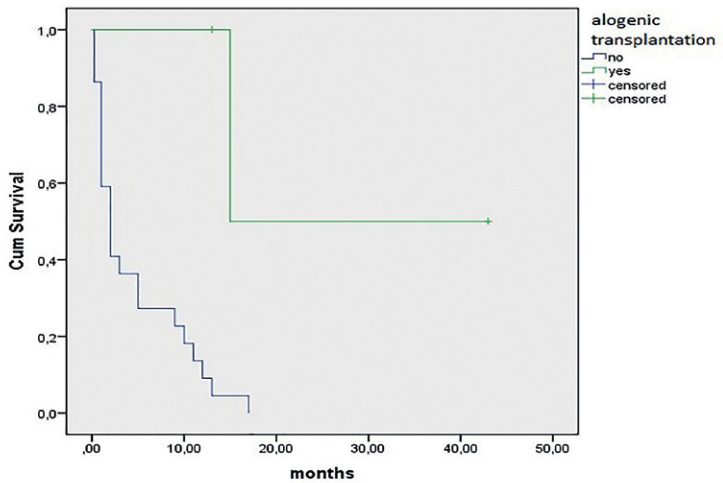

FIGURE 2. Overall survival associated with *IDH* mutations and allogeneic stem cell transplantation in AML-NK patients (p = 0.006 by Kaplan-Meier method).

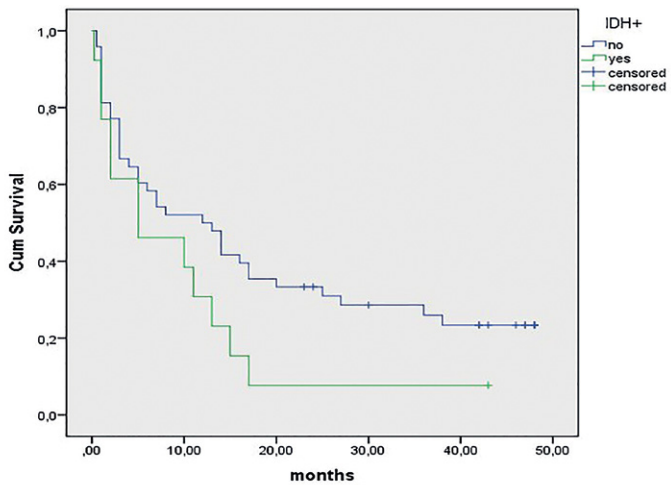

FIGURE 3. Comparison of the overall survival in intermediate group (*NPM1*$^-$/*FLT3*$^-$) between *IDH*$^+$ and *IDH*$^-$ patients (p = 0.050 by Kaplan–Meier method).

TABLE 3. Results of sequential studies of *IDH*+ patients

Patient ID	Age/ sex	*IDH/FLT3/NPM* status on diagnosis	Disease status after induction	*IDH/FLT3/ NPM* status after induction	Disease status after consolidation therapy	*IDH/FLT3/NPM* status after consolidation	Relapse	*IDH/FLT3/NPM* status in relapse
245	53/F	*IDH2*^{R140Q}*/wt/wt*	CR	*wt /wt/wt*	CR	/	yes	/
275	50/F	*IDH2*^{R172K}*/wt/wt*	CR	*wt /wt/wt*	CR	/	yes	*IDH2*^{R172K}*/ FLT3-D835/wt*
280	61/F	*IDH1*^{R132H}*/ FLT3-ITD/Type A*	RD	/	*RD*	/	/	/
291	38/F	*IDH2*^{R140Q} */FLT3-ITD/Type A*	RD	/	RD	/	/	/
305	47/M	*IDH2*^{R140Q}*wt/wt*	CR	*wt /wt/wt*	CR	/	yes	/
320	40/M	*IDH2*^{R140W}*wt/wt*	CR	*wt /wt/wt*	CR	*wt /wt/wt*	/	/
349	39/M	*IDH2*^{R140L}*/wt/wt*	CR	*IDH2*^{R140L} */ wt/wt*	*CR*	*wt /wt/wt*	/	/
378	66/M	*IDH1*^{R132H} */wt/Type A*	ED	/	/	/	/	/
380	44/F	*IDH1*^{R132C}*/wt/wt*	CR	*wt /wt/wt*	CR	/	yes	*IDH1*^{R132C}*/wt/wt*
393	54/M	*IDH2*^{R132C}*/ FLT3-D835/wt*	CR	*wt /wt/wt*	CR	/	yes	/
399	23/F	*IDH2*^{R140Q}*/wt/Type A*	ED	/	/	/	/	/
401	69/M	*IDH2*^{R140Q}*/ FLT3-ITD/wt*	RD	/	/	/	/	/
403	73/F	*IDH1*^{R132C}*/wt/wt*	RD	/	/	/	/	/
412	46/M	*IDH2*^{R140Q}*/wt/Type A*	RD	/	/	/	/	/
418	62/F	*IDH1*^{R132G}*/FLT3-ITD/Type A*	CR	*wt /wt/wt*	CR	/	/	/
423	43/M	*IDH2*^{R140Q}*/wt/wt*	ED	/	/	/	/	/
426	56/M	*IDH2*^{R172K}*/wt/wt*	ED	/	/	/	/	/
469	63/M	*IDH1*^{R132C}*/FLT3-D835/ Type A*	CR	*IDH1*^{R132C}*/ wt/wt*	CR	/	yes	/
487	73/M	*IDH2*^{R140Q}*/wt/wt*	RD	*IDH2*^{R140Q}*/ wt/wt*	RD	/	/	/
556	50/M	*IDH2*^{R140Q}*/wt/Type A*	ED	/	/	/	/	/
612	30/M	*IDH2*^{R140Q}*/wt/wt*	CR	*wt /wt/wt*	CR	*wt /wt/wt*	/	/
615	40/F	*IDH2*^{R140Q}*/wt/Type A*	CR	*wt /wt/wt*	CR	*wt /wt/wt*	/	/
645	43/F	*IDH2*^{R140G}*/ FLT3-ITD/Type A*	RD	/	/	/	/	/
672	33/F	*IDH1*^{R132S}*/wt/Type A*	RD	/	/	/	/	/
680	67/M	*IDH2*^{R140Q}*/FLT3-D835/wt*	ED	/	/	/	/	/

CR = complete remission; ED = early death; RF = refractory disease; wt = wild type

in relapse of disease (patient ID 469). The second one (patient ID 349) achieved CR after of induction therapy but retained *IDH* mutation. The mutation was lost in sequential follow-up sample, but patient died during the consolidation therapy in cyto-morphological remission with bone marrow aplasia from the septic shock (Table 3). Patient with refractory disease (patient ID 487) two months after the beginning of therapy remained *IDH2* positive. Two patients, who lost their *IDH* mutation in CR, regained it in relapse. Two of the nine patients who achieved molecular remission were treated with allogeneic SCT and are still alive. Remaining 7 patients died during therapy and after disease relapse. These results indicate stability of *IDH* mutations during the course of AML.

Discussion

The frequency of *IDH* mutations in patients with AML is 6-19%, but 12-33% in those with AML-

NK.[5,8,17,18,20,33] In our study on adult AML-NK patients, *IDH* mutations were detected in 23% of them. The prevalence of *IDH2* over *IDH1* mutations observed here (15.5% *vs* 7%) was similar to other published results.[5,6,8,22]

Our patients with *IDH* mutations had higher platelet counts and a higher percentage of PB blasts than those without such mutations, which confirms previous findings.[5,6,8,11] We detected *IDH* mutations most frequently in M2 type cases, followed by M1 (36% and 24%, respectively) and M4 type, which is in according with other results.[13,15,19]

Examining correlations between *IDH* mutations and other common genetic alterations in AML, such as *NPM1* and *FLT3* mutations, we found a slight but non-significant prevalence of *NPM1*$^+$ among *IDH*$^+$ patients (*NPM1*$^+$- 26.2% *vs* *NPM1*$^-$- 20.6%; p = 0.496). This is not in line with previous reports.[6, 8 ,9,11,18] The *FLT3* mutations were almost equally distributed between *IDH*$^+$ and *IDH*$^-$ groups of patients, which is in concordance with other studies.[5,7,18]

The prognostic impact of *IDH* mutation is controversial. Most studies have shown that both *IDH1* and *IDH2* mutations confer an unfavourable prognosis in AML-NK, *i.e*. a higher risk of disease relapse and shorter OS.[3,6-8,11,16,17,20] In our study, CR rate was 62.2% in *IDH*$^-$ patients, while in *IDH*$^+$ patients it was somewhat lower (45.8%), but without statistical significance. A similar finding was reported by Nomdedeu *et al*.[17], where the CR rate of *IDH*$^-$ patients was 80% and 63% in *IDH*$^+$ (p = 0.086).

We were able to demonstrate that *IDH* mutations act as an adverse prognostic marker of OS in AML-NK patients. That is, patients with *IDH* mutations had significantly worse OS, with a tendency for shorter DFS. This also confirmed earlier findings.[6-8,11,16,17,20] Among the *IDH*$^+$ patients, OS rate in those who received allogeneic SCT was significantly higher than that in those not given it. This was also observed by Yamaguchi *et al*.[11] and suggests that allogeneic SCT may improve OS in younger patients with *IDH* mutations.

The emergence of new molecular markers in AML-NK has contributed to a better and more precise classification of patients. This group is identified as an intermediate risk group, but because of its heterogeneity in terms of clinical outcome of the disease, more precise allocation is necessary. In addition to the *FLT3* and *NPM1* gene mutations that have already found significance as valuable prognostic factors, the detection of *IDH* mutations has contributed to refined risk classification of AML-NK patients.

When we applied molecular classification based on the presence/absence of *NPM1* and *FLT3* mutations in our cohort of patients, we observed that the presence of *IDH* mutations had an adverse impact on OS in the intermediate risk subgroup (*NPM1*$^-$/*FLT3-ITD*$^-$). This finding, already reported by others[11,16], argues in favour of testing for *IDH* mutations among AML-NK patients.

The frequent co-occurrence of *IDH* mutations with *NPM1* and less often with *FLT3* mutations, indicates that such mutations cooperate in the process of leukemogenesis. *IDH1* and *IDH2* are epigenetic modifier genes involved in DNA methylation and histone modification, and do not completely fit into our current definition of type-I and type-II aberrations, as suggested by the 2-hit theory of cancerogenesis.[34,35] Nevertheless, it has been suggested that *IDH* mutations are an early event in a variety of myeloidneoplasias like myelodisplastic syndrome and myeloproliferative neoplasms (MPN).[35,36] In patients with MPN, the acquisition of *IDH* mutations predicts an increased risk of progression to secondary AML, potentially serving as a marker for early stage transformation.[37-39] Also, the fact that *IDH* mutations are stable during the course of the disease supports the presumption that their emergence is an early event in malignant transformation.

Even though the prognostic significance of *IDH* mutations has been extensively studied, there are only few reports about their value in MRD monitoring. Thus, Gross *et al*.[40] and Jeziskova *et al*.[23] each presented four patients with *IDH1* and *IDH2* mutations, followed by the investigations of Chou *et al*.[15,21] In all three studies, as in our nine *IDH*$^+$ patients who were available for sequential analysis, the mutation was lost during CR and reappeared at relapse of the disease as the same type of mutation. Moreover, none of the patients acquired new *IDH* mutations during relapse.[15,21,23,40] In our study, we registered two *IDH*$^+$ patients retaining the mutation in CR and during the whole follow-up. Chou *et al*.[22] explained a similar finding through the hypothesis that *IDH* mutations are important in maintaining the leukaemia phenotype through cooperation with other oncogenic mutations, but alone are not sufficient for leukaemogenesis *in vivo*.

IDH1 and *IDH2* mutations have significant potential as MRD markers, assuming that the method applied meets the sensitivity criteria for MRD detection. The usual method for discovering *IDH* mutations is PCR-followed by direct sequencing, with a sensitivity of about 20%.[8,14,15,18] Based on this and the fact that *IDH* mutations are heterozygous,

the application of more sensitive methods, such as real-time PCR specific for a given mutation, should be considered for monitoring therapy response and early relapse.

In conclusion, acquired *IDH* mutations are common abnormalities in AML-NK. They confer an adverse effect, especially in patients lacking *NPM1* mutations. In combination with other molecular markers, *IDH* mutational status can lead to an improved risk stratification approach for AML-NK patients. Moreover, *IDH* mutations are stable during the course of the disease and can be potentially used as markers for MRD detection. This could be especially important if specific treatment with *IDH* inhibitors is introduced in everyday practice.

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research

Long-term survival in glioblastoma: methyl guanine methyl transferase (MGMT) promoter methylation as independent favourable prognostic factor

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Background. In spite of significant improvement after multi-modality treatment, prognosis of most patients with glioblastoma remains poor. Standard clinical prognostic factors (age, gender, extent of surgery and performance status) do not clearly predict long-term survival. The aim of this case-control study was to evaluate immuno-histochemical and genetic characteristics of the tumour as additional prognostic factors in glioblastoma.

Patients and methods. Long-term survivor group were 40 patients with glioblastoma with survival longer than 30 months. Control group were 40 patients with shorter survival and matched to the long-term survivor group according to the clinical prognostic factors. All patients underwent multimodality treatment with surgery, postoperative conformal radiotherapy and temozolomide during and after radiotherapy. Biopsy samples were tested for the methylation of MGMT promoter (with methylation specific polymerase chain reaction), IDH1 (with immunohistochemistry), IDH2, CDKN2A and CDKN2B (with multiplex ligation-dependent probe amplification), and 1p and 19q mutations (with fluorescent *in situ* hybridization).

Results. Methylation of MGMT promoter was found in 95% and in 36% in the long-term survivor and control groups, respectively ($p < 0.001$). IDH1 R132H mutated patients had a non-significant lower risk of dying from glioblastoma ($p = 0.437$), in comparison to patients without this mutation. Other mutations were rare, with no significant difference between the two groups.

Conclusions. Molecular and genetic testing offers additional prognostic and predictive information for patients with glioblastoma. The most important finding of our analysis is that in the absence of MGMT promoter methylation, long-term survival is very rare. For patients without this mutation, alternative treatments should be explored.

Key words: glioblastoma; long-term survival; methyl guanine methyl transferase; prognostic factor,

Introduction

Treatment with surgery, conformal radiotherapy and chemotherapy led to modest improvement in the prognosis of patients with glioblastoma (GBM). In the past, surgery alone or combined with less precise techniques of radiotherapy led to median survival of 12 months and only about 20% of pa-

tients survived beyond 2 years.[1,2] After introduction of modern tri-modality treatment, median survival extended to 14 months.[3,4] For the majority of patients, however, the prognosis remains poor and long-term survival beyond 3 years after diagnosis is still rare.[5]

Several clinical characteristics have been confirmed as independent prognostic factors. Worse survival has been associated with advanced age, poor performance status and incomplete surgical resection of the tumour.[6-8] Regarding post-surgical treatment, over 8 weeks of delay with postoperative radiotherapy and omission of postoperative chemotherapy had negative impact upon survival, as well.[9-12]

Study of the prognostic importance of immunohistochemical and genetic characteristics of brain tumours is a relatively recent approach. In low-grade gliomas, mutation of enzyme izocitratedehidrogenaze1 (IDH1) has been found in more than 60% of cases.[13] Patients with mutated IDH1 fare better than those who have IDH1 wild type.[14,15] In anaplastic oligodendroglioma, co-deletion of 1p and 19q chromosomes conferred a significant benefit when compared to patients without this mutation.[16] In GBM, mutation of IDH1 is far rarer than in lower grade gliomas and the prognostic importance of this mutation has not been confirmed.[17-19] However, GBMs often gain methylation of the promoter of methyl guanine methyl transferase (MGMT), an enzyme otherwise un-methylated and as such has been found to diminished its influence on temozolomide therapeutic function and ultimately improve patient survival repair alkylation caused by temozolomide. Some studies have used MGMT methylation status as a stratification tool.[20-23]

The genes cyclin-dependent kinase inhibitor 2A (CDKN2A) and CDKN2B, are sometimes used as a poor prognosis marker in various neoplasms, and the decreased expression of these genes, has been shown to correlate with the poor prognosis.[24] It has been proposed, that as CDKN2A is present in paediatric low grade gliomas, recurring as a high grade one of prognostic factors is CDKN2A deletion and that probably patients expressing could be treated differently at the first presentation.[25]

This study focused on some genetic characteristics of the GBMs and used to test the hypothesis that long-term survival is in association with these genetic alterations. We compare GBM patients with survival beyond 2.5 years with a control group matched by age, extent of surgery, performance status and therapy, but with standard median survival of GBM.

Patients and methods

Selection of patients

In Slovenia, surgery for brain tumours is done in Departments for Neurosurgery of the University Clinical Centre in Ljubljana and in Maribor. In case of indication for radiotherapy and/or chemotherapy, patients are referred to the Institute of Oncology Ljubljana.

The pool of patients covered by this analysis comprises all cases with biopsy-proven GBM and treated at the Institute of Oncology in Ljubljana between 1997 and 2011. Initial analysis included data on demographics, extent of surgery, performance status, post-surgical treatment, time to progression and survival. Follow-up for progression and update on survival were completed on September 2014.

From this series of patients, two groups of patients were selected for detailed immunohistochemical and molecular genetic analysis. All tumours were initially classified and graded according to current WHO 2007 classification of the tumours of the central nervous system.[26] *Long-term survival (LTS) group* were patients with survival beyond 30 months. *Control group* were patients matched to the LTS group according to age, extent of surgery, performance status, radio/chemo therapy and with survival shorter than 30 months.

Immunohistochemical and genetic tissue analysis

From selected paraffin blocks of GBMs with LTS and control groups the samples were taken and tissue microarray (TMA) was made in which the sample of each tissue was 2 mm thick in diameter. After sampling the tissue for TAM 10 sections of 5-μm thickness from each block we additionally cut for molecular epigenetic and genetic analysis for MGMT promoter methylation, IDH1 and IDH2 mutations, presence of 1p/19q co-deletion and CDKN2A, CDKN2Bmutations.

Immunohistochemistry was applied on 4-μm-thick sections of TAM to detect IDH1 R132H mutation using mouse MAb clone H09, diluted 1:50 (Dianova, Hamburg, Germany) on a Bench Mark XT immunostainer (Ventana Medical Systems, Tuscon, AZ, USA).

DNA isolation

Tissue samples were cut at 10 μm from formalin-fixed paraffin-embedded tissue blocks and for the

TABLE 1. Demographics, standard prognostic factors and treatment for long-term survivor (LTS) group and control group

Variable		LTS group	Control group
Mean age (range)		47,5 (21–74)	49, 6 (23–74)
Gender	Male	24	31
	Female	16	9
Surgery	Gross total	22	23
	Reduction	15	15
	Biopsy	3	2
WHO PS	0	9	5
	1	25	26
	2	4	9
	3	2	0
Mean RT dose (Gy)		58	57,9
RT technique	1D	1	0
	2D	3	3
	3D	36	37
No. of fractions		25–33	25–33
Chemotherapy	Yes	37	39
	Adjuvant only	3	1

PS = performance status; RT = radiotherapy

isolation procedure, six to eight 10 μm sections were used. Total DNA isolation was performed using QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's protocol. The DNA was eluted in 60 μl of nuclease-free water. The yield was measured fluorescently using Quant-It (Life Technologies) according to manufacturer instruction and Rotor Gene Q (Qiagen).[27-29]

MGMT methylation detection

For MGMT methylation detection, methyl-specific polymerase chain reaction (MSP) was used in a two-step approach with primers previously described.[28] Briefly, prior to MSP, 500 ng of DNA was used for bisulfite conversion using innuCONVERT Bisulfite Basic Kit according to manufacturer instruction (Analytik Jena) and stored at -20° for subsequent MSP. For MSP, 15 ng of bisulfite converted DNA was used with 0.2 μM of each primer for methylated form and 0.3 μM of primer for unmethylated form, 2 mM of dNTP and 0.25 U of Hot Master Polymerase (5 Prime) in 10 μl reaction. Amplification was performed according to manufacturer instruction using 59 °C for primer annealing. In each run, fully methylated (EpiTect Control DNA, methylated, Qiagen) as well as fully unmethylated controls (EpiTect Control DNA, unmethlyated, Qiagen) were used as assay controls. Results were analysed using 2% agarose gel. The investigator who analysed the glioma samples were blinded to all clinical information.

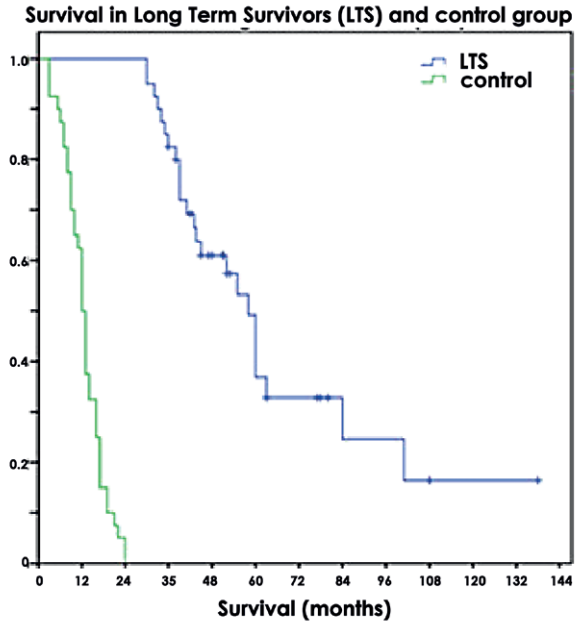
Multiplex ligation-dependent probe amplification (MLPA) analysis was used to detect copy number changes of multiple loci simultaneously (http://www.mlpa.com) and all assays used were prepared by MRC-Holland (Amsterdam, the Netherlands). MLPA assay P088-C1 was used to detect complete or partial losses involving chromosome 1p (19 probes), 19q (11 probes) and genes CDKN2A (3 probes), CDKN2B (2 probes) and identification of the most common IDH1 (R132H, R132C) and IDH2 (R172K, R172M) mutations. MLPA was performed as described by the manufacturer and data analysis was performed with Coffalyser software. Detection thresholds were set at 1.2 and 0.8 for the detection of low-level gains and hemizygous losses, respectively. For chromosome 1pand 19q losses, a distinction was made between complete and partial losses, the latter were defined as a ratio < 0.8 for at least 3 adjacent probes but not of all probes for these chromosome arms.[30]

SPSS 20 statistical package was used for statistical analysis. The prognostic importance of individual parameters for the LPS and control groups were compared with chi-square test and then with non-parametric test package. The difference of corticosteroid dose between groups at different time intervals was calculated using T-test. Cox regression was used for survival analysis.

The study was approved by Ethics and Study Protocol Assessment Committee at the Institute of Oncology Ljubljana and by the Slovenian Ethics Committee for Research in Medicine (approval ref. no. 12/07/2011) and was carried out according to the Helsinki Declaration.

Results

During the period covered by this study, 862 patients (501 male, 361 female) with GBM were treated at the Institute of Oncology in Ljubljana. Median age was 60 years (range: 18 to 86 years). From this series, 40 patients with survival beyond 30 months (LTS group), and 40 patients with shorter survival and matched according to age, extent of surgery, performance status, and therapy (Control group) were selected for further analysis.



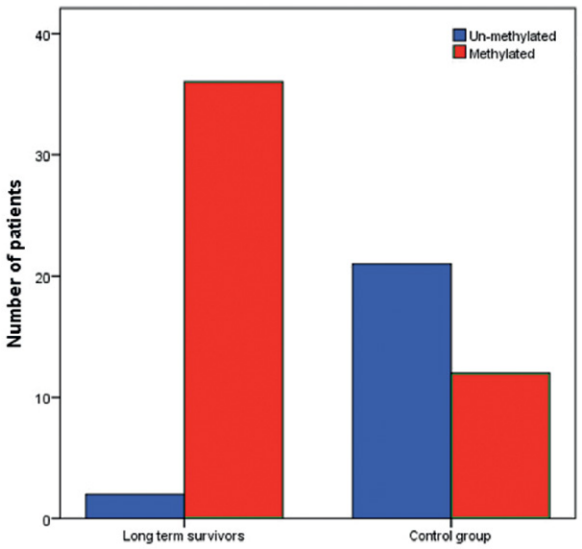


FIGURE 2. Methyl guanine methyl transferase (MGMT) promoter methylation in long term survivors and control group.

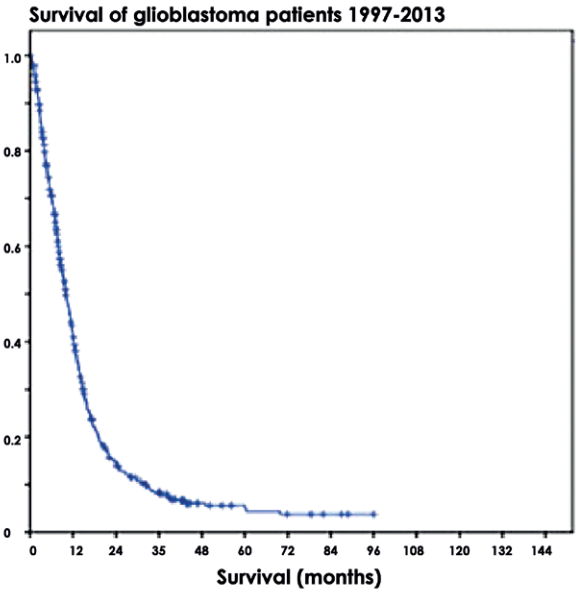


FIGURE 1. Survival of glioblastoma patients.

Data on demographics and basic prognostic factors for the LTS and control groups are presented in Table 1. Regarding standard prognostic factors and treatment, no difference is seen between LTS group and control group.

Median overall survival for all 863 patients was 10 months. Median survival for the LTS group and for control group was 58 and 12 months, respectively (Figure 1).

Results of genetic analysis

MGMT promoter methylation

Using MSP, 48 of 74 (65%) samples from patients with GBM have methylated analysed CpG islands of MGMT. One sample failed to amplify, therefore analysis was not possible, and one sample gave non-conclusive results. Kaplan-Meyer analysis revealed that overall survival was significantly longer in patients with methylated MGMT compared to those with unmethylated MGMT (43 *vs*. 16 months respectively, $p < 0.001$). Similarly, time to progression was significantly longer in patients with methylated MGMT compared to those with unmethylated MGMT (36 *vs*. 11 months respectively, $p < 0.001$).

In 2 cases in the LTS group and 7 cases in the control group, the bioptic material was not sufficient for MGMT promoter methylation analysis.

Methylation of MGMT promoter was confirmed in 36/38 patients in the LTS group and in 12 of 33 patients in the control group (chi square test: $p < 0.0001$) (Figure 2).

IDH1 R132H mutation

Immunohistochemically IDH1 R132H mutation was identified in 6 cases in the LTS group and in 1 case in the control group ($p = 0.043$). The same results were achieved with genetic analysis of IDH1 R132H mutation (Figure 3).

All six IDH1 R132H mutations were present in MGMT methylated GBMs of LTS patients.

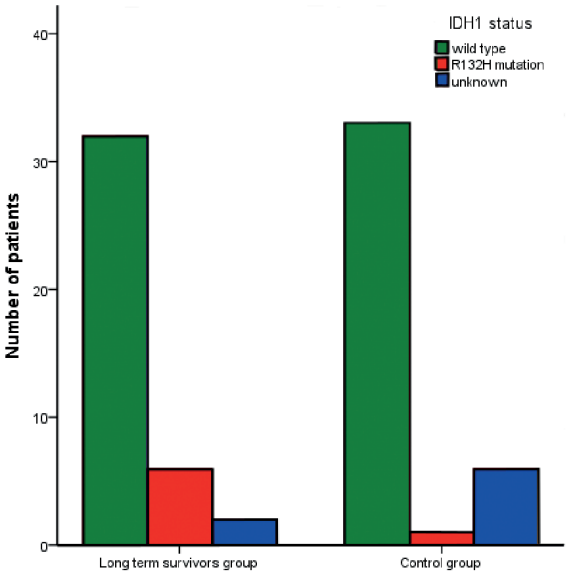


FIGURE 3. Izocitratedehidrogenaze1 (IDH1) mutations in long term survivors and control group.

TABLE 2. Response to primary treatment

	LTS group	Control group	P value
Complete response*	24	4	< 0.001
Partial response	5	2	n.s.
Stable disease	4	13	0.013
Progression	7	20	0.002
Overall response	31/40	19/40	< 0.001
Non evaluable	0	1	

*Includes patients reported by surgeon as gross total resection; LTS = long term survivor

TABLE 3. Molecular and genetic markers

	LTS group	Control group	P value
IDH1 (immunohistochemistry)	6/40	1/34	0.043
IDH1 (genetic)	6/38	1/33	0.043
IDH2 (genetic)	0/38	0/33	n.s.
1p/19q	0/38	1/34	n.s.
1p	3/38	1/34	n.s.
19q	10/38	6/34	n.s.
MGMT methylation	36/38	12/33	< 0.001
CDKN2A (deletion)	29/39	25/34	n.s.
CDKN2B (deletion)	27/39	24/34	n.s.

IDH = izocitratedehidrogenaze; LTS = long term survivor; MGMT = methyl guanine methyl transferase; n.s. = non-significant

We calculated the risk ratio using Cox regression analysis, and in the patients with the IDH1 R132H mutation, the risk of dying of glioblastoma in the observed period was found to be 0.7 of the risk in IDH1 wild type patients. Due to the small number of patients, this was not statistically significant (p = 0.437).

IDH2 mutations

No IDH2 mutations were found.

1p/19q co-deletion

Chromosomal deletions in 1p and 19q were found in 40% of the patients in the LTS group and in 23.5% of patients in control group (p = 0.167), deletions or partial deletions on 1p or 19q were equally distributed in both groups. No significant differences were found when looking for specific deletions and duplications in single or both chromosomes. Only one patient had a 1p/19q co-deletion, thus suggesting GBM arising from previously undiagnosed oligodendroglial tumour.

CDKN2A and CDKN2B

There were no differences between the two groups in the expression of CDKN2A and CDKN2B

The frequencies of the assessed markers are summarized in the Table 3.

Discussion

For any malignancy, prognostic factors are essential when comparing different treatments within a randomised clinical trial or among several separate reports. In addition, predictive factors are helpful in assessing susceptibility to a particular treatment. In combination, identification of prognostic and predictive factors may define categories of patients for whom alternative treatments should be explored.

Regarding glioblastoma, the widely recognized favourable prognostic factors for longer survival are younger age, gross total surgical resection of the tumour, good performance status and initiation of postoperative radiotherapy within 2 months after surgery. Patients over 70 years of age have a median survival of just half a year regardless of the treatment, and the majority of those over 75 don't even reach the end of initial radiotherapy. On the other hand, average survival of patients under 50 exceeds 2 years, as recently reported by our group[4] and by several other authors.[31-33] Type of surgery is also important: median survival is three months

shorter for patients after biopsy, when compared with those after maximal safe resection.[34,35] Performance status after surgery plays a major role in a patient's survival: patients unable to perform daily chores have a median survival of around 3 months.[36-38] Finally, shorter survival has been reported for patients who start radiotherapy after a delay of more than 6 to 8 weeks after surgery.[9]

To ad those known factors into the frame, which can help clinician to decide on the optimal treatment of the glioblastoma patients, recursive partitioning analysis (RPA) of those factors has been performed. Patients are then stratified to RPA classes which closely correspond to median survival.[39-41]

Still, in spite of significant improvement of median survival after tri-modality treatment, the prognosis for most patients remains grim.[42,43]

Our study is an attempt to assess an epigenetic and several genetic characteristics of the tumour as prognostic and predictive factors in glioblastoma. While long-term survivors with this disease are indeed a minority, some patients do survive beyond three years. A group of patients with LTS was therefore compared with a control group of patients with shorter survival. Since the two groups were balanced according to classical prognostic factors, novel prognostic factors would emerge.

Among molecular prognostic factors, MGMT methylation has been the most widely studied.[44-46] MGMT has been proposed as a major factor determining prognosis and also predicting response to temozolomide-based chemotherapy. An overall survival benefit in MGMT promoter methylated patients, even among those treated only with radiotherapy, points to its role as a prognostic factor.[47] Kim with co-workers compared patients with MGMT un-methylated tumours those with MGMT methylated tumours and reported median survival of 20 and 29 months, and 2-year survival of 31% and 54%, respectively.[48]

The most important finding of our study is that MGMT promoter methylation was detected in 95% of patients with LTS, and only in 36% of patients in the control group. The difference is highly significant. On the basis of our data, it seems that with the current treatment, MGMT promoter methylation is a condition without which glioblastoma patients with LTS are rarely seen.

In our analysis, methylated MGMT promoter was confirmed in 36/38 and in 12/33 cases for the LTS and control groups, respectively. It appears that without MGMT promoter methylation, LTS unlikely. Even more importantly, an un-methylated genotype is an ominous portent for the majority of patients.

IDH1 mutations are also significantly more common among patients surviving beyond two and half years. IDH1 mutations were present only in the MGMT promoter methylated patients, As IDH1 mutations in glioblastoma are rare and their independent prognostic impact is still unknown, we would not recommend routine IDH1 testing in all glioblastoma patients.[49]

Treatment of elderly patients with glioblastoma remains a difficult challenge. Recently, it was proposed that elderly patients should be offered treatment, based primarily on the MGMT promoter methylation status: radiotherapy alone for un-methylated and temozolomide or accelerated radiotherapy with temozolomide for patients with MGMT methylated tumours.[50] In our survey, age was not a factor when considering patients for conventional tri-modality treatment and we do have some patients with LTS also among the elderly. In our opinion, an elderly patient in good performance status should be offered conventional tri-modality treatment, regardless of MGMT promoter methylation status.

Conclusions

Molecular and genetic testing offers additional prognostic and predictive information for patients with glioblastoma. The most important finding of our analysis is that in the absence of MGMT promoter methylation, long-term survival is very rare. For patients without this mutation, alternative treatments should be explored.

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research

Mobilization with cyclophosphamide reduces the number of lymphocyte subpopulations in the leukapheresis product and delays their reconstitution after autologous hematopoietic stem cell transplantation in patients with multiple myeloma

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Background. Autologous hematopoietic stem cell transplantation is considered the standard of care for younger patients with multiple myeloma. Several mobilization regimens are currently used, most commonly growth factors alone or in combination with chemotherapy. The aim of our study was to investigate the differences in lymphocyte subpopulation counts between three different mobilization regimens on collection day, in the leukapheresis product and on day 15 after autologous hematopoietic stem cell transplantation.

Patients and methods. In total 48 patients were prospectively enrolled in three different mobilization regimens; (i) filgrastim (20), (ii) pegfilgrastim (19) and (iii) cyclophosphamide + filgrastim (9). Lymphocytes, CD16+/56+ natural killer and CD4+/CD25^{high} T regulatory cells were determined by flow cytometry.

Results. We found a statistically significant difference between the mobilization regimens. Cyclophosphamide reduced lymphocyte and natural killer (NK) cell counts on collection day (lymphocytes 1.08 x 10^9/L; NK cells 0.07 x 10^9/L) compared to filgrastim (lymphocytes 3.08 x 10^9/L; NK cells 0.52 x 10^9/L) and pegfilgrastim (lymphocytes 3 x 10^9/L; NK cells 0.42 x 10^9/L). As a consequence lymphocyte and NK cell counts were also lower in the leukapheresis products following cyclophosphamide mobilization regimen (lymphocytes 50.1 x 10^9/L; NK cells 4.18 x 10^9/L) compared to filgrastim (lymphocytes 112 x 10^9/L; NK cells 17.5 x 10^9/L) and pegfilgrastim (lymphocytes 112 x 10^9/L; NK cells 14.3 x 10^9/L). In all mobilization regimens T regulatory cells increased 2-fold on collection day, regarding the base line value before mobilization. There was no difference in T regulatory cell counts between the regimens.

Conclusions. Mobilization with cyclophophamide reduces the number of mobilized and collected lymphocytes and NK cells as compared to mobilization with growth factors only and results in their delayed reconstitution following autologous hematopoietic stem cell transplantation. We found no difference between filgrastim and pegfilgrastim mobilization.

Key words: mobilization; myeloma; cyclophosphamide; NK cell; stem cell transplantation

Introduction

Novel agents have significantly increased the response rate and overall survival (OS) of patients with multiple myeloma (MM), but high-dose chemotherapy and autologous hematopoietic stem cell transplantation (AHSCT) are still considered the standard of care for younger patients.[1-5] Several

TABLE 1. Patient characteristics

Characteristics	Total number of patients	G-CSF mobilization	Pegfilgrastim mobilization	Cyclophosphamide mobilization
Numbers	48	20	19	9
Sex, M/F	25 / 23	11 / 9	9 / 10	5 / 4
Median age (Range) years	61 (35–71)	60 (35–69)	64 (51–71)	59 (42–63)
ISS stage 1/2/3	13/17/18	3/8/9	7/7/5	3/2/4
Median cycles of chemotherapy	4	4	4	4
Patients with prior radiotherapy	11	3	8	0
Response VGPR/PR/SD		11/9/0	10/8/1	3/5/1
Poor mobilizers	3	1	2	0

F = female; G-CSF = filgrastim; ISS = international staging system; M = male; PR = partial response; SD = stable disease; VPGR = very good partial response

mobilization regimens are currently used, most commonly growth factors alone or in combination with chemotherapy. Considering growth factors, the most commonly used are filgrastim (G-CSF) and the long acting pegfilgrastim.[6-8]

The combination of chemotherapy and growth factors is commonly used for mobilization because of the higher yield of stem cells and fewer collection procedures.[6,9] Usually, chemotherapy regimens include cyclophosphamide at different doses. Intermediate dose cyclophosphamide (3 - 4 g/m^2) significantly increases the proportion of patients achieving the target dose of collected stem cells as compared to low dose cyclophosphamide (1.5 g/m^2) with a significantly faster neutrophil and platelet engraftment, probably due to a higher dose of infused stem cells.[10]

Cyclophosphamide induces bone marrow aplasia followed by delayed reconstitution of lymphocytes resulting in partial recovery on collection day.[11] As a consequence lower doses of lymphocytes are collected and reinfused. There is accumulating evidence that the dose of infused lymphocytes and their recovery on day 15 and 30 after AHSCT are independent prognostic factors for overall survival in patients with non-Hodgkin lymphomas and myeloma.[12-15] Among lymphocyte subpopulations the CD16+/56+ natural killer (NK) cells are particularly strong independent predictors of survival.[12]

T regulatory (Treg) cells express CD4+CD25^{high} and are crucial in the tolerance to self-antigens. Since tumor antigens are derived from self-antigens, Treg cells may decrease the antimyeloma effect. Treg cells directly inhibit NK cell effector function and Treg depletion exacerbates NK cell proliferation and cytotoxicity in in-vitro essays.[16,17] In MM the induction of immunosupression by Treg cells is believed to be associated with myelomagenesis and the progression of MM.[18,19] The effect of cyclophosphamide on Treg cells depends on the dose of cyclophosphamide and the addition of G-CSF. Low doses of cyclophosphamide have a specific toxicity to Treg cells, thereby decreasing their numbers and potentially increasing an immune response against myeloma cells.[20] On the opposite, high doses of cyclophosphamide in combination with G-CSF increase the number of Treg cells by 2-3 fold.[11] The aforementioned data support the role of immunosurveillance on the progression of MM and the concept of an autologous graft-versus-myeloma effect in AHSCT.

The aim of this study was to investigate the influence of three different mobilization regimens (i) filgrastim, (ii) pegfilgrastim and (iii) cyclophosphamide + filgrastim on the lymphocyte subpopulations during the mobilization procedure, in the leukapheresis product and after AHSCT. To the best of our knowledge, ours is the first study to compare the effects of three different mobilization regimens on lymphocyte subpopulations in newly diagnosed patients with MM after novel agent induction.

Patients and methods

In all, 48 patients with newly diagnosed symptomatic MM following induction treatment with bortezomib and dexamethasone were enrolled into this prospective single center study to receive stem cell mobilization with three different regimens; (i) filgrastim, (ii) pegfilgrastim and (iii) cyclophosphamide + filgrastim in a 2 : 2 : 1 ratio. Patients

were enrolled by two of the authors (MS, BS) to achieve same baseline characteristics according to age, number of treatment cycles, sex, ISS stage and response to induction treatment (Table 1). Only newly diagnosed MM patients that received induction treatment with 3 - 6 cycles of bortezomib (1.3 mg/m^2 sc. on days 1, 4, 8 and 11) and dexamethasone (40 mg iv. on days 1–4 of cycle 1 and on days 1, 4, 8 and 11 of the following cycles) were enrolled. Response before enrollment was assessed according to IMWG criteria.[21] Since a bone marrow biopsy is considered mandatory for defining complete response (CR), none of the patients in our study was considered in CR before mobilization, as a bone marrow biopsy was not mandatory at the time of enrollment. Patients with treatment refractory MM were excluded. This study was approved by the Local Ethics Committee (Number 149/04/11) and all patients gave their informed consent according with the Declaration of Helsinki.

Peripheral blood stem cell mobilization

Filgrastim mobilization: Patients received subcutaneous filgrastim 10 mcg/kg (rounded up to 300 mcg or 480 mcg vial size or combination thereof) daily for five days. Peripheral circulating CD34+ cells monitoring was commenced on day five and apheresis was commenced at CD34+ levels greater than 20 × 10^6 /L. Additional filgrastim was allowed on day 6.

Pegfilgrastim mobilization**:** Patients received a fixed subcutaneous dose of 12 mg pegfilgrastim on day one. Peripheral circulating CD34+ cells monitoring was commenced on day five and apheresis was commenced at CD34+ levels greater than 20 × 10^6/L. No additional growth factors were allowed.

Patients received cyclophosphamide 4 g/m^2 intravenous with mesna prophylaxis. Subcutaneous filgrastim 10 mcg/kg was started on day 5. Peripheral circulating CD34+ cells monitoring was commenced on day five and apheresis was commenced at CD34+ levels greater than 20 × 10^6/L. Filgrastim was continued daily until completion of apheresis or for up to day 20.

Blood samples and flow cytometry

Ethylenediaminetetraacetic acid **(**EDTA) anticoagulated peripheral blood samples were obtained from all patients at three time points; (i) before mobilization, (ii) on collection day and (iii) on day fifteen after AHSCT. A sample of the leukapheresis product was obtained on day one of collection. The immunolabeling was performed on samples according to the manufacturer's recommendation. Samples were incubated with 10–20 μL of antibody in the dark for 20 minutes. Red blood cells were then lysed. NK cells were enumerated as CD16+/56+ cells and Treg cells were enumerated as CD4+CD25^{high} cells. Samples were analyzed using Immunotech (Beckman-Coulter) antibodies on the Beckman Coulter Cytomics FC500 analyzer.

Stem cell collection target and definition of poor mobilizers

The collection of at least 2 × 10^6 CD34+ cells/kg recipient body weight was required. Patients with a circulating CD34+ cell count below 20 × 10^6/L up to six days after mobilization with filgrastim and pegfilgrastim or up to twenty days after cyclophosphamide and filgrastim or patients with a yield of less than 2 × 10^6 CD34+ cells/kg in three apheresis procedures were considered poor mobilizers and were excluded from further evaluation.[22]

High-dose therapy and AHSCT

AHSCT was planned 20–40 days after successful stem cell collection. Patients received melphalan (200 mg/m^2) and stem cells were reinfused 24 hours later.[3,5,23] The mean stem cell dose was 2.6 × 10^6 CD34+ cells/kg, 2.6 × 10^6 CD34+ cells/kg and 3.1 × 10^6 CD34+ cells/kg for patients after filgrastim, pegfilgrastim and cyclophosphamide + filgrastim mobilization respectively. Patients received prophylaxis with levofloxacin and posaconazole, and G-CSF support until leukocyte engraftment at the discretion of the treating physician.

Statistical analysis

The SPSS Statistics 21 (IBM, USA) software package was used for the statistical analysis. One-way ANOVA test was used to calculate differences between cohorts. Additional post-hoc analysis using the Tukey's HSD test was used to validate differences between two cohorts. For difference between only two cohorts the t-test for independent samples was used. Results were considered statistically significant if $p < 0.05$.

Results

No differences between the three regimens were noted in lymphocyte, NK cell and Treg cell counts

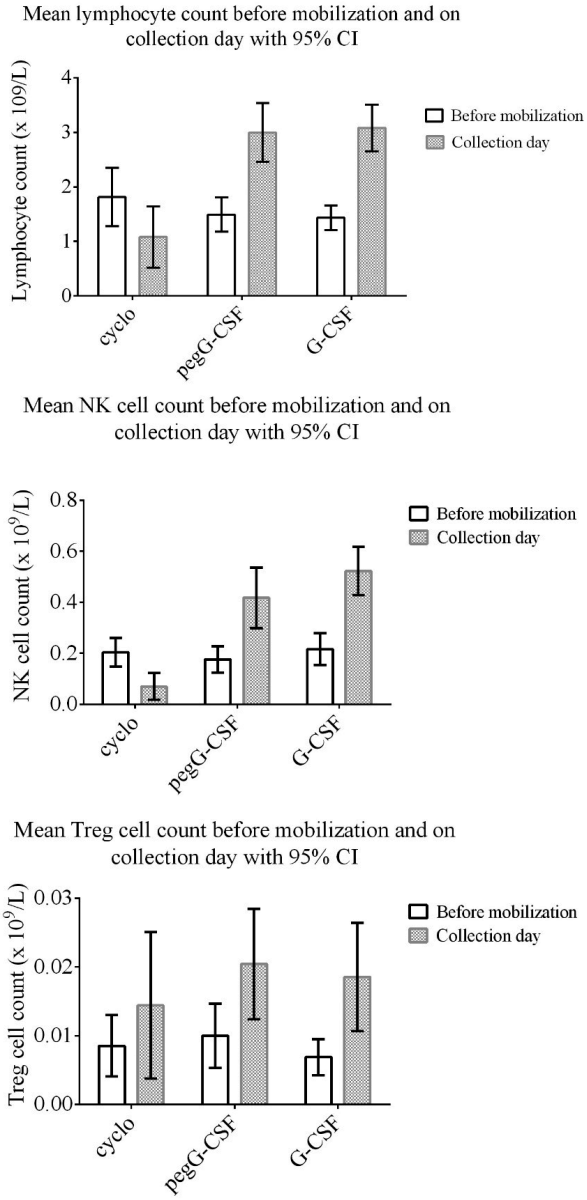

FIGURE 1. The mean lymphocyte, natural killer (NK) cell and Treg cell counts (109/L) before mobilization and on collection day with 95 % confidence interval (CI) for mobilization with cyclophosphamide (cyclo), pegfilgrastim (pegG-CSF) and filgrastim (G-CSF).

before mobilization. Mobilization with cyclophosphamide reduced the lymphocyte and NK cell counts on collection day by 2-fold as compared to baseline value before mobilization. On the contrary mobilization with G-CSF and pegfligrastim induced a 2- fold increase in lymphocyte and NK cell counts on collection day as compared to baseline value before mobilization (Figure 1). This resulted in different lymphocyte and NK cell counts on collection day between the mobilization regimens ($p < 0.001$). In pairwise comparison the differences between mobilization with cyclophosphamide and G-CSF only, and cyclophosphamide and pegfilgrastim only were statistically significant ($p < 0.001$). The differences in lymphocyte and NK cell counts on collection day resulted in a significantly different lymphocyte and NK cell composition of the leukapheresis product (Table 2). All three mobilization regimens increased Treg cell counts on collection day by 2-fold with no differences in absolute values between the three regimens. Comparison between the groups mobilized with G-CSF or pegfilgrastim showed no difference in lymphocyte, NK and Treg cell counts on the collection day, in the leukapheresis product and on day 15 after AHSCT. Because no difference in lymphocyte and NK cell counts during mobilization and in the leukapheresis product was noted between the G-CSF and pegfilgrastim group, and due to the small patient sample, we analyzed lymphocyte counts on day 15 after AHSCT between cyclophosphamide mobilization and growth factors only (G-CSF and pegfilgrastim combined) mobilization. Mobilization with growth factors resulted in a higher lymphocyte count on day 15 post AHSCT compared to cyclophosphamide mobilization ($p < 0.04$).

Three patients failed mobilization with G-CSF or pegfilgrastim and were excluded from further evaluation. All three were salvaged with plerixafor and successfully underwent AHSCT. No patient failed mobilization with cyclophosphamide. All transplanted patients successfully engrafted.

Discussion

The use of cyclophosphamide mobilization in MM patients results in lower mobilization failure, higher stem cell yield and fewer collection procedures but at the cost of higher toxicity.[6,9,10] Furthermore, cyclophosphamide affects lymphocyte subpopulations possibly affecting the response after AHSCT. In our study, we prospectively analyzed the impact of cyclophosphamide, G-CSF and pegfilgrastim mobilization on lymphocyte, NK and Treg cell counts during mobilization, in the leukapheresis product and after AHSCT in newly diagnosed patients treated with bortezomib and dexamethasone.

Our study shows that mobilization with cyclophosphamide resulted in lower absolute lymphocyte and NK cell counts as compared to growth factor only mobilization (Figure 1) and as a conse-

TABLE 2. Mean lymphocyte (Lym), natural killer (NK) and T regulatory (Treg) cell counts with 95 % confidence interval (CI) before mobilization, on collection day, in the leukapheresis product and on day 15 after autologous hematopoietic stem cell transplantation (D+15) for mobilization regimens using cyclophophamide, filgrastim (G-CSF) and pegfilgrastim

Time point	Collection regimen	Cyclophosphamide		Pegfilgrastim		G-CSF		
		Mean value	95% CI	Mean value	95% CI	Mean value	95% CI	Sig.
Before mobilization	Lym (x 10^9/L)	1.81	1.27–2.34	1.48	1.17–1.80	1.43	1.20–1.66	
	NK (x 10^9/L)	0.20	0.15–0.26	0.18	0.12–0.23	0.22	0.15–0.28	
	Treg (x 10^6/L)	8.53	4.1–12.9	10	5.28–14.6	6.86	4.24–9.48	
Collection day	Lym (x 10^9/L)	1.08	0.52–1.64	3	2.46–3.54	3.08	2.65–3.51	**< .001**
	NK (x 10^9/L)	0.07	0.01–0.13	0.42	0.3–0.54	0.52	0.43–0.62	**< .001**
	Treg (x 10^6/L)	14.4	3.73–25.1	20.4	12.4–28.5	18.5	10.6–26.4	.646
Leukapheresis product	Lym(x 10^9/L)	50.1	36–64.2	112	96.6–129	112	94.7–130	**< .001**
	NK (x 10^9/L)	4.18	0.72–7.64	14.3	11.9–16.6	17.5	12.7–22.4	**< .001**
	Treg (x 10^6/L)	1030	392–1670	1030	400–1650	714	430–997	0.531
D+15	Lym (x 10^9/L)	0.31	0.14–0.47	0.47	0.38–0.55	0.47	0.36–0.59	0.115
	NK (x 10^9/L)	0.05	0–0.85	0.10	0.04–0.16	0.10	0–0.34	0.357
	Treg (x 10^6/L)	27.8	4.45–51.1	36.6	0–101	2	0–5.74	0.691

Lym = lymphocytes; NK = natural killer cells; Treg = T regulatory cells

quence the leukapheresis products also contained lower counts of lymphocytes and NK cells. It resulted in slower reconstitution of lymphocytes on day 15 after AHSCT in patients mobilized with cyclophosphamide as compared to those mobilized with growth factors only. The number of lymphocytes and NK cells is important because studies have shown that the infused dose of lymphocytes and the early recovery affects survival in patients after AHSCT suggesting a graft-vs-myeloma effect.[12-15,24] Counts of at least 0.5 x 10^9/L lymphocytes on day 15 after AHSCT predict better OS in patients with MM.[13] In our group 2 out of 9 patients receiving cyclophosphamide mobilization achieved this cutoff value as opposed to 7 out of 19 and 9 out of 17 patients receiving G-CSF and pegfilgrastim mobilization, respectively. Besides a graft-vs-myeloma effect, the early reconstitution of lymphocytes and NK cells protects against viral infections including cytomegalovirus, influenza virus, HIV-1, and hepatitis C virus thereby decreasing infectious complications after AHSCT and possibly increasing the age limit of patients eligibly for AHSCT.[25,26]

Our findings encourage further research because of the expanding use of lenalidomide consolidation and maintenance after AHSCT. Lenalidomide activity is partly exerted through immunomodulation and activation of NK cells.[27,28] Therefore persistent lower lymphocyte and NK cell counts after AHSCT may decrease the efficacy of lenalidomide in cyclophosphamide mobilized patients. Trials on lenalidomide maintenance have not focused on the impact of the mobilization regimen or lymphocyte subpopulations on treatment outcome.[3,29]

Treg cells are associated with MM progression, probably through immunosuppression and inhibition of NK cell function.[16-19] Previous studies show that low doses of cyclophosphamide are highly toxic to Treg cells.[20] In contrast, Condomines in a study of 14 patients mobilized with high dose cyclophosphamide found a 2-fold increase in Treg cells, speculating that a cytokine burst following aplasia due to cyclophosphamide is responsible.[11] Our data shows a 2-fold increase in Treg cells on collection day compared to baseline value before mobilization in all three mobilization regimens. We speculate that growth factors (G-CSF and pegfilgrastim) are responsible for the increase in Treg cell counts and not the cytokine burst following aplasia due to cyclophosphamide. Evidence from animal models show that G-CSF has a strong effect on promotion of Treg cell numbers and function.[30,31] A small clinical trial on 29 patients receiving mobilization with high doses of filgrastim (16 mcg/kg) showed that, in addition to stem cells, Treg cell counts increased during mobilization.[32]

On day 15 after AHSCT Treg cells are the only subpopulation with higher counts compared to baseline value before mobilization and the value on collection day. Our data shows a 4-fold higher Treg cell count on day 15 after AHSCT as compared to baseline value before mobilization and 2-fold higher as compared to the value on the collection day. We speculate that G-CSF application after AHSCT is the major contributor for the increase of Treg cells on day 15 after AHCST and not growth factors given during mobilization. The lower value of Treg cells on day 15 after AHSCT in the G-CSF regimen cannot be properly evaluated due to the small patient group.

In our study we investigated two different growth factors, namely G-CSF and the long acting pegfilgrastim. Stem cell mobilization with both growth factors results in comparable toxic profiles and a similar recovery of leukocytes and platelets after AHSCT.[8] However, results from small trials suggest that pegfilgrastim mobilizes a different CD34+ cell subset due to higher expression levels of various genes indicative of early haematopoiesis.[33,34] Whether this has an impact on lymphocyte subpopulations is not known from the available literature. Our study shows that both growth factors are comparable regarding the increase in lymphocytes, NK and Treg cells during mobilization and in the leukapheresis product. Counts on day 15 after AHSCT are comparable, except Treg cell counts, which are primarily a result of G-CSF application after AHSCT and not during mobilizaton (Table 2).

In the current study we enrolled only patients receiving induction with bortezomib and dexamethasone. Triple agent induction including cyclophosphamide or immunomodulatory drugs is currently the standard induction regimen.[4,35] Weekly applications of cyclophophamide during induction might decrease Treg cells and improve the immune status of patients before mobilization resulting in a different composition of the leukapheresis product and altered reconstitution after AHSCT. In addition, the optimal dose and interval of cyclophosphamide on Treg cell depression is not known. To answer these questions further investigator driven studies are needed.

Our study has some limitations. Other factors may influence the reconstitution of lymphocyte subpopulations after AHSCT. The transplantation of cells expressing dipeptidyl peptidase-4 (CD26) has been shown to influence reconstitution of lymphocyte subpopulations. Monocytes expressing CD26+ improve the reconstitution of helper, suppressor and NK lymphocytes and a higher number of transplanted CD26+ lymphocytes accelerates the reconstitution of NK lymphocytes.[36] Cotransplantation of mesenchymal stromal cells during AHSCT improves lymphocyte recovery after AHSCT.[37]

In summary, we found no differences between G-CSF and pegfilgrastim mobilization regarding the increase in lymphocytes, NK and Treg cells during mobilization and in the leukapheresis product. Therefore mobilization with pegfilgrastim is comparable to G-CSF regarding lymphocyte subpopulations and can be used instead for stem cell mobilization. Our results show that mobilization with cyclophosphamide reduces the number of lymphocytes and NK cells on collection day and in the leukapheresis product thereby reducing the absolute number of lymphocytes on day 15 post AHSCT. Hence, cyclophophamide mobilization might decrease graft-vs-myeloma effect possibly affecting the outcome of AHSCT.

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research

Tracheal cancer - treatment results, prognostic factors and incidence of other neoplasms

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Background. Tracheal cancers (TC) are rare and treatment results that are reported are typically not satisfactory. The purpose of this research was assessment of the results of treatment of TC patients, identification of potential additional surgery candidates, evaluation of prognostic factors, and assessment of the occurrence of other malignancies.

Patients and methods. The Regional Cancer Database and the Hospital Database were searched for patients with tracheal neoplasms. Fifty-eight of 418 patients identified initially, met the inclusion criteria (primary TC with confirmed histology and complete treatment records). Standard statistical tests were used.

Results. Squamous cell carcinoma (SCC; 63.8%) and adenoid cystic carcinoma (ACC; 15.5%) were the most commonly diagnosed histological types of TC. Radiotherapy was delivered in 48 cases, surgery or endoscopic resection in 20, and chemotherapy in 14. TC was diagnosed as a second cancer in 10 patients, in 1 patient it occurred prior to the lung cancer, and in 1 was diagnosed simultaneously. During the median follow-up of 12.7 months, 85.5% of the patients died because of the disease. Local recurrence occurred in 17% cases. In univariate analysis, patients with ACC had statistically better five-year overall survival (77.8%) than those diagnosed with SCC (8.4%, p = 0.0001). Radiotherapy, performance status and haemoptysis were factors significantly influencing overall survival (OS) in the multivariate analysis. Among patients who were not treated surgically, 15–26% were found to constitute additional surgery candidates, depending on the selection criteria.

Conclusions. The diagnostic workup should be focused on the identification of TC patients suitable for invasive treatment and radiotherapy. Respiratory system cancer survivors can be considered a risk group for tracheal cancer. Radiotherapy constitutes an important part of the treatment of patients with TC.

Key words: tracheal cancer; metachronous neoplasms; adenoid cystic carcinoma; squamous cell carcinoma; radiotherapy

Introduction

Primary tracheal carcinomas (TC) are rare and account for 0.01–0.4% of all malignancies with annual incidence of merely 0.2 cases per 100,000 people.[1-4] In most patients, TC are symptomatic, but often misdiagnosed as asthma, chronic pulmonary disorder or tumours of the thyroid or lungs.[3-12] Two most common histological types are squamous cell carcinoma (SCC), and adenoid cystic carcinoma (ACC) - these two types account for over 75% of primary TC.[6,7,13,14]

Knowledge on tracheal cancer is limited only to the results of retrospective reviews of institutional and national databases. Most of the studies suggest that standard methods of treatment should include extensive segmental resection with adjuvant radiotherapy (RT) or chemoradiotherapy.[1,3,4-7,13,15] Modern techniques of tracheal surgery are associated with low perioperative risk when performed

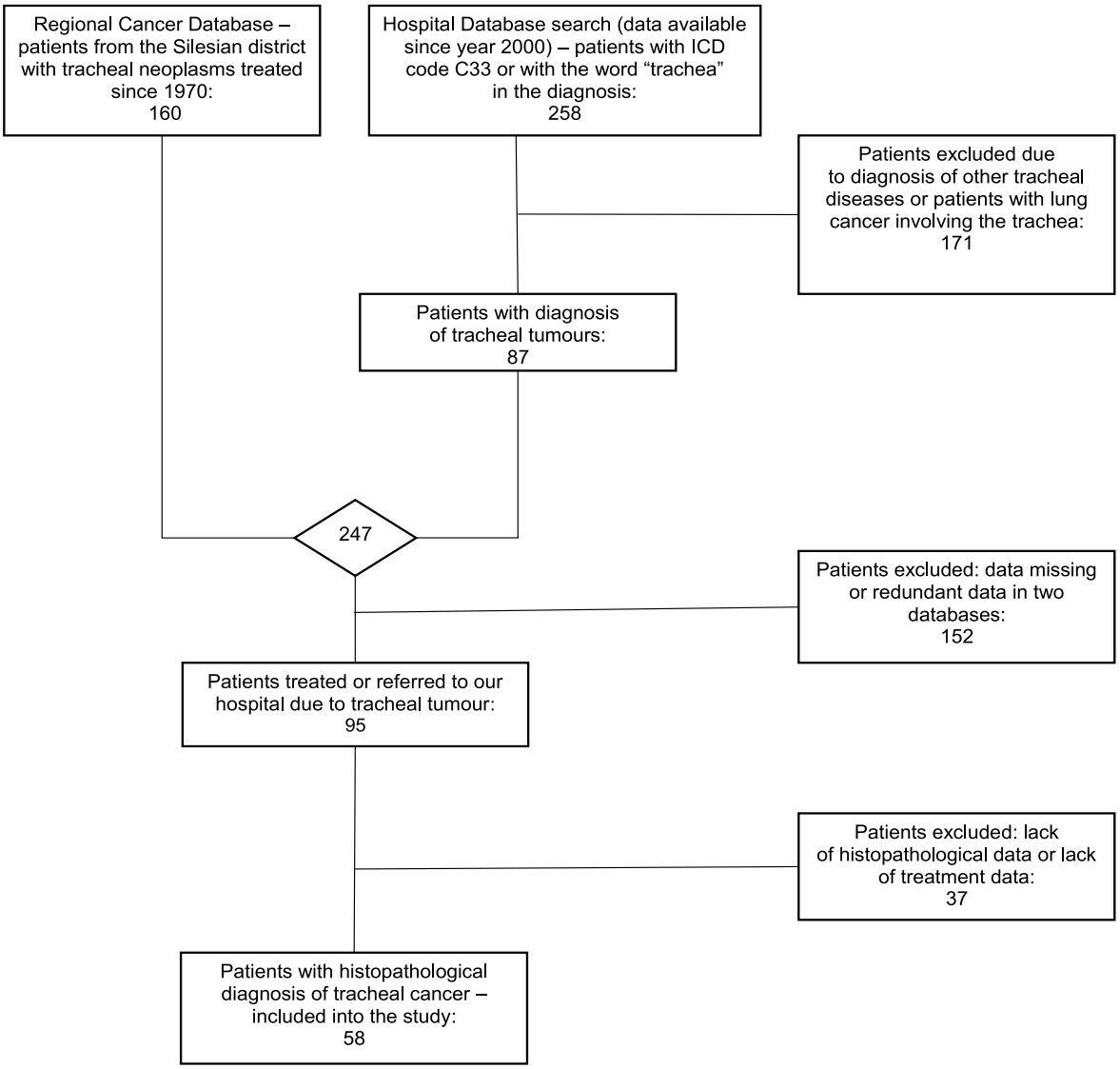

FIGURE 1. Patient identification algorithm and inclusion criteria

in specialized centres, and some researchers indicate that resection should be possible in more than 25–50% of patients.[3,14,16,17]

Adjuvant RT (3–6 weeks after the surgery with total dose of 60 Gy or a biologically equivalent dose of neutrons) or primary curative RT treatment in inoperable cases (doses ranging between 68 and 80 Gy) have also been proven effective in some studies.[3,18,19] There is a lack of information on the late effects occurring after definitive RT for TC. The quantitative analysis of normal tissue effects in the clinic (QUANTEC) report suggests that limiting the dose to 80 Gy or lower using standard fractionation may reduce the risk of central airway stenosis. Therefore, most patients with TC are treated with lower doses (although some radioresistant tumours, such as ACC, may require higher doses to achieve local control and a small increase of the risk of treatment toxicity is acceptable).[20]

According to Grillo *et al*., approximately 40% of all tracheal SCCs are reported to occur before, together with or after carcinoma of the oropharynx, larynx, or lung.[13] In spite of the above, second cancers are not very often described among patients with TC and only one large study (270 patients) reported second cancers in 19.6% of TC patients.[8,13,15,21-23]

The aim of our study was to assess the outcome of patients treated for TC, search for potential prognostic factors, evaluate the occurrence of second malignancies, and, finally, identify those pa-

tients who potentially could have been additional surgery candidates.

Patients and methods

We conducted a retrospective study on patients diagnosed with TC in our institution between 1997 and 2015. The Regional Cancer Database and the Hospital Database were searched for patients with tracheal neoplasms. Among 418 patients (160 from the Regional Cancer Database and 258 from the Hospital Database), we found 58 cases that met the inclusion criteria (Figure 1).

Medical records of patients were reviewed to search for second malignancies. All patients with lung or larynx cancer invading trachea were excluded from the study. Second cancer was diagnosed if one of the following criteria were met: histopathology of the second cancer was different from tracheal cancer histopathology or disease-free interval between those two cancers exceeded three years.

Additional research was performed to identify those patients, who potentially could have been candidates for surgery. Our criteria for a theoretical surgery candidate were: [1] good performance status (Eastern Cooperative Oncology Group [ECOG] 0–1), [2] no nodal or distant metastases, [3] no infiltration of surrounding organs and [4] age below 70 (due to possible surgery contraindications not associated with TC).

The overall survival (OS) was evaluated using the Kaplan-Meier method. To verify the significance of variables influencing the OS in the univariate analysis, the Cox proportional hazard model was employed. P of less than 0.05 value was considered to indicate statistical significance. Follow-up was defined as the period of time from the date of diagnosis of tracheal cancer to the date of the cut-off or death. The cut-off date for survival was December 14, 2015. Follow-up was calculated separately for the whole group and for patients who were alive at December 14, 2015. Time to progression was measured from the date of the end of the treatment to the date of local or distant progression. The study was performed according to the Helsinki Declaration and the Institutional Review Board Committee.

Results

Patients' characteristics

We found 58 cases diagnosed in 41 males and 17 females (gender ratio: 2.4:1). The age at diagnosis varied from 27 to 79, mean 57.6 (SD ± 12.3). The details of histopathological diagnoses and treatment employed are shown in Table 1.

TABLE 1. Distribution of histology types and methods of treatment summary

Histology	Number of patients
Squamous cell carcinoma	37 (63.8%)
Adenoid cystic carcinoma	9 (15.5%)
Microcellular carcinoma	5 (8.6%)
Non-microcellular carcinoma	4 (6.9%)
Adenocarcinoma	2 (3.5%)
Papillary carcinoma	1 (1.7%)
Methods of treatment	**Number of patients**
Radiotherapy	25
Radiotherapy + endoscopic resection	10
Radiotherapy + surgery	4
Radiotherapy + chemotherapy	6
Radiotherapy + endoscopic resection + chemotherapy	3
Endoscopic resection	3
Chemotherapy	5
Symptomatic	2

Pathologic grade was poorly documented (19 cases) and in most cases for which it was reported the neoplasm was poorly differentiated or undifferentiated (14; 74%). All patients were diagnosed on the basis of histopathological examination, bronchoscopy, and chest computed tomography (CT). In 50 cases, bronchoscopy or CT showed a mass protruding into tracheal lumen. Stricture of the trachea was found in 43 patients, whereas infiltration of surrounding tissues was present in 28 cases.

Fourteen patients had ECOG performance status of 0, thirty three patients had the status of 1, while in eleven cases it was 2. Tumour size was difficult to assess due to retrospective nature of the study. The available data were obtained from CT scans and bronchoscopy. Only ten patients had a tumour smaller than 2 cm in its maximum diameter (T1 according to T-stage by Bhattacharyya).[7] Nodal status was assessed in 57 of 58 (98.3%) patients. In 23 of them, the lymph nodes were involved. Metastases in other organs were found in four patients before the treatment and occurred in eleven more patients during the follow-up.

TABLE 2. The characteristics of radiotherapy

	Number of patients	Total dose range (Gy_2)	Median total dose (Gy_2)	Mean total dose (Gy_2)	SD (Gy_2)	Fraction dose (Gy)	Median fraction dose (Gy)	Mean fraction dose (Gy)
Radiotherapy	48	14.0–82.6	33.2	45.0	±21.5	1.6–8.0	2.5	3.0
Curative	23							
External beam radiotherapy	23	42.5–82.6	66.0	66.4	±8.8	1.6–3.0	2.0	2.0
Brachytherapy boost	3	12.5–16.0	16.0	14.8	±2.0	5.0–6.0	6.0	5.6
Palliative	24	18.7–48.5	24.0	28.2	±7.4	2.0–8.0	4.0	3.7
External beam radiotherapy	20	18.7–42.0	23.3	27.1	±5.7	2.0–4.0	4.0	3.4
Brachytherapy	5	16.0–29.7	24.0	23.5	±4.9	6.0–8.0	6.0	6.6

Gy_2 = doses converted to 2-Grey equivalent doses (EQD2) using linear-quadratic model

Symptoms

Clinical symptoms occurred in 95% of patients. The most common symptom was dyspnoea (72.4%), cough (53.4%), haemoptysis (32.8%), and hoarseness (27.6%). Other symptoms included weight loss (22.4%), pain (15.5%), dysphagia (12.1%), weakness (10.3%), stridor (5.2%), fever (8.6%), the feeling of having an obstacle in trachea (10.4%), and neck tumour (6.9%). Forty-two patients (72.4%) were current or former smokers, and among them 32 persons were diagnosed with SCC, 3 patients with ACC, 2 others with microcellular carcinoma, and 5 patients with other histological types. As for the number of pack-years, it varied from 8 to 135 with the median of 40 (mean 46.2).

Treatment characteristics

Radiotherapy

Forty-eight out of 58 patients were treated with the ionizing radiation in our Institution. In 25 cases, RT was the only treatment modality used, 11 patients were irradiated with curative intent and 14 patients had palliative radiotherapy. Brachytherapy (BT) was a part of the treatment in 8 cases, and in 3 of them it was used as an additional boost to radical external beam RT. External beam radiotherapy was a part of therapy in 48 patients (Table 2).

To sum up the doses of various RT courses delivered with different fraction doses (FD), the physical doses were converted to 2-Gray equivalent doses (EQD2) using linear-quadratic model.[29] TD (Gy_2) (α/β = 10) in palliative RT ranged from 18.7 to 48.5 Gy_2 (mean 28.2; median 24) delivered in 2 to 16 fractions of 2.0 to 8.0 Gy (mean 4.1; median 4). In radical RT, the fraction dose (FD) varied from 1.6 to 3 Gy (median 2) and the tumour was irradiated to TD ranging from 42.5 to 82.6 Gy_2 (mean 66.4, median 66.0). TD of BT boost ranged between 10 and 12 Gy (EQD2 = 12.5–13.3) delivered in two fractions (FD 5–6 Gy). In all patients irradiated with curative or palliative intent, the treated volume included the tumour of trachea and margins added to create the clinical and planning target volumes. In 15 cases, lymph nodes were included in the clinical target volume (mediastinal lymph nodes in 9 cases; mediastinal and supraclavicular lymph nodes in 6 cases), and the treatment comprised of two phases - in the first phase the irradiated volume encompassed the primary tumour and lymph nodes with margins, whereas, in the second phase - only the tumour with margins. In one case, RT was delivered after radical surgery, and neck nodes were irradiated with TD of 42.5 Gy_2. In one case, the exact data on the RT volume was not available.

Most of the patients treated with radiotherapy in our hospital had their treatment delivered with linear accelerators with 6–20 MV photons. Five patients treated in earlier years of the study (before 2004) were treated on Cobalt-60 machines. Six patients were irradiated outside our Institution and the detailed data on the treatment machines used were not available. Data on energies used in the treatment were available in 54 cases: fifteen patients received treatment with 6 MV photons, sixteen persons with 20 MV photons, three patients with 6 and 20 MV photons, two patients with 15 MV photons, and five others with 60-Cobalt (1.25 MeV). During the treatment, the position of the patient was verified based on skin marks prepared during simulation and MV portal imaging. Image guided radio-

therapy (IGRT; orthogonal X-ray images acquired before every fraction) was used in later years of the study (after the year of 2006). Most of the patients had conventional, 3D conformal plans, four were treated with intensity modulated radiotherapy (IMRT), and one with tomotherapy.

Eight patients received high dose rate (HDR) brachytherapy with Iridium-192. All patients had bronchoscopy before application. The applicator was placed in close proximity of the tumour. The size of the applicator depended on tumour size and pattern of infiltration. In all the cases, the treated volume consisted of the gross tumour volume with a margin.

Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) criteria were used to assess toxicity of radiotherapy delivered. The entries in the medical records of the patients were re-evaluated, because in the majority of cases the radiation-induced reaction was rather presented in a descriptive form than given exactly according to the EORTC/RTOG grading system. Thirty-one patients did not declare any side effects. Eight reported dysphagia, three indicated dyspnoea, two patients suffered from oedema, additional two patients experienced pain and one person suffered from deterioration of the general condition. In most cases, the toxicity was minor (9 cases) or mild (6 patients), however one patient died due to haemorrhage in the course of brachytherapy.

Surgery and systemic therapy

Surgery or endoscopic resection of the tumour was combined with RT in 17 cases; however, only two patients had radical excision of the tumour. Thirteen patients had only endoscopic resection of the tumour. Seventeen patients had tracheotomy and stents placed inside the trachea as a part of the palliative treatment. Chemotherapy (CTH) was applied in 14 cases (in 13 cisplatin-based), and in 5 of them it was the only treatment given. CTH regimens used included cisplatin alone or combined with: adriamycin, navelbine, etoposide, mitomycin C or 5-fluorouracil. Patients received one to six cycles of CTH. As all the patients received CTH outside our institution, the assessment of toxicity of the systemic treatment could not have been performed.

Follow-up

The median follow-up of all patients was 12.7 months. The cut-off date for survival was December 14, 2015. Of 58 patients, 49 patients (84.5%) died because of the disease and 9 were still alive. For the nine patients who were alive, the median follow-up was 27.4 months (range 0.5 to 183.8 months).

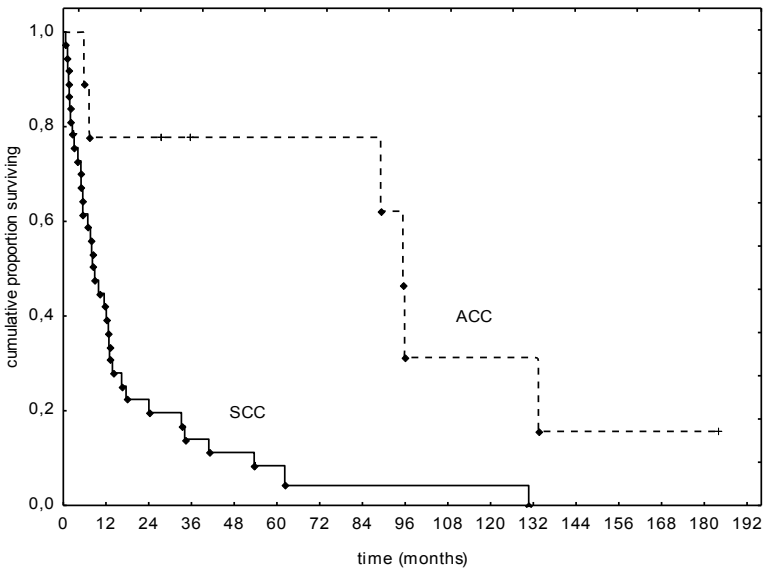

FIGURE 2. Overall survival of patients with adenoid cystic carcinoma (ACC) and squamous cell carcinoma (SCC).

Thirty-two patients had control visits in our institution. Within this group, 11 cases of local recurrence of the disease from 4 to 67 months (median 49) after the treatment was observed. In 5 patients, it was a recurrence of SCC, in one patient - ACC, in one case microcellular carcinoma, in 2 cases adenocarcinoma, and in one patient - non-microcellular carcinoma. In all the cases of the recurrent disease, RT was a part of the therapy, whereas surgery was performed in 2 cases. BT was delivered in 3 cases as a part of the treatment of the recurrence. Five patients had been treated with cisplatin-based CTH, and three had symptomatic treatment.

A second recurrence was diagnosed in 5 cases. In 2 patients, BT was delivered, two had external beam radiotherapy, and one patient received symptomatic treatment. In 5 of 6 patients who received BT with palliative intention local control was achieved for a median time of 9.7 months (range 3–23 months).

Survival data and prognostic factors

In the univariate analysis, patients with ACC had statistically better overall survival (OS) than those diagnosed with SCC ($p = 0.0001$). Five-year OS for ACC and SCC were 77.8% and 8.4% respectively (Figure 2). Patients with performance status of 0 or 1 had better OS than

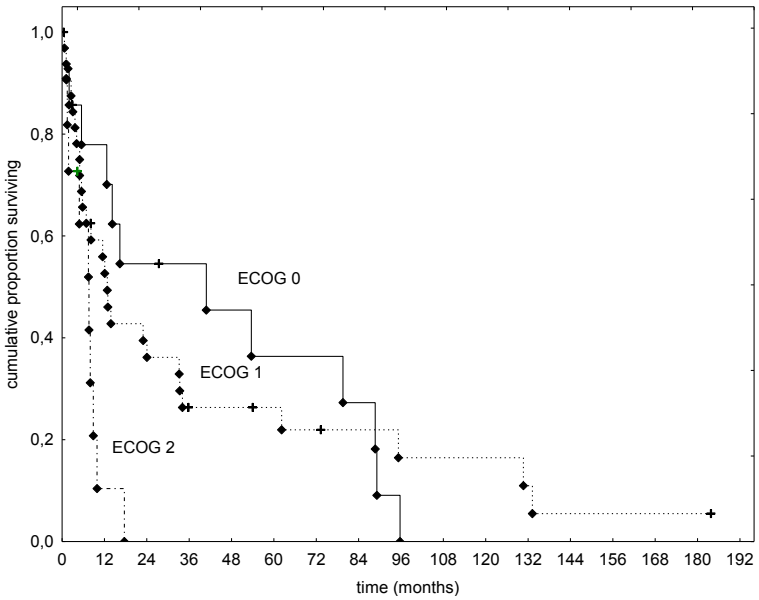

FIGURE 3. Overall survival of patients with Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, and 2.

those with performance status of 2. Five-year OS for patients with ECOG 0 and 1 was 36% and 26%, respectively, compared to 0% for patients with ECOG 2 (Figure 3). OS was lower in the group of smokers (p = 0.016). In addition, haemoptysis was a symptom associated with shorter OS (p = 0.049). The presence of other symptoms did not influence the overall survival. Patients who received radiotherapy had better OS (p = 0.026). Excision of the tumour (surgery or endoscopic resection) also had a positive influence on OS (p = 0.030). The use of systemic treatment was not proven efficient (p = 0.361). The variables significantly influencing the OS in the Kaplan-Meier analysis were then included into a multivariate analysis which confirmed significant influence of radiotherapy (p = 0.013), performance status (p = 0.033) and haemoptysis (p = 0.003) on OS. The histological type of cancer and surgical excision of the tumour did not reach the threshold of statistical significance (p = 0.068, and p = 0.324, respectively). The multivariate analysis did not confirm the independent significance of the smoking status (p = 0.109).

In 11 patients (19%, 8 men and 3 women), another cancer apart from the TC was diagnosed (Table 3). In one case, tracheal and renal tumours were diagnosed simultaneously. TC was diagnosed as a second cancer in 9 cases and in 1 as the first neoplasm. Five of them were located in the respiratory system (3 cancers of the larynx and 2 lung cancers). The others were: one renal cancer, one thyroid cancer, one prostate cancer, one breast cancer and one myelodysplastic syndrome.

Time between diagnoses ranged from 0 to 33.5 years with the median of 4.4 (mean 8.4). Treatment of the other cancer included: surgery in 8 cases, RT in 3 cases, and hormonal therapy in one case. Eight of these patients were diagnosed with SCC of the trachea, one patient with ACC, one patient with microcellular carcinoma, one patient with non-microcellular carcinoma. Six patients were current or former smokers with mean pack-years of 66.2 (range from 25 to 135).

Based on the predefined criteria we found additional 8 patients (13.8% of the whole group) as theoretically possible surgical candidates. If nodal involvement was not considered a contraindication for surgery, the total of 15 patients met the remain-

TABLE 3. The characteristics of other cancers among patients with tracheal cancer (TC)

Patient No	HP of TC	Year of diagnosis of TC	HP of the other cancer	Year of diagnosis of the other cancer
(1)	SCC	2011	Myelodysplastic syndrome	2009
(2)	SCC	2006	Larynx cancer - SCC	1998
(3)	SCC	2005	Clear cell renal carcinoma	2005
(4)	SCC	2011	Thyroid adenocarcinoma	1978
(5)	ACC	2005	Lung cancer - SCC	2006
(6)	Non-micro	2013	Clear cell renal carcinoma	1995
(7)	SCC	2008	Larynx cancer - SCC	1999
(8)	SCC	1999	Lung cancer - SCC	1995
(9)	SCC	2003	Larynx cancer - SCC	2000
(10)	Micro	2010	Prostate cancer - adenocarcinoma	2008
(11)	SCC	2015	Breast cancer - mucinous carcinoma	2005

ACC = adenoid cystic carcinoma; HP = histopathological type; Micro = microcellular carcinoma; No = number; Non-micro = non-microcellular carcinoma; SCC = squamous cell carcinoma; TC = tracheal cancer

ing criteria and the percentage of potential surgery candidates rose to 25.9%.

Metastases were initially diagnosed in 4 patients. During the follow-up they occurred in twelve more persons. In eleven of them, they were located in lungs, in two cases in bones, in one patient in spleen, in one case in liver and in one patient in cerebellum. In six of these patients, TC was diagnosed as SCC, in 5 patients as ACC, in two patients as microcellular carcinoma, in one patient as non-microcellular carcinoma, in one case as adenocarcinoma, and in one patient as papillary carcinoma.

Discussion

Epidemiological studies in Europe, USA and Japan have shown that most patients with primary malignant tracheal tumours present with an advanced local disease, have SCC tumours and are treated non-surgically.[3,8] Numerous authors point out that the diagnosis of TC is often delayed due to highly uncharacteristic symptoms, such as "frog in the throat" sensation reported by Howard *et al*.[8,10,13,18,23] In our series, one patient had been treated with surgery and RT for thyroid carcinoma until the second histopathological examination was performed and diagnosis of TC was made. Misdiagnosis of TC as thyroid tumour was also described by other authors.[9,21] In their report, Licht *et al*. described 109 cases of TC among which 17 were diagnosed incidentally at autopsy and 21% of treated patients received asthma medication due to symptoms present at the time of diagnosis.[13]

ACC is usually diagnosed in younger patients than SCC and, in the current series, the median age of diagnosis of SCC and ACC also differ: 59 and 46, respectively.[4,8,10,13,21,30] ACC histology was a prognostic factor correlated with better OS in our research, which is in agreement with studies in which patients with SCC and ACC were compared.[2,7,13,15,26,27,29,31] According to these reports, five-year OS for SCC ranged from 7% to 46%, and for ACC from 10 to 89.4%. In our group, the 5-year OS for SCC and ACC were 8.4% and 77.8%, respectively.

The other factors found to correlate with better OS were: surgery or complete resection[4,7,15,27-32], radiotherapy[2,4,13,28,29,31], the status of lymphatic nodes[7,13,14,32,33], stage[7,12,34,35], negative airways margins[14,15,32] and the performance status.[28,34]

The performance status of 0 or 1 was associated with better prognosis than PS 2 in our series which is in line with previous reports. We found statistically significant influence of the smoking status on OS only in the univariate analysis, however, this difference was not statistically significant in the multivariate analysis. Other studies also did not report OS to be influenced by the smoking status.

Number of patients who had undergone surgery as the first-line treatment varied among studies from 5% to 100%.[2,4-8,12-17,25-27,29] Licht *et al*., in a nationwide study of TC in Denmark, and Honings *et al*., in a nationwide study of TC in the Netherlands, reported that the number of patients that undergo surgery is 10% and 11.6%, respectively.[12,27] Honings and colleagues, in their multidisciplinary audit concerning undertreatment of TC, found 32% additional surgery candidates.[8] They pointed out that over 50% of TC could be treated surgically if properly diagnosed and referred to a tertiary oncology centre with multidisciplinary experience in the treatment of this disease. This is confirmed in our study. Only 25% of patients were treated invasively in our series, whereas this number could rise to about 39–51%, depending on the eligibility criteria employed. Due to retrospective nature of our research, it cannot be stated that all these patients were indeed undertreated. There might have been objective factors precluding surgery which were not mentioned in the documentation available for the authors of this report. Nevertheless, this finding should be a premise to meticulous assessment of the indications and contraindications for surgery to ensure the optimal treatment of TC patients. The centralization of the care for patients with this rare airway tumour may result in selection of a greater number of patients for surgical resections and, therefore, potentially better outcomes.[8]

In many studies, surgery was followed by adjuvant radiotherapy (in 20–100% patients), and those who were not eligible for surgery received radiotherapy as the main treatment. The overall survival depended on whether surgery was performed, resection was complete or radiotherapy followed surgery.[2,13-15,25-27,29] Our findings indicate that surgical treatment or endoscopic resection of the tumour did not influence OS in the multivariate analysis. This somewhat surprising result can be explained by a bias introduced by low number of complete resections and low number of patients with any invasive procedures in our series. It is remarkable, however, that five-year OS for non-surgery and surgery/endoscopic resection group were 10% and 48%, respectively.

Radiotherapy as a sole or main treatment method was described by a number researchers.[8,11,18,19,30-31,33-34,36] Only four studies present the

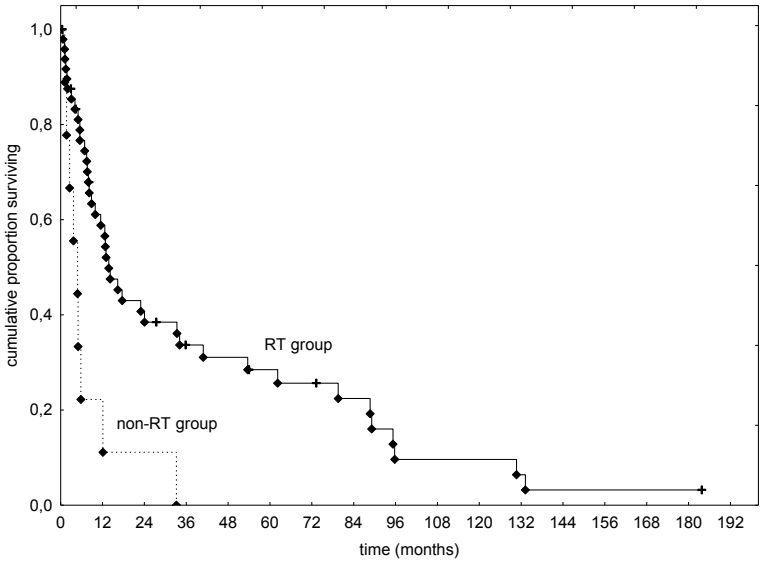

FIGURE 4. Overall survival in the irradiated (RT) and non-RT group.

outcome of at least 50 patients.[8,28,31,34] The median total doses usually ranged from 40 to 60 Gy_2. In our series, the doses delivered ranged from 14 to 82.6 Gy_2 and the median total dose delivered in radical radiotherapy was 66.0 Gy_2. In some reports, an additional brachytherapy boost of 6 to 30 Gy was added to escalate the dose delivered to the tumour.[19,28,34,37,38] In our series, three patients had a brachytherapy boost and the BT total dose ranged from 12.5 to 16 Gy_2. In the studies cited above, five-year OS for patients treated only with radiotherapy ranged from 8 to 30.4%.[19,28,31,33,34] Mornex *et al*. reported that the total dose over 56 (up to 70 Gy in their series) correlated with better outcomes - 12% for the higher TD compared to 5% for the lower.[28] Hetnał *et al*. reported that patients with ACC histology treated with radiotherapy had better outcomes than SCC patients, and five-year OS were 80% and 9% for ACC and SCC, respectively.[34] In our series, radiotherapy was a part of the treatment in 48 patients, and those patients who received RT had better overall survival in spite of large heterogeneity of the group (both radical and palliative treatments included), various fractionation schemes used, and no uniform target volume definition throughout the analysed period of time (Figure 4).

Only one of 8 patients who received brachytherapy had a haemorrhage (all others did not develop any toxicity after this treatment). Brachytherapy can be considered a part of palliative treatment like in case of bronchial infiltration in carefully selected patients. Analysis of the outcome in the current series cannot give a clear answer whether BT should be part of the treatment in all patients (for example as a boost to radical radiotherapy) because of the low number of patients in whom this treatment was employed.

There is no consensus about CTH use in TC treatment. Chemotherapy was a part of the therapy in 0% to 29% of patients in large studies.[2,12,14,25,26,28,29,31] Cisplatin was the most commonly used drug followed by cyclophosphamide and etoposide. In our series, CTH was used in 14 patients (various regimens were employed according to diagnosed histopathologic type of TC) and 13 of them (93%) received cisplatin-based therapy. CTH did not improve OS in our series which is similar to observation made by Manninen *et al*.[31] However, the retrospective nature of the study and heterogeneity of the group precludes drawing unequivocal conclusions.

In the current series, TC was diagnosed as a second cancer in 10 cases, and in 1 case it was the first neoplasm. Five of the other cancers were located in the respiratory system (3 cancers of the larynx and 2 lung cancers). Vrabec found multiple primary carcinomas in 11.5% of 1518 patients presenting with upper aerodigestive cancers.[39] Li *et al*. pointed out that there is a greater likelihood of developing multiple primary carcinomas in the same system than in unrelated tissues. The head and neck region and the lung are often involved with multiple primary cancers and patients with one tumour are more likely to develop a second one.[21] Gaissert *et al*. found second cancers in almost 20% of TC patients, and most of them (70%) were airway and lung cancers.[15] Other authors found second cancers in 8 to 44% of patients (groups over 50 patients), mostly located in lungs or larynx.[8,13,15,21-23,29] Histological type of the second cancer is usually the same as primary, and the SCC histology predominates.

As the number of second cancers among TC patients is relatively high, physicians engaged in the care of patients with cancer of the head and neck area should be prepared not only to treat the first malignancy, but also to be constantly vigilant for later development of subsequent primary carcinomas.[39]

To sum up, the diagnostic workup in TC patients should be concentrated on identification of those suitable for an invasive treatment and radiotherapy as it positively influences the outcome. Respiratory system cancer survivours can be considered a risk group for the tracheal cancer. Radiotherapy is an important part of the treatment of patients with TC, whereas the role of chemotherapy requires further investigation.

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research

Survival of patients with intermediate stage hepatocellular carcinoma treated with superselective transarterial chemoembolization using doxorubicin-loaded DC Bead under cone-beam computed tomography control

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Background. The purpose of this retrospective study was to evaluate treatment response, adverse events and survival rates of patients with intermediate stage HCC treated with superselective doxorubicin-loaded DC Bead transarterial chemoembolization (DEBDOX) under cone beam computed tomography (CBCT) control.

Patients and methods. Between October 2010 and June 2012, 35 consecutive patients with intermediate stage HCC (32 male, 3 female; average age, 67.5 ± 7.8 years; 22 patients Child-Pugh class A, 8 class B, 5 without cirrhosis) were treated with DEBDOX TACE. Portal vein thrombosis was observed in 6 (17.1%) patients. DEBDOX TACE was performed by superselective catheterization of feeding vessels followed by embolization with 100-300 μm microspheres loaded with 50-100 mg of doxorubicin. In all cases, CBCT was used during chemoembolization. Tumor response rates were defined according to mRECIST criteria.

Results. Overall, 120 procedures were performed (mean, 3.2 per patients). We treated 97 lesions with an average diameter of 4.9 ± 1.9 cm. There were 32 minor and 2 (1.6%) major complications (one liver abscess and one cerebrovascular insult). After a mean follow-up of 27.7 ± 10.5 months, 94.3% of patients achieved an objective response to treatment (42.4% complete response and 57.6% partial response). Mean time to progression was 10.9 ± 5.3 months. Mean overall survival was 33.9 months (95% CI; 28.9 – 38.9 months), with 1- and 2- year survival of 97.1% and 65.7%, respectively.

Conclusions. Superselective DEBDOX TACE performed under CBCT control is a safe and effective method with high rates of tumor response and overall survival.

Key words: hepatocellular carcinoma; chemoembolization; doxorubicin; drug eluting bead; cone-beam computed tomography

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer mortality in the world.[1] According to the Barcelona Clinic Liver Cancer (BCLC) staging system, curative therapies (resection, transplantation, and percutaneous ablation) can improve survival in HCC patients diagnosed at an early stage and offer potential long-term curative effects.[1] About 20% of HCC patients are classified as BCLB-B, or intermediate-stage HCC.[1] Transarterial chemoembolisation

(TACE) is the standard treatment for patients with intermediate-stage HCC, but due to heterogeneity of the patient population in this stage, tumor response and survival rates are variable and scattered across the literature.[2-4] Several clinical studies have confirmed the benefits of doxorubicin-loaded DC Bead (DEBDOX; drug-eluting bead doxorubicin] with respect to improved tumor response, reduced adverse events and improved survival.[5-10] Furthermore, recent data shows that superselective TACE is associated with lower adverse events, higher rate of tumor response and increased survival.[11-13] In superselective TACE, proper identification of the tumor feeding arteries and detection of the target tumor is crucial.[11,12,14] However, angiography frequently cannot identify HCC lesions because of their small size or decreased hypervascularity. This often results in lobar or segmental TACE of relatively large liver areas, and in turn to lower rate of tumor response and potential increase in adverse events due to high repetition of TACE. Cone-beam CT (CBCT) is a novel technique that is increasingly used during TACE for inoperable HCC.[15-20] This imaging technique uses a flat-panel detector angiographic system to produce CT-like soft-tissue images without the need to transfer the patient to a CT unit.[15,17,19] It can be used to visualize tumor-feeding vessels and parenchymal stain during TACE, achieving detection accuracies significantly superior to standard 2D angiography.[17] In this respect, the advantage of using CBCT during TACE improves the safety and effectiveness of the procedure, and has been suggested to have a benefit on local recurrence rates and overall survival during conventional TACE.[20,21]

The purpose of this retrospective study was to evaluate treatment response, adverse events and survival rates of patients with intermediate stage HCC treated with superselective DEBDOX TACE under CBCT control.

Materials and methods

Patient selection

This single institution study included 35 patients with intermediate stage HCC that were treated with DEBDOX TACE under CBCT control between October 2010 and June 2012. Clinical examination, laboratory evaluation and CT and/or MR imaging were performed in each patient at baseline at least one month before the TACE session. The inclusion criteria for DEBDOX-TACE were intermediate stage HCC according to BCLC system. Portal vein thrombosis was not an exclusion criterion.

Written informed consent of patients was obtained before the treatments. Ethics committee approval for treatment was provided for data analysis. Ethics committee approval for treatment was not required because TACE is approved as a standard of care for intermediate stage HCC. All the procedures followed the Helsinki declaration.

TACE technique

Treatment with chemoembolization was based on the consensus of the Liver Multidisciplinary Team Meeting, held weekly at our institution. All patients underwent at least two sessions of DEBDOX TACE. After local anesthesia, a short 5 F introducer sheath (Terumo Europe N.V., Belgium) was put in place via the common femoral artery. Digital subtraction angiography (DSA) of the celiac and superior mesenteric arteries was routinely performed via a 5 F catheter (Sidewinder®, Terumo Europe N.V.) to determine vascular anatomy and variants and to assess portal flow. DSA was performed with the administration of non-ionic contrast agent iopromide 370 mg/ml (Ultravist 370®, Bayer HealthCare, Germany) through a power injector (Avanta®, Medrad, Bayer HealthCare).

The injection rate was normally 5 mL/s with a total of 20 mL injected. A 2.4 F microcatheter (Progreat®, Terumo Europe N.V.) was then superselectively positioned in the tumor feeding arteries before delivery of the DC Bead (DEBs) (DC Bead®, Terumo Europe N.V.). Prior to DEBs delivery, DSA and CBCT were performed with the administration of iopromide through a power injector to confirm complete coverage of the targeted lesion(s). DEBs with a diameter of 100-300 μm were loaded with 50 mg of doxorubicin per vial (maximum dose of 100 mg of doxorubicin). In patients with multifocal tumors, the position of the microcatheter was changed within the same session to ensure superselective DEBs delivery in each lesion (Figure 1). Delivery of the mixture was continued until a near stasis end point or the antegrade blood flow was achieved. After DEBDOX TACE, completion arteriography was performed by manual injection to minimize reflux into non-targeted areas. All patients were kept under observation for a period of 24-48 h. Second DEBDOX TACE was performed after a period of 4-6 weeks in all patients. Additional chemoembolization procedures were performed if the multifocality of the disease did not allow for complete targeting of the tumor in the first two treatment sessions (Figure 2). DEBDOX TACE treatment was repeated on demand, that is, in pa-

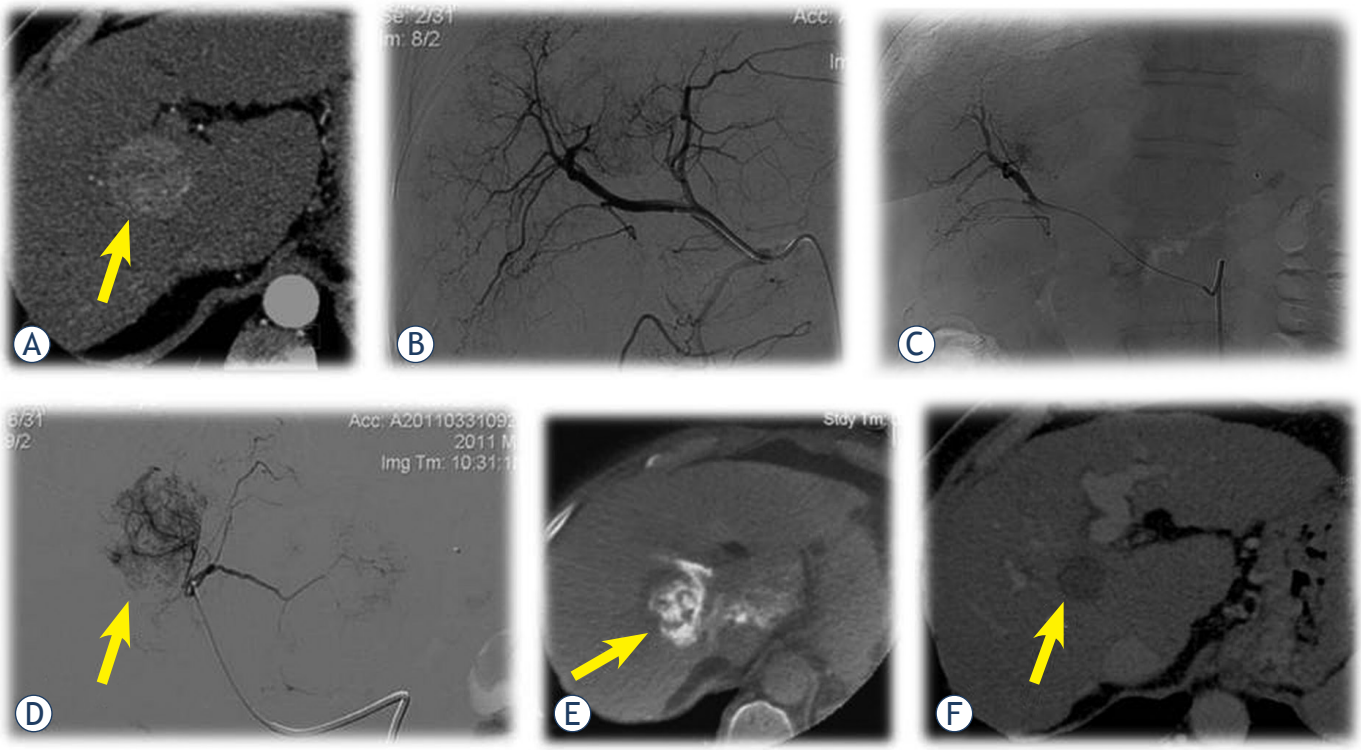

FIGURE 1. A 56-year-old female with HCC. **(A)** Contrast-enhanced CT shows tumor (arrow) between right and left liver lobe. **(B)** Initial angiography shows tumor in the liver (arrow). **(C)** Superselective contrast injection throught microcatheter into segmental branches for eight liver segment confirmes the tumor. **(D)** Superselective contrast injection throught microcatheter into segmental branches for first liver segment also confirmes the tumor (arrow). **(E)** CBCT after contrast injection throught microcatheter into segmental branches for first liver segment confirmes that the target lesion (arrow) is dominantely suplied from this artery and superselective DEBDOX TACE was performed from both artery. **(F)** Two months after the DEBDOX TACE, control CT shows complete devascularization of the target lesion (arrow) (complete response).

tients with residual or recurrent tumors observed by CT or MRI, according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) and in agreement with recent expert opinions.[22]

Cone beam CT technique

All patients underwent C-arm single phase CBCT. Imaging was performed by using a commercially available angiography system (Allura Xper FD20®; Philips HealthCare). This system was equipped with the XperCT option, enabling C-arm CBCT acquisition and volumetric image reconstruction (Feldkamp back projection). For each CBCT scan, the area of interest was positioned in the system isocenter, and, over approximately 10 seconds, 310 projection images were acquired with the motorized C-arm, covering a 240° clockwise arc at a rotation speed of 20° per second. As the images were being acquired, the projections were transferred via fiber-optic connection to the workstation (Philips Xtravision, Rel 6.2; Dell Precision 670; Round Rock, TX/USA). The two-dimensional projection images were reconstructed by using Feldkamp back projection into three-dimensional volumetric images with isotropic resolution of 0.98 mm for a 250 × 250 × 194-mm field of view (matrix size, 256 × 256 × 198). Typically, a 2.4-French microcatheter (Progreat®, Terumo Europe N.V.) was advanced into either a subsegmental or a segmental hepatic artery, depending on the location of the targeted tumor. CBCT was performed with the administration of non-ionic contrast agent (Ultravist 370®, Bayer HealthCare) through a power injector (Avanta®, Medrad, Bayer HealthCare). The injection rate was typically 1mL/s with a total injected volume of contrast agent 10 mL and delay time 15 seconds. The patients were instructed to be at end-expiration apnea during each CBCT acquisition. The final position of the microcatheter for delivery of the DEBs was based on the results of C-arm CBCT. This was done to superselectively deliver the bead to the tumors (Figure 3).

Follow-up

Follow-up after DEBDOX TACE included clinical evaluation, laboratory data, tumor treatment re-

sponse and serious adverse events registration. Treatment response was evaluated with contrast enhanced four-phase computed tomography (CT) or magnetic resonance (MR) imaging with hepatospecific contrast media 2 months after DEBDOX TACE according to mRECIST.[22] A viable tumor was defined by contrast agent uptake in the arterial phase and washout in the portal phase and/or late phase. Radiological follow up was performed every 3 months with either contrast enhanced four-phase CT or MRI with hepatospecific contrast media. CT was performed on a 64-row multidetector CT scanner (Siemens Medical Systems®, Erlangen, Germany) and 16-row multidetector CT scanner (Siemens Medical Systems®) with a four-phase protocol (non-enhanced, arterial, portal and late venous phase), which involved the administration of 100 to 130 ml of non-ionic contrast agents (Ultravist 370®, Bayer HealtCare; Visipaque 320®, GE Healthcare) at a rate of 5 ml/s via a power injector by using a bolus tracking algorithm. Images were reconstructed at 5- and 2-mm thickness in axial and coronal planes.

MR imaging was performed on 3 T MAGNETOM Trio, A Tim Sistem scanner (Siemens Medical Systems®). All images were acquired with a phased array abdominal coil. Routine protocol included imaging by using T2 weighted fast spin echo sequences, T1 weighted fat suppressed in- and out- of phase GRE, diffusion weighted imaging and dynamic enhanced multiphasic breath-hold T1 imaging by using hepatospecific contrast agent (Primovist®, Gd-GD-EOB-DTPA, Bayer Schering Pharma AG, Germany). Images were acquired before contrast material application and during the arterial, portal, equilibrium, delayed and hepatospecific phase (after 20 minutes or more); 0.2 mM/ kg of contrast agent was injected intravenously at a rate of 1 ml/s followed by 20 ml of saline.

Tumor response, time to progression and survival assessment

Tumor response, mean time to progression and overall survival (OS) were calculated according to a follow-up time defined as the number of months from first DEBDOX TACE to 12th August 2014, last imaging control of the patient, last contact with the patient or patient's death. Treatment response was determined at every follow-up imaging control, according to mRECIST.[22] Patients with complete response or partial response were classified as having an objective response to treatment.

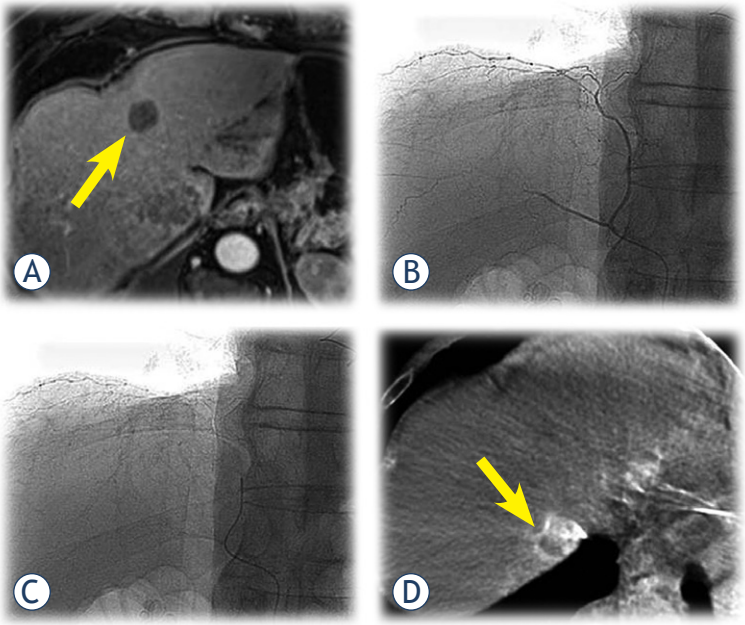

FIGURE 2. A 64-year-old male with HCC. **(A)** Control MR after chemoembolization shows complete response of the target lesion (arrow) in fourth segment and partial response of the target lesion in eight segment. **(B)** Superselective contrast injection throught microcatheter into the righr phrenic artery. **(C)** Position of microcatheter prior CBCT. **(D)** CBCT shows that this artery suplied the target tumor in seventh segment. Superselective DEBDOX TACE was performed.

Statistical analysis

Quantitative variables were expressed as mean ± standard deviation and categorical as count and proportions. Survival rates and curves were determined using the Kaplan-Meier methods. Differences in the survival rate, regarding Child class were assessed using the log rank test. Last date for collection of data and calculating survival was 12th August 2014. A p value less than 0.05 was considered statistically significant.

Potential prognostic variables were evaluated as predictors of survival in the Cox proportional hazards model. All variables with a p value < 0.05 at univariate analyses were included in the multivariate analyses. All calculations were performed with statistical software (SPSS package version 19.0; SPSS Inc, Chicago, IL, USA).

Results

Patient characteristics at baseline

The baseline demographic, clinical, laboratory and tumor staging characteristics of the patients included in the analysis are summarized in Table 1. Mean

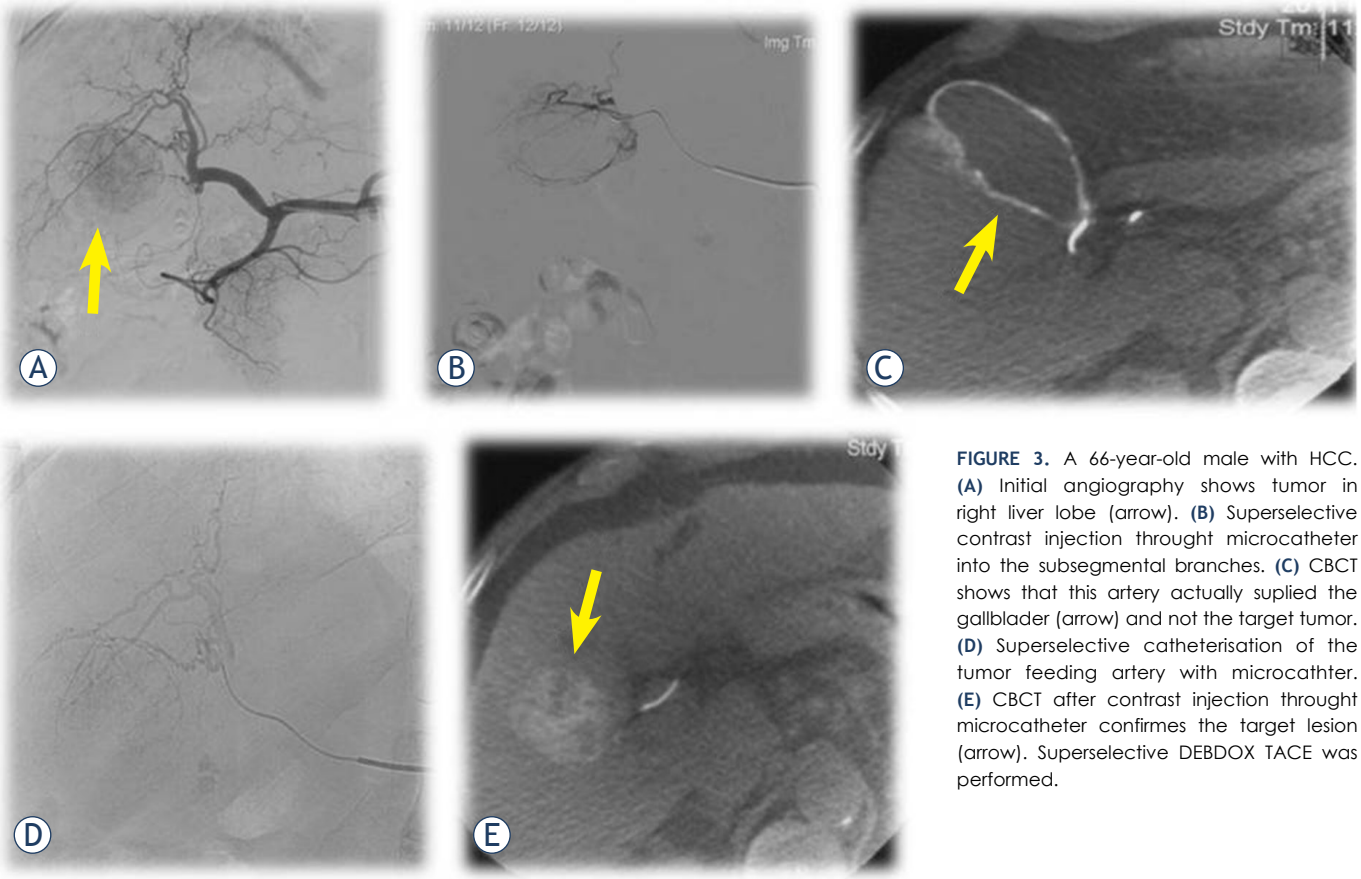

FIGURE 3. A 66-year-old male with HCC. **(A)** Initial angiography shows tumor in right liver lobe (arrow). **(B)** Superselective contrast injection throught microcatheter into the subsegmental branches. **(C)** CBCT shows that this artery actually suplied the gallbladder (arrow) and not the target tumor. **(D)** Superselective catheterisation of the tumor feeding artery with microcathter. **(E)** CBCT after contrast injection throught microcatheter confirmes the target lesion (arrow). Superselective DEBDOX TACE was performed.

patients age was 67.5 ± 7.8 years, the majority were male (32 of 35 patients). Five of 35 (11.4%) patients were not cirrhotic. The most frequent etiology of cirrhosis was ethanol (43.3%). Twenty-two patients were classified as Child-Pugh class A (73.3%) and the remaining 8 as class B (26.7%). Twenty-four of 35 (68.6%) patients had unilobar, predominantly right lobe disease. Portal vein thrombosis was observed in 6 (17.1%) patients.

Procedure and complications

Overall, 120 DEBDOX TACE procedures were performed in 35 patients. Mean number of procedures per patient was 3.2 ± 1.5. Superselective catheterization of feeding vessels was followed by embolization with 100-300 μm microspheres loaded with 50 mg of doxorubicin. The maximum cumulative dose of doxorubicin was 100 mg. All procedures were performed under CBCT control.

Complications occurred in 34 of 120 procedures. There were two (1.6%) major complications: an ischemic cerebrovascular insult to the cerebellum and an infection of the necrotic tumor that resolved after antibiotic treatment and resulted in prolonged hospitalization. Minor complications occurred in 32 procedures (26.6%), of which 4 procedures resulted in two complications simultaneously, which were, however, etiologically different (Table 2).

Response rate and time to progression

Thirty-three of 35 patients (94.3%) achieved an objective response after two sessions of DEBDOX TACE. Fourteen of 33 (42.4%) patients achieved complete response, while 19 (57.6%) achieved a partial response. Of the remaining two patients with no objective response, one patient had stable disease and the other had progressive disease (appearance of numerous new lesions in both liver lobes) despite continuous treatment with DEBDOX TACE. These patients were treated with sorafenib.

Twenty of 33 patients who had already achieved an objective response developed disease progression over time. Mean time to progression (TTP) was 10.9 months ± 5.3 months (range, 5.8 – 24.8 months).

Overall survival

After a mean follow-up period of 27.7 months ± 10.5 months, 17 patients died. One patient died of acute respiratory infection with acute respiratory insufficiency, two of spontaneous bacterial peritonitis with sepsis, one of respiratory arrest, two of toxic encephalopaty, four of progression of HCC and the remaining of liver failure. Mean OS of the whole cohort was 33.9 months (95% CI: 28.9 – 38.9). 1-year survival was 97.1% and 2-year survival was 65.7%. There was no significant difference in survival between patients with cirrhosis Child A and B (p = 0.417). Cox regression analysis showed that none of the clinical and laboratory data were statistically significant independent risk factors for survival.

TABLE 1. Baseline demographic, clinical, laboratory and tumor staging characteristics of patients

Characteristic	Value
Age, [years]	67.5 ± 7.8
Gender (M/F), n [%]	32/3 [91.4/8.6]
Cirrhosis (yes/no), n [%]	30/5 [85.7 /14.3]
Etiology of cirrhosis, n [%]:	
Ethanol	13 [43.3]
HBV	4 [13.3]
HCV	4 [13.3]
other	9 [29.9]
Albumin [g/l]	38.4 ± 4.6
INR	1.2 ± 0.2
Total bilirubin [μmol/l]	25.6 ± 17.4
Child-Pugh score (points)	6.0 ± 0.7
Child-Pugh score (classes), n [%]:	
A	22 [73.3]
B	8 [26.7]
Creatinine [μmol/l]	81.7 ± 23.4
ASAT [μkat/l]	1. 1 ± 0.7
ALAT [μkat/l]	0.8 ± 0.6
γGT [μkat/l]	2.3 ± 1.6
aFP [kIE/l]	152.5 ± 310.2
Portal vein thrombosis (yes/no), n [%]	6/29 [17.1/82.9]
Bilobar disease, n [%]	11 [31.4]
Unilobar disease, n [%:]	24 [68.6]
right lobe, n [%]	20 [83.3]
Overall number of nodules, n	97
Number of nodules per pt, n	2.8 ± 2.2
Maximum diameter of HCC nodule per pt. [cm]	4.7 ± 1.9

HBV = hepatitis B virus, HCV = hepatitis C virus; INR = international normalised ratio; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; γGT = gamma glutamyl transpeptidase; aFP = alpha-feto protein; pt = patient. Quantitative variables expressed as mean ± standard deviation.

Discussion

The purpose of this retrospective study was to evaluate treatment response, adverse events and survival rates of patients with intermediate stage HCC treated with superselective DEBDOX TACE under CBCT control. By facilitating tumor targeting and super-selective therapy delivery, our results support the hypothesis that CBCT plays a key role in achieving satisfactory tumor response and OS.[20,21]

TACE is the most widely used loco-regional therapy for patients with intermediate stage HCC. The number of treatment sessions depends on the response of the tumor and whether serious side effects are seen.[3-5,7] The overall response rate for this treatment is about 50%, with the lowest reported around 15% and the highest around 85.6%.[2-5,16] In a prospective study of 67 consecutive patients (122 nodules, all < 5 cm), Golfieri *et al.* showed that, when compared with lobar conventional TACE (cTACE), selective/superselective cTACE was associated with higher mean levels of necrosis (75.1% versus 52.8%; p = 0.002) and a higher rate of complete necrosis (53.8% versus 29.8%, p = 0.013).[11] These findings suggest that selective/superselective cTACE may determine a higher rate of tumor necrosis than lobar TACE. With improved treatment efficacy and tolerance, DEBDOX TACE represents a major advancement in treatment of intermediate stage HCC.[2,6,9,10,23] In a recent prospective, randomized phase II study comparing cTACE with DEBDOX TACE, the DEBDOX TACE group showed a trend for a higher objective response rate than the cTACE group (51.6% versus 43.5%, respectively), together with better tolerability.[8,24] In another prospective, randomized study, DEBDOX TACE resulted in a better objective response than bland embolization with non-loaded bead (73.1% versus 55.9%, respectively).[10] Similarly, Boatta and colleagues showed a 72% objective response rate in 154 patients treated with DEBDOX TACE.[24] In a study by Suk *et al.*, where CBCT after DEBDOX TACE was used to predict short-term tumor response according to mRECIST, objective response was achieved in 85.6% of patients (complete response 63.8%, partial response 21.8%), and objective response rates at one month after CBCT-guided TACE was also reported in a study by Loffroy *et al.* in 78% of patients (complete response 46.3%, partial response 26.8%).[2,16] In a retrospective study of 116 consecutive patients Georgiades *et al.* showed that delivery

of two treatment sessions also lead to higher tumor response.[25]

Our results show a very high percentage (91.9%) of objective response defined according to mRECIST. The most plausible reasons for high objective response rate in our study were the routine utilization of CBCT control to guide superselective DEBDOX TACE in all patients and the delivery of at least two treatment sessions for all lesions.

TACE is also associated with OS benefits, reported in a meta-analysis of randomized controlled trials.[3,26] Reported 1-, 2- and 3-year survival rates ranged from 24% to 90%, 59% to 81% and 29% to 47%, respectively.[3,13,21,25,27] However, most of these studies assessed cTACE.[3,4,6] Studies reporting on survival of patients treated with DEBDOX TACE are scarce. A retrospective case–control study from a single academic institution in the United States demonstrated a survival benefit for patients undergoing DEBDOX TACE versus cTACE.[23] One-year and 2-year survival were superior with DEBDOX TACE.[28]

In a recent study from Burrel *et al.* that analyzed strictly selected patients with intermediate stage HCC treated with DEBDOX TACE in a highly specialized center, reported 1- and 3-year OS rates equal to 89.9% and 66.3%, respectively. Median survival time in this study was 48.6 months.[6] The overall favourable efficacy of DEBDOX TACE was further corroborated by a recent international, long-term (5 years of follow-up) study, where OS at 5 years was 22.5% and the mean OS was 43.8 months.[29] To our knowledge there is only one study determining survival of HCC patients treated with TACE under CBCT control. This study showed that patients receiving CBCT-assisted cTACE had significantly higher OS rates than those receiving cTACE with angiography alone. OS rates of patients who underwent chemoembolization under CBCT assistance were 94%, 81% and 71% at 1, 2, and 3 years, respectively.[21] OS rates in our study were 97% at 1 year, 66% at 2 years, with an average survival of 33.9 months. In particular, 1-year survival rate of our patients was higher than in most other studies reported in the literature, while 2-year survival was only marginally lower than the results reported by Iwazawa *et al.*, where cTACE was also performed under CBCT control (2-year survival, 81%).[21] The high 1-year OS reported in our study may be related to the high objective response rates achievable with DEBDOX TACE, supported by the fact that objective response to treatment correlates with prolonged OS.[30] In addition, the most common reason for death in our study was not related to progression of HCC but rather to liver cirrhosis, which may explain the significant drop in 2-year survival.

TABLE 2. Number and type of minor complications after DEBDOX TACE.

Type of complication	No. of complications
Post-embolization syndrome*	23
Rise in blood pressure	4
Gastric erosions or ulcers	2
Chest pain	2
Hematoma at puncture site	1

*Post-embolization syndrome was defined as elevated body temperature, pain in the abdomen, nausea and/or vomiting, leukocytosis and elevated liver enzymes.

TACE-associated adverse events, although usually transient and manageable, occur in a significant proportion (35 – 100%) of patients, and may include post-embolization syndrome (comprising of fever, abdominal pain and a moderate degree of ileus), relevant liver function deterioration, ascites and gastrointestinal bleeding.[11] Our analysis shows that DEBDOX TACE under CBCT control is a safe treatment method. The majority of complications were minor, either self limited or managed conservatively. One of the two major complications, infection of tumor necrosis, was due to the fact that necrotic tissue is an optimal culture medium for bacteria.[6] The other major complication was a cerebrovascular insult. This was likely due to the reflux of the contrast and DEB particles mixture from an extrahepatic feeding artery (mammary artery) into the basilar artery that resulted in ischemic injury to the cerebellum. To our knowledge, 12 other cases with 25% mortality are reported in the literature.[31] Our patient, however, died of liver failure 5 months after the procedure. Thus, no treatment-related deaths were reported.

We speculate that the utilization of intraprocedural CBCT and delivery of at least two treatment sessions for all lesions contributed to the high response rates, low occurrence of adverse events and good OS reported in our study. This imaging technique provides cross sectional, soft-tissue, CT-like images without the need to move the patient to a CT room and offers many advantages over classic 2D angiography alone.[15,17,19,32] First, CBCT detection accuracy of HCC lesions is equivalent to multidetector CT and MRI and superior to 2D

angiography.[15,17,19] Second, it is the most accurate imaging technique to identify tumor-feeding arteries.[14] Third, selective CBCT during hepatic angiography may be used to rule out non-target embolization to non-tumor feeding extrahepatic arteries and ensure that the selected branch supplies the tumor safety margin.[17,18,33,34] In this respect, CBCT can also help with catheter placement when synchronous non-tumor and tumor enhancement are seen and can distinguish between tumor and non-tumor staining, most frequently occurring with feeding from the inferior phrenic artery.[18] Finally, effective radiation doses for CBCT have been reported to be lower than with conventional.[2,18]

CBCT also has some limitations. Its contrast resolution is lower than that of conventional CT, thus providing less differentiation between HCCs and surrounding liver parenchyma.[18,19] Furthermore, the detection of HCCs under diaphragm may be impaired by motion artefacts, while streak artefacts from the catheter and contrast material may also degrade image quality.[15,18,19] The field of view of CBCT is smaller than that of conventional CT and liver truncation may occur.[18] Finally, the acquisition of CBCT images requires additional contrast administration. This can be balanced with the use of CBCT guidance software to guide micro-catheter positioning to the targeted arteries, which may in turn help to reduce both radiation exposure and contrast administration.[17]

There are some limitations in our study. First, this study was a retrospective analysis and lacks a control group, thus failing to provide robust data of a prospective randomized controlled trial. In particular, we decided to avoid randomization between superselective DEBDOX TACE under CBCT and superselective DEBDOX TACE without CBCT, because of the anticipated clinical advantages of CBCT. Second, our small sample size may have introduced a selection bias. The BCLC B stage includes a heterogenous population of HCC patients and there is lack of a standard treatment methodology and patient selection criteria for TACE.[25] Therefore, our patient selection criteria were strict in order to achieve the best outcome. Third, histopathologic correlation with CT or MR imaging regarding tumor necrosis after treatment with DEBDOX TACE was not performed since previous reports already showed good correlation between the percentage of tumor necrosis obtained at histopathologic examination and the tumor enhancement assessed with imaging.[11,16]

In conclusion, superselective DEBDOX TACE performed under CBCT control is a safe and effective method with high tumor response and survival rates. Further prospective studies with a larger number of patients are required to elucidate the incremental benefit when compared to conventional techniques.

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research

The ratio of weight loss to planning target volume significantly impacts setup errors in nasopharyngeal cancer patients undergoing helical tomotherapy with daily megavoltage computed tomography

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Background. Changes in head and neck anatomy during radiation therapy (RT) produce setup uncertainties of nasopharyngeal cancer (NPC) irradiation. We retrospectively analyzed image guidance data to identify clinical predictors of setup errors.

Patients and methods. The data of 217 NPC patients undergoing definitive RT on a helical tomotherapy (HT) unit were analyzed. Factors including tumor stage, body mass index, weight loss, and planning target volume (PTV) were assessed as predictors of daily megavoltage computed tomography (MVCT) setup displacements, which were automatically registered using software.

Results. Mean daily setup displacements (in mm) were 1.2 ± 0.6, 1.8 ± 0.8, 3.4 ± 1.4 in the medial-lateral (ML), superior-inferior (SI), and anterior-posterior (AP) directions, respectively. Mean weight loss was 4.6 ± 3.3 kg (6.8 ± 4.9%). Patients with weight loss > 5% had significantly larger setup displacements in the AP (3.6 ± 1.5 *vs.* 2.9 ± 1.1 mm, $p < 0.001$) and SI (1.6 ± 0.7 *vs.* 1.9 ± 0.9 mm, $p = 0.01$) direction, but not in the ML direction ($p = 0.279$). The AP setup error increased 0.06 mm ($y = 0.055x + 2.927$, x: percentage of weight loss/PTV, y: AP displacement) per one percent increase in weight loss normalized to PTV.

Conclusions. Patients with weight loss > 5% and smaller PTVs, possibly because of small body frame or neck girth, were more likely to have increased setup errors in the AP direction.

Key words: nasopharyngeal cancer; intensity-modulated radiotherapy; setup errors

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant neoplasm of mucosal origin.[1] It has unique racial and geographic distribution with higher incidence in Southeast Asia, North Africa, and the Middle East.[2] Since the nasopharynx is located posterior to the nasal cavity and surrounded by critical structures, radical surgical resection of NPC is very challenging.[1] Radiation therapy (RT) is the mainstay of treatment for NPC due to its radiosensitivity.[3,4] Compared to three-dimensional conformal

TABLE 1. Patient characteristics (n = 217)

Variable	Number (%)	
Age		
Median	46	
Range	17–76	
Sex		
Male	160	(73.7)
Female	57	(26.3)
T stage		
1	97	(44.7)
2	32	(14.7)
3	50	(23.0)
4	38	(17.5)
N stage		
0	51	(23.5)
1	67	(30.9)
2	67	(30.9)
3a	18	(8.3)
3b	15	(6.9)
AJCC stage		
I	29	(13.4)
II	47	(21.7)
III	76	(35.0)
IVA	32	(14.7)
IVB	33	(15.2)
Chemotherapy		
No	27	(12.4)
Yes	190	(87.6)
Fraction		
33 (2.12 Gy/fraction)	182	(83.9)
35 (2 Gy/fraction)	35	(16.1)

AJCC = American Joint Committee on Cancer

radiation therapy, intensity-modulated radiation therapy (IMRT) yields superior parotid gland sparing and reduces xerostomia.[5-7]

IMRT delivers a conformal and steep dose gradient. Precise target localization is critical for IMRT delivery. During the course of RT to NPC, the primary tumor and surrounding anatomy commonly undergo significant volume changes secondary to tumor shrinkage and weight loss.[8,9] Changes in head and neck anatomy during treatment can potentially compromise the accuracy of radiation delivery.[8] The aim of image-guided radiation (*i.e.*, image-guided radiotherapy; IGRT) is to detect and correct the set-up errors prior to treatment of head and neck cancers, including NPC.[10-13]

No set-up error study with large patient numbers has identified factors affecting set-up errors in radiation delivery to patients with NPC. Among IGRT modalities, helical tomotherapy with daily megavoltage computed tomography (MVCT) is the one most frequently used to evaluate and correct daily online interfraction setup errors.[11,14] Using daily MVCT setup data, our aim was to determine which clinical and treatment factors (including tumor stage, body mass index [BMI], weight loss, and planning target volume [PTV]) predict setup errors in NPC patients treated with IMRT by tomotherapy.

Patients and methods

Patients

We retrospectively reviewed the clinical and RT data of 217 NPC patients (160 males and 57 females; median age 46 years [range 17–76]) who underwent definitive RT by helical tomotherapy between September 2008 and May 2013 at the National Taiwan University Hospital. Patients who received RT as salvage or with palliative intent were excluded. A total of 29 (13.4%), 47 (21.7%), 76 (35%), and 65 (29.9%) patients had American Joint of Cancer Committee (AJCC 7th edition) stage I, II, III, and IV, respectively (Table 1). Concurrent cisplatin and/or tegafur-uracil (UFUR) was used in 190 (87.6%) patients at the discretion of medical oncologists.

This study followed the Helsinki Declaration and complied with the ethical standard guidelines. No patient identifying information was used or reported. Our institutional review board approved this retrospective study with the approval number 201605075RINC on 6/16/16.

Treatment planning

All patients underwent CT simulation with thermoplastic mask immobilization. CT scan images of 3-mm slices were obtained with and without intravenous contrast medium. IMRT doses were delivered to three target volumes defined for each patient in 33 to 35 treatment fractions. The primary tumor and involved lymph nodes with safety margin were treated to 70 Gy fractionated into 2–2.12 Gy daily doses. The clinical target volume (CTV) of areas at risk for microscopic involvement (nasopharynx, retropharyngeal nodal regions, skull

base, clivus, pterygoid fossae, parapharyngeal space, sphenoid sinus, nasal cavity, maxillary sinuses, and levels I through V cervical nodes) were treated with 59.4–64 Gy. Clinically negative parts of the lower neck were treated with 54 Gy. A margin of 3 mm was used for the PTV. Margins were reduced if the CTV was near critical structures including the brain stem and eyes. The prescribed dose covered 95% of the PTV. Doses to the organs at risk including brainstem, spinal cord, eyes, optic nerves, pituitary gland, parotid glands, submandibular glands, thyroid glands, cochlea, brachial plexus, and oral cavity were minimized without compromising PTV coverage.

Daily MVCT and setup error

Prior to each treatment, MVCT images were acquired after positioning the patient using wall lasers and external markings, and were reconstructed with "Normal" (4 mm) slice thickness. Setup displacements were determined with autoregistration software using the "Bone and Soft tissue" option. Setup errors of the target in the medial-lateral (ML), superior-inferior (SI), anterior-posterior (AP) directions as well as rotational errors were obtained. If a rotational displacement was larger than 3 degrees, the therapists would re-position the patient and repeat MVCT. Translational errors were corrected by automatic couch positioning.

Data analyses

Clinical and treatment factors including T and N stages, BMI, weight loss percentage, and PTV were used for data analyses. We performed the Student *t*-test and linear regression to correlate these factors with setup errors. Statistical analyses were done using the software SPSS Version 22. P values less than 0.05 were considered statistically significant.

Results

The patient cohort had an average weight (±standard deviation) before RT of 67.9 ± 13.1 kg (range: 40.0–128.8), average BMI before RT of 24.4 ± 3.5 kg/m^2 (range: 16.4–40.3), mean weight loss following 6 to 7 weeks of RT of 4.6 ± 3.3 kg (range: –3.8 to +14.5), which represented 6.8 ± 4.9% (range: –8.3 to +18.0) of weight before RT, and mean PTV of 848.0 ± 210.2 cc (range: 415.7–1584.3). A total of 7231 MVCT images were evaluated for setup errors, with a median number of 33 (range: 33–35) MVCT images per patient.

Mean daily setup displacements (in mm) were 1.2 ± 0.6, 1.8 ± 0.8, and 3.4 ± 1.4 in the ML, SI, and AP directions, respectively (Table 2). The displacement was significantly larger in the AP direction than in the ML ($p < 0.001$) and SI ($p < 0.001$) directions. The displacement was also significantly

TABLE 2. Setup error by clinical factors

	ML error	SI error	AP error
Overall	1.2 ± 0.6	1.8 ± 0.8	3.4 ± 1.4
T stage			
1,2 (n = 129)	1.2 ± 0.6	1.8 ± 0.9	3.3 ± 1.5
3,4 (n = 88)	1.1 ± 0.5	1.7 ± 0.8	3.5 ± 1.3
p value	0.241	0.814	0.414
N stage			
0, 1 (n = 117)	1.1 ± 0.6	1.7 ± 0.8	3.4 ± 1.3
2, 3a, 3b (n = 100)	1.2 ± 0.6	1.9 ± 0.9	3.4 ± 1.5
p value	0.133	0.239	0.890
BMI			
< 25 (n = 138)	1.2 ± 0.6	1.7 ± 0.8	3.2 ± 1.3
≥ 25 (n = 79)	1.2 ± 0.5	1.9 ± 0.9	3.6 ± 1.5
p value	0.845	0.177	0.053
PTV			
< 850 cc (n = 113)	1.2 ± 0.7	1.9 ± 0.9	3.6 ± 1.5
≥ 850 cc (n = 104)	1.2 ± 0.5	1.6 ± 0.7	3.2 ± 1.3
p value	0.292	0.021	0.03
Weight loss			
≤ 5% (n = 84)	1.1 ± 0.4	1.6 ± 0.7	2.9 ± 1.1
> 5% (n = 133)	1.2 ± 0.6	1.9 ± 0.9	3.6 ± 1.5
p value	0.279	0.010	<0.001

AP = anterior-posterior; BMI = body mass index; ML = medial-lateral; PTV = planning target volume; SI = superior-inferior.

TABLE 3. Setup errors by the specified threshold

	ML error (%)	SI error (%)	AP error (%)
< 1.0 mm	3549 (49.1)	2471 (34.2)	525 (7.3)
1.0~1.9 mm	2315 (32.0)	2076 (28.7)	939 (13.0)
2.0~2.9 mm	925 (12.8)	1390 (19.2)	1492 (20.6)
3.0~3.9 mm	314 (4.3)	729 (10.1)	1800 (24.9)
4.0~4.9 mm	85 (1.2)	330 (4.6)	1310 (18.1)
≥ 5.0 mm	45 (0.6)	237 (3.3)	1167 (16.1)

AP = anterior-posterior; ML = medial-lateral; SI = superior-inferior

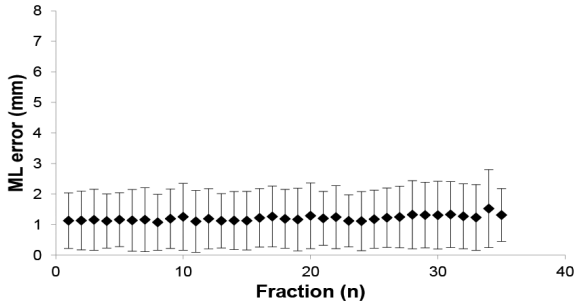

FIGURE 1. Setup error of the medial-lateral (ML) direction at each treatment fraction.

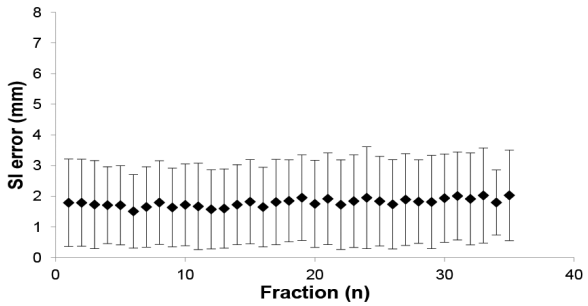

FIGURE 2. Setup error of the superior-inferior (SI) direction at each treatment fraction.

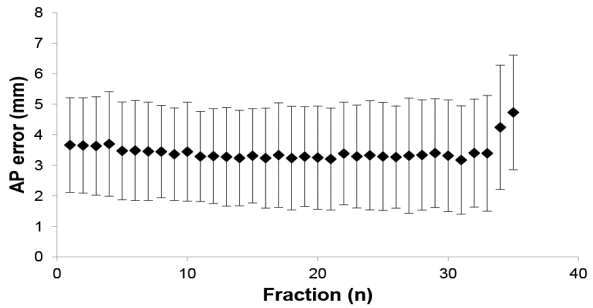

FIGURE 3. Setup error of the anterior-posterior (AP) direction at each treatment fraction.

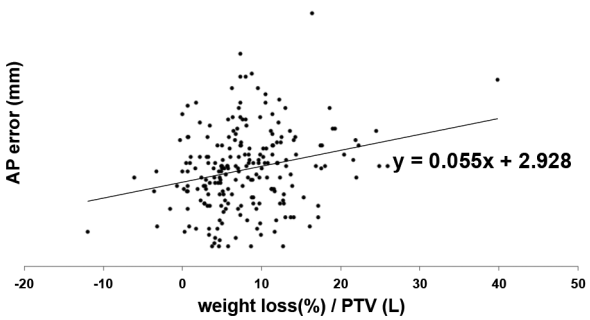

FIGURE 4. Linear regression graph of anterior-posterior (AP) setup error and weight loss normalized by planning target volume (PTV) ($R^2 = 0.059$, $p < 0.001$).

larger in SI direction than in the ML direction ($p < 0.001$). Setup errors greater than 3 mm occurred for 6.1%, 18.0%, and 59.1% of treatment fractions in the ML, SI, and AP directions, respectively (Table 3). There was a trend of increased setup errors toward the end of treatment course, especially in the AP direction (Figures 1–3).

Next, we determined whether T stage, N stage, BMI, PTV, or weight loss were factors affecting setup errors using the Student *t*-test. Setup displacements in all directions were not significantly different between patients with different T stage (1–2 *vs.* 3–4) and N stage (0–1 *vs.* 2–3). There was a strong trend indicating that patients with larger BMI (≥ 25) before RT had an increased setup displacement in the AP direction (3.6 ± 1.5 mm *vs.* 3.2 ± 1.3 mm, $p = 0.053$) during treatment. Smaller PTV (< 850 cc) was associated with larger setup displacement in the ML direction (1.9 ± 0.9 *vs.* 1.6 ± 0.7 $p = 0.021$) and AP direction (3.6 ± 1.5 mm *vs.* 3.2 ± 1.3 mm, $p = 0.03$). Patients with weight loss > 5% had a significantly larger setup displacement in the AP (3.6 ± 1.5 mm *vs.* 2.9 ± 1.1 mm, $p < 0.001$), and SI (1.9 ± 0.9 mm *vs.* 1.6 ± 0.7 mm $p = 0.01$) directions but not in the ML ($p = 0.279$) direction.

Treatment volume strongly depended on gross disease volume, neck girth, and target margins. To control the effect of treatment volume on weight loss, we normalized the amount of weight loss to PTV. We calculated the normalized weight loss by the ratio of the percentage of weight loss over PTV (cc). As shown in Figure 4, normalized weight loss correlated significantly with setup displacement in the AP direction ($R^2 = 0.059$, $p < 0.001$). For every one percent increase in weight loss normalized to PTV, AP setup error was increased by 0.06 mm ($y = 0.055x + 2.927$, x: percentage of weight loss/PTV, y: AP setup displacement).

Discussion

Setup uncertainties greatly impact the accuracy of intensity-modulated radiation delivery. CT image guidance is routinely used to correct setup errors prior to radiation delivery. A prior small study of ten nasopharynx and nasal cavity cancer patients treated with RT has shown a large setup error of 3.6 ± 1.0 mm without daily CT image guidance.[11] In our study, the daily set-up variations were assessed in NPC patients treated with definitive IMRT delivered by helical tomotherapy. A large setup displacement in the AP direction with error greater than 3.0 mm occurred for 59.1% of treat-

ment fractions. The nasopharynx is situated immediately anterior to the clivus and brainstem. For nasopharyngeal cancer with clival involvement, the posterior margin and PTV expansion was often small or absent in order to avoid the brainstem. Therefore, our study suggests that daily IGRT is crucial in ensuring adequate coverage of nasopharyngeal gross tumor volume and protection of the brainstem.

To our knowledge, our study produced the largest dataset of daily setup errors by helical tomotherapy during NPC RT. Helical tomotherapy-based MVCT uses the same X-ray source for image acquisition and treatment.[15] No surrogate telemetry systems are required to register image space to treatment space. In contrast, the kilovoltage (kV) cone-beam computed tomography (CBCT) on a linear accelerator can produce systematic errors due to misalignment. Compared with MVCT, kV CBCT offers superior spatial and contrast resolution because of less Compton scattering and pair production interaction with its kV photon source.[16] Nevertheless, helical tomotherapy MVCT can provide sufficient contrast to delineate many soft tissue structures.[17] The cone beam geometry of CBCT systems can generate a larger scattered radiation component affecting image quality indices including homogeneity, contrast, and noise in the reconstructed CBCT images.[18] Furthermore, MVCT scan contrast is linearly related to the electron density of the material imaged and is not associated with scatter artifacts produced by dental prosthesis.[17] Given these potential shortcomings of kV CBCT, helical tomotherapy MVCT might be a more suitable choice for daily IGRT.

NPC is a lymphoid-rich neoplasm that is radiation-sensitive and undergoes dramatic shrinkage in response to radiation.[19] Shrinkage of the gross tumor volume can lead to anatomy shifts and increased setup errors. A previous study using electronic portal imaging in 20 patients undergoing definitive IMRT showed setup displacements of > 3 mm occurred more frequently in patients with bulky nodal disease.[13] In contrast to the previous finding, our data indicated no worsening of setup errors in patients with advanced T stage or N stage NPC. Furthermore, weight loss greatly impacts setup error magnitude, and is consistent with previous findings in patients treated with head and neck RT.[13,20,21] Weight loss during RT for head and neck cancers is multi-factorial. Ionizing radiation, along with concurrent systemic chemotherapy, induces acute mucositis and taste change.[6] Radiation-induced xerostomia contributes to swallowing difficulty. Inadvertent radiation dose to the brainstem and vestibule as well as platinum-based chemotherapy can induce intractable nausea.[22] These factors combine to contribute to severe malnourishment and weight loss during NPC RT.

Several potential strategies can minimize weight loss-induced setup errors during RT. Rigorous weight monitoring and nutrition support with enteral feeding can ameliorate malnutrition and help preserve head and neck muscle mass and subcutaneous fat.[23] Aggressive management of mucositis and nausea with supportive care can alleviate discomfort during food intake.[24] Optimization of radiation treatment planning can help reduce mucositis and nausea by minimizing the dose to the mucosal surface and brainstem.[22,25] As observed in our study, there is a trend toward enlarged setup errors with increasing number of fractions. The use of a shorter radiation treatment course with altered fractionation scheme may also help reduce setup errors. Adaptive RT can potentially decrease radiation dose to normal organs and thereby reduce xerostomia and nausea.[26-30]

Volume of irradiated normal tissue is correlated with RT toxicity. Size of the PTV can be a crude predictor of acute RT toxicity.[31,32] However, we found that patients with larger PTV actually had smaller setup errors in the SI and AP directions. Our image guidance procedure allows the observers to apply additional shifts to ensure adequate target coverage following automatic correction of setup displacements. It is possible that the larger PTV achieves smaller setup error by reducing these shifts. In our study, weight loss normalized to PTV accounts for the observed effect of PTV on setup errors. Importantly, weight loss normalized to PTV is a more precise measure of "tissue density loss" and correlates better with setup errors due to weight change.

Our study had several limitations including the absence of post-treatment images that can reveal the extent of intrafraction displacement. Nonetheless, a prior study that employs post-treatment cone-beam CTs has shown a small intrafraction motion of 1.2 mm during RT delivery to NPC with standard thermoplastic mask immobilization.[33] Our study also lacks treatment toxicity data that would further determine the causes of weight loss. The retrospective nature of this analysis poses the risks of patient selection bias and uncontrolled confounding factors. Despite these limitations, using our large daily MVCT dataset, we were able to identify weight loss as an independent risk factor for setup errors. Future prospective study is need-

ed to determine if weight loss intervention reduces interfraction setup errors in NPC RT.

In conclusion, weight loss is an independent risk factor for setup error during RT delivery to NPC. Patients with a moderate weight loss of more than 5% may be susceptible to increased interfraction AP and SI setup errors. Ameliorating weight loss during RT to NPC by close dietary monitoring and appropriate interventions may improve target coverage and reduce treatment toxicity by reducing the frequency and magnitude of setup errors.

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research

Virtual modelling of novel applicator prototypes for cervical cancer brachytherapy

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Background. Standard applicators for cervical cancer Brachytherapy (BT) do not always achieve acceptable balance between target volume and normal tissue irradiation. We aimed to develop an innovative method of Target-volume Density Mapping (TDM) for modelling of novel applicator prototypes with optimal coverage characteristics.

Patients and methods. Development of Contour-Analysis Tool 2 (CAT-2) software for TDM generation was the core priority of our task group. Main requests regarding software functionalities were formulated and guided the coding process. Software validation and accuracy check was performed using phantom objects. Concepts and terms for standardized workflow of TDM post-processing and applicator development were introduced.

Results. CAT-2 enables applicator-based co-registration of Digital Imaging and Communications in Medicine (DICOM) structures from a sample of cases, generating a TDM with pooled contours in applicator-eye-view. Each TDM voxel is assigned a value, corresponding to the number of target contours encompassing that voxel. Values are converted to grey levels and transformed to DICOM image, which is transported to the treatment planning system. Iso-density contours (IDC) are generated as lines, connecting voxels with same grey levels. Residual Volume at Risk (RVR) is created for each IDC as potential volume that could contain organs at risk. Finally, standard and prototype applicators are applied on the TDM and virtual dose planning is performed. Dose volume histogram (DVH) parameters are recorded for individual IDC and RVR delineations and characteristic curves generated. Optimal applicator configuration is determined in an iterative manner based on comparison of characteristic curves, virtual implant complexities and isodose distributions.

Conclusions. Using the TDM approach, virtual applicator prototypes capable of conformal coverage of any target volume, can be modelled. Further systematic assessment, including studies on clinical feasibility, safety and effectiveness are needed before routine use of novel prototypes can be considered.

Key words: cervical cancer; brachytherapy; applicators

Introduction

Brachytherapy (BT) is an essential component of treatment for locally advanced cervical cancer. Historically, X ray-based BT has been used, resulting in suboptimal disease control and toxicity rates, especially in advanced tumours.[1-5] Currently, image guided adaptive brachytherapy (IGABT) is being increasingly implemented worldwide. This modern technique is based on geometric individualization of the BT implant, sectional imaging, computerized treatment planning and remote afterloading technology.[6] The concept of IGABT enables tight control over dose distribution in the tissues and personalized adaptation of treatment according to individual tumour size, topography and relation to the organs at risk (OAR). Consequently, dose escalation to the target volume is achieved,

while respecting OAR dose constraints. Dosimetric advantages of IGABT over conventional 2D BT are well documented and are reflected in superior clinical outcomes.[7-16]

Even with the most sophisticated treatment planning technology, optimal placement of source channels in the target volume remains a precondition for the success of BT. The ability to compensate for inadequacies of suboptimal insertion by optimization of source dwell-times and dwell-positions is limited. Standard intracavitary (IC) applicators for BT of cervical cancer do not always enable an acceptable balance between the coverage of the target volume and dose to the OAR.[17] In patients with unfavourable tumour topography and/or gross residual disease extending beyond the reach of standard dose distribution, parts of the tumour cannot be covered with the prescribed dose without exceeding OAR tolerance. This leads to dosimetric cold spots in the tumour that predispose to treatment failure.[18,19] Recently developed intracavitary and interstitial (IC/IS) applicators for transvaginal placement of parallel parametrial needles allow for moderate lateral displacement of prescribed isodose.[20-23] While this leads to favourable therapeutic ratio in majority of cases, there is substantial proportion of challenging tumours which require alternative techniques for optimal outcome. Systematic assessment of dosimetric characteristics of currently available IC and IC/IS applicators or novel prototypes has not been performed so far. Theoretically, an ideal BT applicator would enable standardized and reproducible insertion of the IC and IS channels in optimal geometries, allowing for complete coverage of any tumour encountered in clinical practice. In this manuscript we present an innovative approach to evidence-based applicator development, using the method of Target-volume Density Mapping (TDM). TDM methodology was consequently applied in multi-institutional setting and promising preliminary outcome was presented.[24] Publication of the final results of this analysis is pending.

We conducted this study as a part of a wider applicator development project, performed by the international Gyn GEC ESTRO network. The work presented here is entirely technical, focusing merely on the software development and specification of related methodology. No patient information was used during this work. The developed methodology was consequently used in substudies on patients, included in the multi-institutional EMBRACE and retroEMBRACE protocols. These protocols were approved by the institutional review boards / ethical committees of participating centres. Promising preliminary results of this analysis were presented, while the publication of the final results is pending.[24] In the present manuscript, only the theoretical concept of software-based methodology for TDM generation is detailed.

Methods

Multidisciplinary task group from the Institute of Oncology Ljubljana and the University of Primorska, Faculty of Mathematics, was formed in 2009 to propose methodological framework for evidence based applicator modelling. The group consisted of an experienced radiation oncologist, a medical physicist and a computer scientist. The activities of the group were arbitrarily divided into (1) TDM software development and (2) Applicator modelling.

Development of software for target density mapping

Development of TDM software was identified as core priority of the task group. The coding process reflected three main domains of requests regarding application functionalities:

Individual input data should consist of single-case DICOM (Digital Imaging and Communications in Medicine) files containing target and applicator structures.

Sequential import of DICOM data-sets from multiple cases should be enabled, followed by rigid co-registration of structures, using the IC applicator as the reference.

Software output should be an object in DICOM format, containing pooled information from individual cases.

Validation and geometric accuracy check of the software application was based on the import and processing of a series of phantom DICOM data-sets. The phantom sets consisted of virtual tandem & ring applicators and structures of known shapes, sizes and topographic relations with the applicator.

Modelling of virtual applicator prototypes

Standardized model for quantitative analysis of dosimetric effects of existent and prototype applicators on the TDM was developed. This task included the definition of concepts, terms and workflow for post-processing of the TDM. Applying

these definitions, the workflow for computer based modelling of applicator prototypes was developed.

Results

Software for target density mapping

Dedicated software (Contour Analysis Tool 2 - CAT 2) was developed by the task group. The principles of CAT 2 application are outlined in Figure 1. The method is based on sequential import of DICOM structure files from a representative sample of individual cases, containing the target volume and the tandem & ring applicator. CAT 2 performs rigid co-registration of the imported structures, based on the applicator as the frame of reference. This implies rotational and translational shifts of the target volume and the applicator, resulting in alignment of the (1) centre of the ring, (2) tandem and ring axes and (3) axis connecting the centre of the ring with the first ring-source position. Consequently, TDM is created as a hybrid data set, containing pooled information from a sample of cases. Individual target volumes in the TDM are aligned to the single reference applicator, maintaining unchanged relative topography to the applicator axes as in the original data set. The differences in tandem length and ring diameter are not taken into account during co-registration process. Each voxel of the TDM is assigned a Target Density Value (TDV), corresponding to the number of target volume contours that encompass that voxel. Finally, TDVs are converted to grey levels and the data set is transformed to DICOM image, containing the reference applicator and the TDM in an "applicator-eye-view".

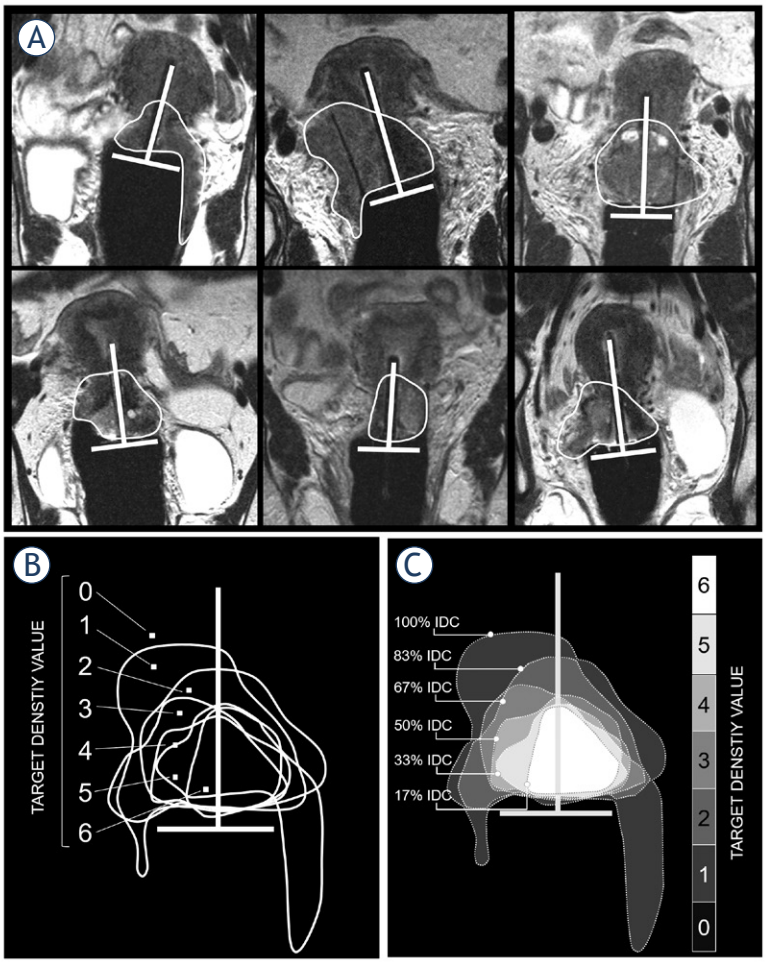

FIGURE 1. Principles of Target-volume Density Map (TDM) generation and post-processing on an example of 6 cervical cancer cases. **(A)** Contours of high risk clinical target volumes (CTV_{HR} – thin white lines) are shown on mid-coronal T2 weighted MRI with the applicator in place. Source channels are depicted as thick white lines. **(B)** CAT 2 generates the TDM by rigid co-registration of individual CTV_{HR}s on a reference applicator. TDM voxels are assigned target density values, corresponding to the number of encompassing CTV_{HR}s. These values are transformed to grey levels. **(C)** Resulting TDM DICOM image is exported to treatment planning system where isodensity contours (IDC) are auto-segmented (white dotted lines).

Modelling of virtual applicator prototypes

This workflow starts with the import of the TDM DICOM object to the treatment planning system. Post-processing steps are summarized below:

Generation of Iso-Density Contours and Residual Volumes at Risk

Iso-density contours (IDC) are generated using the auto-segmentation functionality of the treatment planning system. IDCs are defined as delineations, connecting voxels with the same TDV and are labelled as percentage of encompassed TDVs. Labels of individual IDCs are calculated by using the following formula:

$$IDC_{TDV} = \frac{TDV_{max} - TDV + 1}{TDV_{max}} \; x \; 100 \; [\%]$$

For example, the label of outermost IDC in Figure 1C (TDV=1 and TDV_{max}=6) is:

$$IDC_1 = \frac{6 - 1 + 1}{6} \times 100 = 100\%$$

And the label of innermost IDC in Figure 1C (TDV=6 and TDV_{max}=6) is:

$$IDC_1 = \frac{6 - 6 + 1}{6} \; x \; 100 = 17\%$$

Therefore, outermost IDC encompasses voxels with TDVs of 1 to 6 (100% of TDVs) while the in-

nermost IDC encompasses only the voxels with TDV of 6 (17% of TDVs) (Figure 1C). Following auto-segmentation of IDCs, residual volume at risk (RVR_{TDV}) is generated for each IDC_{TDV}, using the Boolean operators of the treatment planning system. RVR_{TDV} is defined as volume of virtual patient minus the volume of the IDC_{TDV}. It corresponds to the potential volume in which the tissues and OAR could be located in a hypothetical patient with the target volume of IDC_{TDV}.

Application of the standard treatment plan on TDM

Conventional treatment plan, based on the IC applicator with standard loading and dose specification at Manchester point A is applied to the TDM (Figure 2A). Dose volume histogram (DVH) parameters are recorded for individual IDC_{TDV}s and RVR_{TDV}s. Recommended minimal set of DVH parameters for IDC_{TDV} includes volume receiving the prescribed dose (V100) and dose received by the 90% and 98% (D90 and D98) of the IDC_{TDV} volume. For RVR_{TDV}, minimum doses received by 2 cm^3 of the most irradiated RVR_{TDV} (D2cc) are recorded. Characteristic curves for the IC applicator with standard loading are generated by plotting the DVH parameters against IDC_{TDV} and RVR_{TDV} levels (Figure 2D).

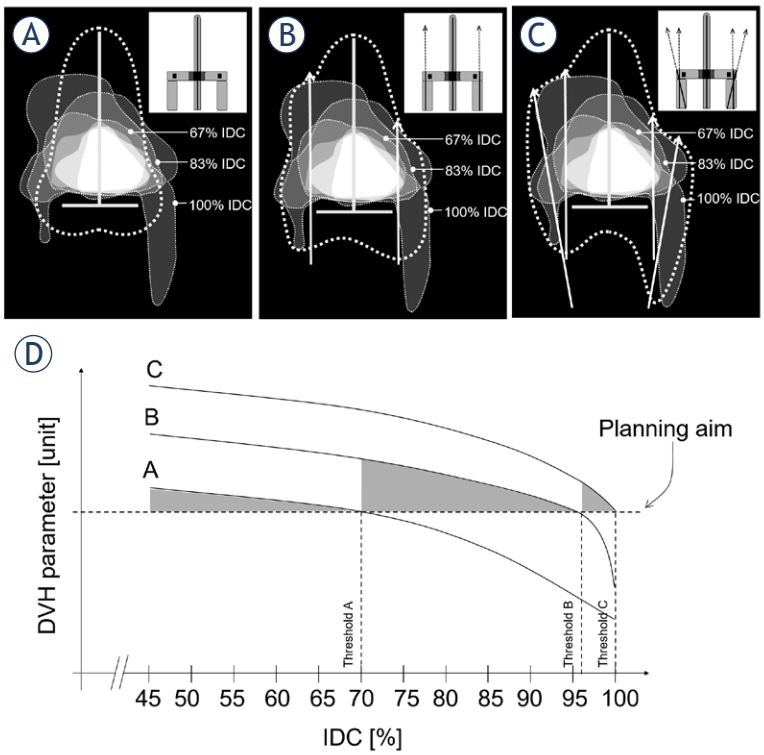

FIGURE 2. Schematic representation of applicator modelling based on our theoretical example of Target-volume Density Map (TDM). *Above:* Three virtual applicators are reconstructed on the TDM. Dose distribution is optimized based on a set of specific planning aims for the isodensity contours (IDC) and for residual volumes at risk (RVR). The optimized prescription isodoses for individual applicators are shown as thick dotted lines. **(A)** *Standard intracavitary applicator:* limited possibility for optimization. The planning aim is achieved for ≈70% IDC. **(B)** *Combined intracavitary and interstitial applicator with parallel parametrial needles (Vienna-type)*: planning aim is achieved for ≈95% IDC. **(C)** *Combined intracavitary and interstitial applicator with parallel and oblique parametrial needles:* planning aim as achieved for ≈100 % IDC. **(D)** Characteristic curves for the IDC of the three applicator types. RVR curves are not shown. Shaded areas under characteristic curves represents IDC ranges in which certain applicator is able to achieve the planning aim. Arbitrary planning aim and applicator thresholds are marked by dotted lines.

Modelling of class solution IC+IS applicators

During this step of the process, characteristic curves are used to assess the limitations of standard IC plans. TDV threshold at which the standard plan fails to meet the planning aims for IDC and dose constraints for RVR are identified (Figure 2). Consequently, qualitative assessment of dose distributions is performed for treatment plans above the threshold. This includes topographical analysis of under-dosed and over-dosed regions of IDC and RVR, respectively. Based on the topographic analysis, virtual IS channels are added to the standard IC applicator to achieve optimal implant geometry for the corresponding IDC and RVR (Figure 2B and 2C). Next, source dwell-positions and dwell–times inside the IC and IS channels are adjusted to optimize the dose distribution. These steps are repeated in an iterative manner until class-solution applicators, capable of covering complete TDM are modelled. Determination of optimal implant geometry and loading and specification of planning aims (IDC) and dose constraints (RVR) may vary between or within planners, leading to various applicator solutions. For the purpose of this project, we applied our institutional standard pre-planning and optimization procedure which was described recently.[25] Finally, characteristic curves of the modelled prototypes are generated and compared (Figure 2D).

Discussion

In our present project, we developed CAT 2 software for TDM generation and related post-processing methodology. Our method enables evidence-based modelling of novel IC/IS applicator prototypes and in-silico testing of existent devices. The proposed approach is based on analysis of pooled spatial information of target volumes from a representative sample of patients in the "applicator-eye view".

Lessons learned from historical evolution of applicators are essential for optimal utilization of modern development strategies, including our TDM method. Historical Paris and Stockholm techniques date back approximately 100 years.[26-28] The applicators consisted of a rubber tandem and a pair of colpostats (Paris technique) or flat vaginal box (Stockholm method), pre-loaded with ^{226}Ra. In the 1930's, the Manchester system was developed, introducing the point A and the applicator which was geometrically similar to its predecessors.[29] Nowadays, fixed geometry of uterine tandem and vaginal components (ovoids, cylinders or ring) enables reproducible insertion and reliable treatment using small sealed sources and afterloading technology. Standard loading patterns of modern applicators mimic the historical patterns based on milligram-hours of ^{226}Ra tradition.[30] In topographically unfavourable tumours, optimization of loading can expand the prescribed dose for up to approximately 4 mm at the point A level.[17] Target volumes that extend beyond this region and cases with unfavourable OAR topography pose a challenge to standard IC techniques. Applicators with static shielding in the ovoids were developed to improve the therapeutic ratio by reducing the irradiation of the OAR and their dosimetric impact was studied extensively.[31-34] Use of shielded devices outside the context of IGABT and model-based dose calculation algorithms may result in unintentional shielding of the target volume and uncertainties regarding the OAR doses.[32] Emerging technologies, including static adjustable shielding and dynamic modulated BT with rotating paddle, can achieve highly conformal dose distributions in large targets of complex shapes.[35-37] However, when used in pure IC BT, directional modulation of intensity may lead to excessively high doses near the applicator along the emission window axis.[36] These techniques need to stand the test of further studies before their routine implementation. Dynamic shielding may play an important role in the future, especially if applied in combination with IC/IS insertion technique.[37] In summary, the exciting technological developments which we have witnessed over the past century failed to overcome the inherent physical limitations of IC BT.

Manufacturing of non-ferromagnetic applicators allowed for implementation of MRI based IGABT.[38-41] This can be regarded as the single most important advancement in the field of IC applicator development over last decades. IGABT offered an unprecedented opportunity to assess limitations of standard IC applicators and design improved IC/IS prototypes. TDM methodology described in this manuscript is based on estimation of the impact of individual applicator adjustments on a single "virtual patient", containing pooled information from a sample of patients. In contrast, the traditional paradigm of empirical development is based on analysis of applicator limitations in individual cases, followed by iterative process of technical improvements. Using the traditional paradigm, novel IC/IS applicators were developed recently. Dimopoulos *et al*. introduced the *Vienna tandem & ring applicator* in which the ring serves as template for insertion of parallel needles into the parametria.[20] In their first report, 170 needles were inserted in 22 patients. The mean depth of insertion was only 37 +/- 7 mm and the mean difference between planned and actual needle tip positions was sub-millimetric. Due to the use of blunt needles and short needle tracks, no organ perforations were identified and only 8.8% of the needles were outside the $CTV_{HR.}$[20] Tandem & ovoids applicator underwent similar evolution, resulting in the *Utrecht applicator* with comparable geometric, clinical and dosimetric characteristics.[22,23] IGABT with these application techniques enables moderate expansion and individual shaping of the isodose distribution, leading to improved dosimetric and clinical outcome.[13,14,17,22,23] However, tumours with major extra-cervical infiltration at time of BT that extends beyond dosimetric reach of these applicators remain to pose a special challenge (*i.e.* infiltration of sacrouterine ligaments and distal parametria).

TDM-based methodology allows for objective quantification of IC and IC/IS applicator characteristics. By studying the impact of implant-geometry adjustments, prototypes of novel applicators that are theoretically capable of covering any target volume can be generated. Our method has been endorsed by the Gynaecological GEC ESTRO Network and preliminary results of its implementation in a multi-centre setting have been published in abstract form.[24] In this study, CTV_{HR} delineations from a representative sample of 264 patients were used to generate a TDM. Standard IC and Vienna IC/IS applicators achieved coverage of around 60% and 95% IDC, respectively. Prototypes, able of covering the remaining 5% of most challenging IDCs were proposed by adding virtual oblique needles to the Vienna applicator.[24] The impact of applicator geometries and dose optimization on RVR envelopes was not assessed in this preliminary report.

In the final analysis of this study, which is currently underway, two dose optimization models

will be applied: standard and optimized. (1) In the standard model of dose planning, no RVRs will be assumed around the IDCs and no RVR-based optimization / reporting will be performed. This model therefore represents the most favourable theoretical scenario in terms of IDC dose-coverage. (2) In the optimized model, each IDC will be entirely surrounded by an RVR envelope and achieving the dose constraints for the RVR will be the absolute priority of dose optimization process. Optimized model is therefore the theoretical worst case scenario in terms of IDC coverage and it is practically never encountered in real clinical practice. In optimized model, the reported D2cc parameters for all the RVR envelopes will be the same, reflecting the RVR dose constraints. The utility of the proposed two-model approach is as follows: the two extreme planning approaches are the limiting scenarios, between which the actual clinical situations are expected. Consequently, the area between the two limiting IDC characteristic curves can be regarded as the "useful area" of a specific applicator. We believe that the development of new applicator prototypes, based on the TDM method will lead to further testing in clinical studies and finally commercial availability of safe, feasible and dosimetrically optimal devices, capable of covering the BT target volumes in any clinical situation. It can be expected that this will translate into further improved clinical outcomes of locally advanced cervical cancer management.

The emerging technology of personalized 3D printing of single-use IC/IS BT applicators is an exciting alternative to commercially available devices.[42] Individual tailoring of the applicator size, shape and source channel geometry according to patient's anatomy and tumour topography can be considered as the ultimate method to adaptive BT insertion. Advanced level of expertise, routine availability of pre-BT MRI, access to 3D printer and dedicated clinical time are required for implementation of 3D printing approach. Thus, standard IC/IS applicators remain to play an important role, especially in clinical settings with high incidence of cervical cancer and limited resources, making the TDM method presented here highly relevant.

In our method, the differences in tandem length and ring diameter are not taken into account during co-registration process, leading to a minimal overlap between the central parts of some target volumes with the reference applicator. Since central voxels are located in vicinity of the sources and high dose regions, this inconsistency bears minimal significance and is of limited interest for applicator development. The relevance of applicator development in the context of IGABT depends on accuracy of contouring of the target volume and organs at risk. There is a growing body of evidence that contouring uncertainties may be the weakest link in the process of IGABT.[43] Since our TDM methodology relies on import of individual target volume delineations, contouring uncertainties can undermine the validity of TDM-based applicator prototyping. Therefore, quality assurance of imported contours is essential for clinically relevant utilization of our method. There are three major aspects that need to be considered in this regard: (1) selection of target volume for TDM generation, (2) experience of radiation oncologists and (3) imaging modality for target volume delineation.

Selection of target volume for target density mapping

CTV_{HR} was recently identified as the most robust target volume for dose prescription and optimization in cervical cancer IGABT.[44] When comparing delineations of ten observers with the expert consensus contours, the mean conformity index for the GTV, CTV_{IR} and CTV_{HR} was 0.58, 0.68 and 0.72, respectively. Corresponding mean inter-delineation distances were 4.2 +/- 3.5 mm, 5.2 +/- 5.6 mm and 3.8 +/- 3.4 mm, respectively.[44] Other published studies revealed similar degree of inter-observer variation for the $CTV_{HR.}$[45-47] and confirmed more favourable results for CTV_{HR} when compared with GTV and $CTV_{IR.}$[45] In conclusion, we recommend using CTV_{HR} as the target structure for TDM generation.

Experience of radiation oncologists

CTV_{HR} contouring is a challenging procedure with a long learning curve.[43] The observers in published inter-observer studies on cervical cancer IGABT were experts, participating in the EMBRACE protocol (IntErnational study on MRI-guided BRAchytherapy in locally advanced CErvical cancer) or contouring instructors.[44-48] GEC ESTRO recommendations were strictly applied in these studies.[38,50] In spite of the favourable circumstances, the magnitude of "residual" uncertainties at expert level was substantial and was reflected in clinically relevant uncertainties of the reported DVH parameters.[44,48] Therefore, complete elimination of contouring uncertainties from clinical practice is not-realistic. This needs to be taken into account when interpreting the results of studies and is one of the major inherent limitations of the TDM

method presented here. To ensure optimal validity of the TDM method, the imported contours should ideally originate from clinical studies with expert-based peer review or plausibility checking for quality control.

Imaging modality

MRI is gold standard for cervical cancer IGABT and GEC ESTRO recommendations on various aspects of its use in IGABT were published.[38-41] Excellent clinical results of mono- and multi-institutional studies can be regarded as indirect confirmation of validity of the concepts and terms, defined in these recommendations.[9-16] While guidelines for CT-based contouring recently became available, physical limitations of CT for soft tissue depiction remain a major impediment.[50] Not surprisingly, inter-observer studies on contouring variation demonstrate superior results for MRI-based delineation when compared with CT-based approach.[44,51-54] Ultrasound and PET CT may add complementary information during delineation, but their role as eventual independent modality for contouring remains to be defined. In summary, TDM-guided applicator prototyping should be based on target volumes, delineated on MRI.

Conclusions

We developed methodology for applicator modelling based on pooled analysis of target volumes in reference applicator-eye-view. The system was validated using the phantom geometries. Recommendations to minimize uncertainties resulting from contouring variation were proposed. Using our method, virtual applicator prototypes capable of covering any target volume can be developed. Computer-based modelling represents the basic step in development of applicator prototypes which need to undergo further systematic assessment, including studies on clinical feasibility and safety. Our methodology was applied in multi-institutional setting and promising preliminary outcome published.[24] Final analysis of the data is currently underway.

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research

Outcome of severe infections in afebrile neutropenic cancer patients

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Results of this analysis were in part presented at the MASCC/ISOO International Symposium on Supportive Care in Cancer in New York City in 2012 (oral presentation of the abstract #734).

Background. In some neutropenic cancer patients fever may be absent despite microbiologically and/or clinically confirmed infection. We hypothesized that afebrile neutropenic cancer patients with severe infections have worse outcome as compared to cancer patients with febrile neutropenia.

Patients and methods. We retrospectively analyzed all adult cancer patients with chemotherapy-induced neutropenia and severe infection, who were admitted to the Intensive Care Unit at our cancer center between 2000 and 2011. The outcome of interest was 30-day in-hospital mortality rate. Association between the febrile status and in-hospital mortality rate was evaluated by the Fisher's exact test.

Results. We identified 69 episodes of severe neutropenic infections in 65 cancer patients. Among these, 9 (13%) episodes were afebrile. Patients with afebrile neutropenic infection presented with hypotension, severe fatigue with inappetence, shaking chills, altered mental state or cough and all of them eventually deteriorated to severe sepsis or septic shock. Overall 30-day in-hospital mortality rate was 55.1%. Patients with afebrile neutropenic infection had a trend for a higher 30-day in-hospital mortality rate as compared to patients with febrile neutropenic infection (78% *vs*. 52%, p = 0.17).

Conclusions. Afebrile cancer patients with chemotherapy-induced neutropenia and severe infections might have worse outcome as compared to cancer patients with febrile neutropenia. Patients should be informed that severe neutropenic infection without fever can occasionally occur during cancer treatment with chemotherapy.

Key words: afebrile infection; fever; neutropenia; hypothermia; cancer

Introduction

Infections are important cause of morbidity and mortality in cancer patients.[1] Neutropenia is a major risk factor for the development of infection in cancer patients undergoing treatment with chemotherapy.[2,3] Fever remains the most prevalent and evident sign of infection in neutropenic cancer patients and early initiation of broad-spectrum antibiotic therapy significantly reduces mortality in these patients.[4-7]

Fever is a phylogenetically ancient host defense response to variety of infectious and non-infectious triggers. It is a result of upregulation of the hypothalamic thermostatic set point due to the complex interplay between endogenous and exogenous pyrogens, enzymatic and neuronal pathways and endogenous antipyretic neuropeptides and hormones.[8-11] Pyrogenic cytokines concurrently trigger production of acute phase reactants and stimulate activation of various metabolic, endocrinologic and immunologic systems.[12] Data from clinical

observations and observational studies show that fever may have a beneficial effect on morbidity and mortality caused by the non-life-threatening infections.[8,11,12] However, in some neutropenic cancer patients fever may be absent despite microbiologically and/or clinically confirmed infection.[5] According to the Infectious Disease Society of America (IDSA) and some other guidelines afebrile neutropenic patients with new signs or symptoms of infection are considered to be at high-risk for complications and therefore prompt use of empirical antimicrobial agents is recommended.[13-15] Nevertheless, the level of evidence for this recommendation is weak (B-III) and is based on the extensive clinical experience of the Panel members.[13]

Afebrile infections are not unique to neutropenic cancer patients. Even more, published data shows that non-neutropenic patients with afebrile severe infections, especially those with hypothermia, have higher mortality rates compared to febrile non-neutropenic patients.[8,16,17]

We conducted a retrospective study to evaluate the incidence and characteristics of severe infections in afebrile neutropenic cancer patients undergoing treatment with chemotherapy at our cancer center. Furthermore, we hypothesized that afebrile neutropenic cancer patients with severe infections have worse outcome as compared to cancer patients with febrile neutropenia.

Patients and methods

Study population and data collection

We performed an electronic database search to identify all adult cancer patients, who were transferred from the Department of Medical Oncology to the Intensive Care Unit (ICU) at the Institute of Oncology Ljubljana due to the severe neutropenic infection between January 2000 and December 2011. All eligible patients for this study were required to have a severe infection due to a chemotherapy-induced neutropenia. Multiple episodes of neutropenic infections in one patient were considered as separate events. All information on relevant patients' characteristics, cancer treatment and episodes of infections were retrieved retrospectively from patients' charts. All data were extracted by a single author.

Febrile neutropenic infections were defined as at least one axillary body temperature measurement of ≥ 38°C in the presence of neutropenia (absolute neutrophil count < 1 x 10^9/l at the time of diagnosis of infection with further decline to < 0.5 x 10^9/l during the following days). Afebrile neutropenic infections were defined as a maximum axillary body temperature < 38°C within 72 hours of diagnosis of neutropenic infection, which had to be microbiologically and/or clinically documented. Severe neutropenia was defined as an absolute neutrophil count ≤ 0.1 x 10^9/l.

Infection was considered microbiologically documented when a positive culture was yielded from the site of infection, and clinically documented when there were clinical and/or radiological signs of infection without microbiological confirmation. Characteristics of organisms isolated from blood cultures were recorded. At least two positive blood culture sets were required when *coagulase-negative staphylococci* or other potential skin contaminants (e.g. *Corynebacterium* spp., *Propionibacterium* spp., *Bacillus* spp.) were isolated. Polymicrobial bacteremia was defined as more than one organism isolated from blood culture specimens obtained within 24 hours of the first positive blood culture specimen in a single patient.[18]

We calculated the Multinational Association for Supportive Care in Cancer (MASCC) Risk Index score for each patient as described by Klastersky *et al*.[19] Comorbidities were evaluated using the adult comorbidity evaluation-27 index (ACE-27) and Charlson comorbidity index (CCI).[20,21] Severe sepsis and septic shock were defined according to the established 2001 International Sepsis Definitions Conference criteria.[22]

Our retrospective study was approved by the Institutional Review Board and Ethics Committee (#ERID-KESOPKR/74).

Statistical analysis

We used descriptive statistics to describe relevant patients' characteristics. The outcome of interest was 30-day in-hospital mortality rate, as proposed by Feld *et al*.[23] Association between the outcome and different prognostic factors and comparison of different characteristics between febrile and afebrile patients were evaluated using Chi-square or Fisher's exact test as appropriate. To compare mean time from the diagnosis of neutropenic infection to the ICU admission between afebrile and febrile patients we used independent-samples t-test. Due to the small number of events a multivariate analysis was not feasible. All significance tests were two-sided using an alpha level of 0.05. No correction was applied for multiple statistical testing.

TABLE 1. Baseline patients' characteristics, cancer type and treatment

Characteristics		N (%) (n = 69)
Median age (range)		58 years (31–86)
	Elderly (> 65 years)	28 (40.6%)
Sex	Male	42 (60.9%)
	Female	27 (39.1%)
Cancer type	Lymphoma	52 (75.4%)
	Solid tumors	17 (24.6%)
Chemotherapy treatment intent	Neo/adjuvant solid or 1st-line lymphoma	35 (50.7%)
	1st-line solid or 1st-relaps lymphoma	13 (18.8%)
	2nd-line solid or 2nd-relaps lymphoma	12 (17.4%)
	≥ 3rd-line solid or ≥ 3rd-relaps lymphoma	9 (13.1%)
CCI	Low (0)	28 (40.6%)
	Medium (1–2)	30 (43.5%)
	High (3–4)	8 (11.6%)
	Very high (≥ 5)	3 (4.3%)
ACE-27	None (G0)	17 (24.6%)
	Mild (G1)	16 (23.2%)
	Moderate (G2)	13 (18.9%)
	Severe (G3)	23 (33.3%)
WHO performance status	0–2	54 (78.3%)
	3–4	15 (21.7%)
Bone marrow infiltration	Yes	22 (31.9%)
	No	29 (42.0%)
	Unknown	18 (26.1%)
Previous neutropenic infection	Yes	24 (34.8%)
	No	45 (65.2%)
Site of infection[a]	Bloodstream	25 (36.2%)
	Lower respiratory tract[b]	25 (36.2%)
	Gastrointestinal tract[c]	13 (18.8%)
	Upper respiratory tract	2 (2.9%)
	Urinary tract	1 (1.5%)
	Skin and soft tissue	1 (1.5%)
	Unexplained fever[d]	2 (2.9%)
Time period of ICU admission (year)	2000–2003	8 (13.6%)
	2004–2011	51 (86.4%)

ACE-27 = adult comorbidity evaluation-27 index; CCI = Charlson comorbidity index; ICU = Intensive Care Unit; WHO = World Health Organization

[a] Includes 53 episodes of microbiologically documented infection (MDI) and 16 episodes of clinically documented infection (CDI)

[b] Pneumonias with 13 cases of MDI and 12 cases of CDI with radiological signs only

[c] Includes cases of cholecystitis, peritonitis, neutropenic enterocolitis

[d] CDI with fever and signs of septic shock

Results

Study population

We identified 69 episodes of neutropenic infections in 65 cancer patients treated with chemotherapy, who required admission to the ICU during the 11-year period. Four patients had two separate episodes of febrile neutropenic infection. Baseline patients' characteristics, cancer type and treatment are summarized in Table 1.

Patients received a median of one cycle (range 1–9 cycles) of treatment with chemotherapy prior to the episode of neutropenic infection. Median overall length of hospitalization and hospitalization in the ICU were 14 days (range 1–52 days) and 5 days (range 1–34 days), respectively.

A median MASCC score was 14 (range 0–21). Only two episodes were considered low-risk according to the MASCC score. In the remaining 67 high-risk episodes, 35 had the MASCC score < 15. Thirty patients (43.5%) had severe neutropenia at the onset of neutropenic infection. In 21 (30.4%) and 19 (27.5%) episodes patients received either prophylactic or therapeutic granulocyte-colony stimulating factors (G-CSF), respectively.

Infections were microbiologically documented in 53 (76.8%) episodes. Out of the remaining 16 clinically documented infections, 13 were confirmed radiologically or with ultrasound (12 pneumonias and one cholecystitis), two patients had fever with clinical signs of septic shock and one had cellulitis. Details about the sites of infection are listed in Table 1.

Bloodstream and lower respiratory tract were the most common sites of infection. The most common microbiological isolates from blood cultures were gram-negative bacteria in 72% (*E.coli* in eight, *Pseudomonas aeruginosa* in three, *Stenotrophomonas maltophilia* in three, *Klebsiella pneumoniae* in two, *Acinetobacter baumannii* in one and unidentified non-fermenting gram-negative bacteria in one episode). Gram-positive bacteria were isolated from three out of 25 positive blood cultures (*Enterococcus faecalis*, *Streptococcus pneumoniae* and *methicillin-resistant Staphylococcus aureus*). In one case anaerobic bacteremia was identified (*Clostridium tertium*). There were two cases of polymicrobial blood infection (with *E.coli*, *Enterococcus faecalis* and *Candida albicans* in one, and *Enterobacter asburiae*, *Proteus mirabilis* and *Enterococcus faecalis* in the other). Candidemia with *Candida albicans* was seen in one case.

Median time from the diagnosis of neutropenic infection to the ICU admission was 10 hours (range 0 hours – 27 days). Reasons for ICU admission were septic shock in 39 (56.5%) patients, severe sepsis in 20 (29%) patients, pulmonary thromboembolism in three patients and seven patients were already in the ICU at the onset of neutropenic infection. In 61 (88.4%), 30 (43.5%), 6 (8.7%) and 11 (15.9%) episodes vasopressors and/or inotropes, mechanical ventilation, dialysis and surgery were required, respectively.

Afebrile neutropenic infections

Sixty (87%) episodes of neutropenic infections were febrile and only 9 (13%) episodes, which oc-

curred in 9 patients, were afebrile. Median age of patients with afebrile infection was 56 years (range 41–82 years). All were considered high-risk with a median MASCC score of 13 (range 7–18). There were no differences between patients with afebrile and febrile neutropenic infection with regard to age, sex, comorbidities, status at the time of diagnosis of neutropenic infection, MASCC score, severity of neutropenia, type of infection, rate of Gram negative or polymicrobial bacteremias and time to the ICU admission. However, patients with afebrile neutropenic infection were more likely to have solid tumors (Table 2).

Five patients were afebrile throughout the episode of infection, in two patients there was a history of fever at home, and two patients developed fever on the 5th and 7th day of hospitalization, respectively, due to a secondary, health care-associated infection. The highest recorded temperature within 72 hours of diagnosis of neutropenic infection was 37.2°C or lower in five patients, and between 37.4 and 37.8°C in the remaining four patients.

Six patients (66.7%) received drugs with potential antipyretic effect (non-steroidal anti-inflammatory drugs, paracetamol, corticosteroids, metamizole) at least once within 48 hours prior and 72 hours after the diagnosis of neutropenic infection, five intermittently and only one continuously. Similarly, 56.7% of patients with febrile neutropenic infection received drugs with potential antipyretic effect (p = 0.73). There was no difference in the rate of afebrile neutropenic infections in patients who received drugs with antipyretic effect compared to patients who did not (15% *vs*. 10.3%, respectively, p = 0.72).

Presenting clinical signs and symptoms of infection in afebrile neutropenic patients were hypotension, severe fatigue with inappetence, shaking chills, altered mental state and cough, in descending order of frequency. All of them eventually deteriorated to severe sepsis or septic shock.

Eight patients (89%) had microbiologically documented infections: four had gram-negative bacteremias (two *E.coli*, one *Klebsiella pneumoniae*, one *Pseudomonas aeruginosa*), two had polymicrobial peritonitis, one had a gram-positive pneumonia and one had a gram-negative urinary tract infection but without blood culture drawn. One patient had a radiologically documented pneumonia.

Outcome

In all patients with severe neutropenic infection 30-day in-hospital mortality rate was 55.1%. In four patients, who experienced two separate episodes of severe neutropenic infection, two survived both episodes and two had fatal outcome during the second episode. Thirty (79%) deaths occurred in the ICU and the remaining deaths occurred after the ICU discharge. Cause of death was attributed to the uncontrolled infection in 31 patients, pulmonary thromboembolism in three, comorbidities in two, and cancer progression in two patients. In patients with afebrile neutropenic infection episode 30-day in-hospital mortality rate was 78%. While in six patients death occurred due to the uncontrolled infection, one patient died due to the cancer progression. The remaining two patients who survived had radiologically documented pneumonia and *Pseudomonas aeruginosa* bacteremia.

Prognostic factors associated with 30-day in-hospital mortality are listed in Table 3. Patients with afebrile neutropenic infection had a trend for a higher 30-day in-hospital mortality rate as compared to patients with febrile neutropenic infection (78% *vs*. 52%, p = 0.17). MASCC score < 15 (71% *vs*. 38%, p = 0.006), lactic acidosis (100% *vs*. 53%, p = 0.04) and use of mechanical ventilation (87% *vs*. 31%, p < 0.001) were all associated with significantly higher 30-day in-hospital mortality rates. We did

TABLE 2. Comparison between patients with afebrile and febrile neutropenic infection

Characteristics		Afebrile (n = 9)	Febrile (n = 60)	p-value
Age (years)	> 65	4	24	1.0
	≤ 65	5	36	
Sex	Male	4	38	0.28
	Female	5	22	
Cancer type	Lymphoma	3	49	0.005
	Solid tumors	6	11	
CCI	Low (0)	5	23	0.47
	Medium - very high (≥ 1)	4	37	
ACE-27	None - mild (G0–1)	5	28	0.73
	Moderate - severe (G2–3)	4	32	
Status at diagnosis	Inpatient	3	34	0.29
	Outpatient	6	26	
MASCC score	< 15	5	30	1.0
	≥ 15	4	30	
Severe neutropenia	Yes	3	27	0.72
	No	6	33	
Type of infection	MDI	8	45	0.33
	CDI	1	15	
Bloodstream infection[a]	Gram negative[b]	4	16	0.55
	non-Gram negative	0	5	
Mean time to the ICU (hours)		41	54	0.73

ACE-27 = adult comorbidity evaluation-27 index; CCI = Charlson comorbidity index; CDI = clinically documented infection; ICU = Intensive Care Unit; MASCC = Multinational Association for Supportive Care in Cancer; MDI = microbiologically documented infection

[a] n = 25

[b] also includes polymicrobial bacteremias with at least one Gram negative bacteria

TABLE 3. Prognostic factors associated with 30-day in-hospital mortality

Prognostic factors		Died (n = 38)	Survived (n = 31)	p-value
Age (years)	> 65 ≤ 65	17 21	11 20	0.44
Cancer type	Lymphoma Solid tumors	29 9	23 8	0.84
CCI	Low (0) Medium - very high (≥1)	17 21	11 20	0.44
ACE-27	None - mild (G0-1) Moderate - severe (G2-3)	20 18	13 18	0.38
G-CSF prophylactic	Yes No	14 24	7 24	0.2
G-CSF therapeutic	Yes No	12 26	7 24	0.41
Fever status	Febrile Afebrile	31 7	29 2	0.17
MASCC score	< 15 ≥ 15	25 13	10 21	0.006
Bloodstream infection	Yes No	15 23	10 21	0.54
Mechanical ventilation	Yes No	26 12	4 27	P < 0.001
Lactic acidosis[a]	Yes No	7 31	0 27	0.04
Time period of ICU admission (year)	2000–2003 2004–2011	4 34	4 27	1.0

ACE-27 = adult comorbidity evaluation-27 index; CCI = Charlson comorbidity index; G-CSF = granulocyte-colony stimulating factors; ICU = Intensive Care Unit; MASCC = Multinational Association for Supportive Care in Cancer;

[a] n = 65

not find any significant association between the mortality and age, cancer type, comorbidities as assessed by the two different comorbidity indexes, presence of bloodstream infection, use of G-CSF and time period of ICU admission.

Discussion

Fever in neutropenic cancer patients with infections is only rarely absent and may predict worse outcome.[13-15] In our study we identified 69 episodes of severe neutropenic infection in 65 cancer patients, among which 9 (13%) episodes were afebrile. All afebrile neutropenic infection episodes were either microbiologically and/or radiologically documented and all progressed to severe sepsis or septic shock. Patients with afebrile neutropenic infection had a trend for a higher 30-day in-hospital mortality rate as compared to patients with febrile neutropenic infection (Table 3).

Majority of published studies to date, which evaluated cancer patients with neutropenic infections, included only patients with febrile neutropenia. Sickles *et al*. studied clinical signs of the five most common localized infections (pharyngitis, skin infection, pneumonia, anorectal infection and urinary tract infection) in neutropenic and non-neutropenic cancer patients. They reported that fever was much more common in neutropenic patients than in non-neutropenic ones. When analyzing all infection sites together, fever (defined as > 38°C) was absent in 2–10% of all patients, depending on severity of neutropenia (in those with absolute neutrophil count ≤ 0.1 x 10^9/l fever was absent in only 2%).[5]

Published data show that 18–40% and 10–20% of non-neutropenic patients develop normothermia or hypothermia, respectively, despite infection with severe sepsis or septic shock.[9,16,24-29] However, definitions of fever, normothermia and hypothermia differed in published studies. Recently published study by Weinkove *et al*. reported 23% incidence of afebrile neutropenic sepsis in subgroup of patients with hematological malignancies (20% of these had a peak body temperature below 36.5°C and the remaining between 36.5 and 37.4°C during first 24 hours in the ICU).[30]

According to our results, overall mortality rate in the ICU was 55.1% and was in line with published results of other studies.[31-35] Lower MASCC score (MASCC score <15), need for mechanical ventilation and lactic acidosis at the time of the ICU admission were associated with significantly higher 30-day in-hospital mortality (Table 3). These observations are in line with previously reported studies in patients with severe infections.[32,34,36-39] Although studies have reported improved survival rates of critically ill cancer patients during the past decade as compared to later time periods, in part due to implementation of the Surviving Sepsis Campaign guidelines, we did not find any significant difference in mortality rates between patients treated in the ICU before and after 2004 (50 *vs*. 55%, respectively, p = 1.0) (Table 3).[40,41] Furthermore, we observed a trend for a higher mortality rate in patients with afebrile neutropenic infections as compared to patients with febrile neutropenic infections (78 *vs*. 52%, p = 0.17) (Table 3). Published data show that patients with severe infections and hypothermia more likely develop septic shock as compared to those who are not hypothermic.[25,27,29] Furthermore, reported mortality rates in this subpopulation are consistently higher compared to normothermic or febrile patients, and are in range between 50–80%.[24-29,42] In contrast, data on patients with normothermic response to severe infection are inconsistent.[24-28] Results of recently published large

multicentric retrospective registry study, which included 4027 patients with neutropenic sepsis, showed that patients with neutropenic sepsis and peak temperature below 36.5°C (hypothermia) within the first 24 hours of admission to the ICU had approximately two-fold higher risk for in-hospital death as compared to normothermic (defined as temperatures between 36.5 and 37.4°C) patients. They did not observe any significant difference in mortality between normothermic and febrile (peak temperature of ≥ 37.5°C) patients. Similar was observed in a subgroup of patients with neutropenic sepsis and hematological malignancy.[30] Due to the retrospective nature of our study and due to the small number of patients with afebrile infections further subset-analyses (hypothermic *vs*. normothermic *vs*. febrile) were not feasible.

Use of drugs with antipyretic effect (e.g. some drugs used for the management of pain, corticosteroids) could potentially block the development of fever, which may delay presentation of these patients with serious infections to medical services. However, in our study there were no differences in the use of drugs with antipyretic effect in the febrile and afebrile subgroups of patients. Also, we did not find any differences in the rate of afebrile neutropenic infections in patients who received drugs with antipyretic effect and those who did not. Results of recently published study showed that feeling unwell in the absence of fever, which is a key marker of neutropenic sepsis, might discourage patients from contacting a doctor.[43] Delay of treatment with effective antimicrobial therapy in patients with severe neutropenia and bacteremia or septic shock is associated with increased mortality rates.[44,45] Patients on chemotherapy treatment should be informed that, although rarely, fever may be absent even in severe neutropenic infections and warned of possible other signs and symptoms of infection. In our study we observed that patients with afebrile neutropenic infection can present with hypotension, severe fatigue with inappetence, shaking chills, altered mental state or cough.

Our study has several limitations. First, this was a retrospective, single center study with a small sample size of heterogeneous patient population and therefore our findings are only hypothesis-generating. Only patients with solid tumors and lymphomas are treated at our center; patients with acute leukemias who frequently experience neutropenic infections were thus excluded from our analysis. Second, due to the small number of patients with afebrile infection a multivariate analysis as well as further subset comparisons (e.g. hypothermic *vs*. normothermic *vs*. febrile patients) were not feasible. Third, due to retrospective nature of our study we were not able to assess the potential impact of delayed effective antimicrobial therapy on the outcome of febrile and afebrile patients. Fourth, definition of febrile and afebrile episodes in our study may be flawed as axillary body measurement, used in our study, correlates least accurately with core body temperature with as much as 0.9°C variation.[46] According to the international guidelines for the management of infections in neutropenic cancer patients body temperature should be measured orally, while axillary measurements are discouraged.[13,15] Finally, we evaluated all episodes of severe neutropenic infections (with four patients having two separate episodes of febrile neutropenic infections), as occurring in real life practice, and thus risked potential statistical bias.[23]

In conclusion, afebrile infections in neutropenic cancer patients are rare. However, in cancer patients with chemotherapy-induced neutropenia and severe infections, afebrile patients might be at higher risk of death due to the complications of infection as compared to patients with febrile neutropenia. In addition, we identified lower MASCC score (MASCC score < 15), need for mechanical ventilation and lactic acidosis at the time of the ICU admission as negative prognostic factors for outcome. Patients on potentially myelosuppressive systemic cancer treatment should be informed that occasionally fever may be absent during the episode of neutropenic infection, and that they should immediately seek medical attention if signs or symptoms suggestive of infection occur, even in the absence of fever.

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research

Red blood cell transfusion and skeletal muscle tissue oxygenation in anaemic haematologic outpatients

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Background. Stored red blood cells (RBCs) accumulate biochemical and biophysical changes, known as storage lesion. The aim of this study was to re-challenge current data that anaemia in chronically anaemic haematology patients is not associated with low skeletal muscle tissue oxygen (StO_2), and that RBC storage age does not influence the tissue response after ischaemic provocation, using near-infrared spectroscopy.

Patients and methods. Twenty-four chronic anaemic haematology patients were included. Thenar skeletal muscle StO_2 was measured at rest (basal StO_2), with vascular occlusion testing (upslope StO_2, maximum StO_2) before and after transfusion.

Results. Basal StO_2 was low (53% ± 7%). Average RBC storage time was 10.5 ± 3.9 days. Effects of RBC transfusions were as follows: basal StO_2 and upslope StO_2 did not change significantly; maximum StO_2 increased compared to baseline (64 ± 14% *vs.* 59 ± 10%, p = 0.049). Change of basal StO_2, upslope StO_2 and maximum StO_2 was negatively related to age of RBCs. The decrease of maximum StO_2 was predicted (sensitivity 70%, specificity 100%), after receiving RBCs ≥ 10days old.

Discussion. Resting skeletal muscle StO_2 in chronic anaemic patients is low. RBC storage time affects skeletal muscle StO_2 in the resting period and after ischaemic provocation.

Key words: skeletal muscle; tissue oxygenation; red blood cells; transfusion; storage lesion

Introduction

Anaemia is state of decreased blood oxygen carrying capacity.[1] Acute anaemia is associated with increased tissue oxygen extraction.[2] On the other hand, with chronic anaemia human body has time to at least partially adapt to decreased blood oxygen carrying capacity.[3,4]

Near-infrared spectroscopy (NIRS) is non-invasive method to assess tissue oxygenation (StO_2) and estimate tissue haemoglobin (THb) levels.[5] We have studied skeletal muscle StO_2 in critically ill patients with preserved oxygen (*i.e.*, cardiogenic shock) and with impaired oxygen extraction (*i.e.*, septic shock).[6-9] In addition to measuring resting StO_2, we performed vascular occlusion tests to stop arterial blood flow, to estimate oxygen consumption, and at the end of the occlusion, it was also possible to estimate vascular reactivity and maximal reperfusion capability.[10]

Under acute blood loss in trauma patients, the skeletal muscle StO_2 measured by NIRS correlates with blood haemoglobin (Hb) and delivery of oxygen, and can easily detect latent stage of haemorrhagic shock.[11] Unexpectedly, chronically anaemic haematology patients with preserved oxygen ex-

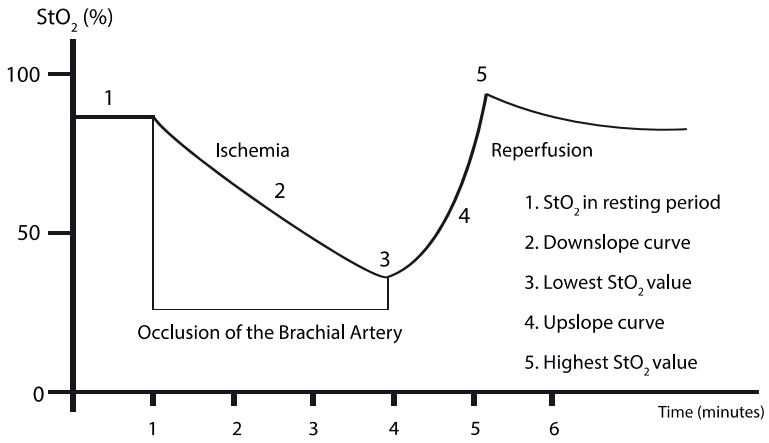


FIGURE 1. Schematic presentation of thenar skeletal muscle StO_2 before, during and after the vascular occlusion tests. Before the vascular occlusion, the StO_2 is measured in the resting period (1, basal StO_2). During the vascular occlusion, the StO_2 gradually decreases. The rate of this decrease is determined from the curve as the downslope StO_2 (2; %/min), as a surrogate of the tissue oxygen consumption. After reaching the predetermined minimum StO_2, present here as 40% StO_2 (3), the vascular occlusion is released, and the StO_2 begins to rise again. The rate of this increase is determined from the curve as the upslope StO_2 (4; %/min), as a surrogate marker of the microcirculatory reactivity. After the release of the occlusion, the StO_2 increases to higher values compared to the basal StO_2 due to post-ischaemic vasodilatation (5, maximum StO_2). The StO_2 then slowly returns to the basal StO_2.

traction capability did not show low skeletal StO_2 and THb index despite their severe anaemia, which is what would be predicted from normal physiological responses.[12]

It is known that there are structural and functional changes to RBCs during storage. Recent study detected deleterious effects of RBC storage on microvascular responses to transfusion in stable anaemic trauma patients.[13]

The aim of the present study was to re-challenge the current data that anaemia in chronically anaemic haematology patients is not associated with low skeletal muscle StO_2, and that the age of RBCs does not influence tissue responses. We investigated these aspects using improved technology NIRS devices, for deeper tissue penetration and removal of the superficial signal from the skin.

Materials and methods

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (N°117/06/12, 29.06.2012). All of the patients were informed of the goals of the study, and signed their written consent. This study was carried out according to the Helsinki-Tokyo Declaration.

Patients

This prospective observational study included patients that were treated in the Outpatient Haematology Clinic of Clinical Department of Haematology, University Medical Centre Ljubljana. All of the patients included were in need of a blood transfusion, which was ordered by treating physicians who were not involved in the study. The exclusion criteria were age <18 years, and patient rejection of participation.

Transfusions

Leucodepleted RBC units in saline-adenine-glucose-mannitol additive solution and with maximal allowed haemolysis of 0.8% were acquired from the Blood Transfusion Centre of Slovenia. The patients were transfused with two units of RBCs with maximal age difference of 3 days.

Near-infrared spectroscopy measurements and analysis

The thenar skeletal muscle StO_2 and THb concentrations were measured with tissue spectrometer (Equanox 7600; Nonin Medical, Minnesota,USA). The electrode (8004CA, Equanox Advance Sensor, Nonin Medical) was placed on the thenar eminence to measure the maximum resting StO_2. During measurements, there were no additional treatment procedures in place, except for the RBC transfusion. All of the patients were positioned in a semi-recumbent position.

In this resting period before the transfusion and after StO_2 signal stabilisation, the basal StO_2(%) and THb(g/l) were determined. Then vascular-occlusion test was performed, as reported previously.[6] In short, a sphygmomanometer cuff was placed over the brachium, and the pressure cuff inflation was taken to 60 mmHg over systolic blood pressure, to stop the blood flow in brachial artery. The StO_2 decreased during this arterial occlusion, which was measured as the downslope StO_2 (%/min). After reaching a StO_2 of 40% (the minimum StO_2) the cuff was released, and the StO_2 and THb continuously measured for an additional 5 min (Figure 1). During this reperfusion, the StO_2 increased rapidly, as the upslope StO_2 (%/min), and usually reached values higher than the basal StO_2, to give the maximum StO_2 (%). The same procedure was carried out 30 min after the RBC transfusion.

These time-dependent StO_2 values were continuously saved using the RealTerm software

(http://realterm.sourceforge.net), which allowed a 1-Hz sampling rate. The data acquired were further analysed off-line using the Microsoft Excel 2010 software (Microsoft, WA, USA).

All of the NIRS measurements were carried out without knowing the exact age of RBCs.

Vital functions measurements

Heart rate and systolic and diastolic blood pressures were measured (IntelliVueMP30, Philips Healthcare, Netherlands) before and after the transfusions, 5 min before NIRS measurements. Blood pressure was measured on the opposite hand to that used for NIRS measurements. Thenar skin temperature was measured immediately before NIRS measurements with a non-contact infrared clinical thermometer (Geratherm Medical AG Germany) (measuring range 34.0°C to 42.2°C, accuracy of ± 0.2°C).

Laboratory measurements

The Hb(g/L) and haematocrit before the transfusions were acquired according to routine laboratory tests (CoulterLH750 Haematology Analyser, Beckman Coulter Inc, USA).

Statistics

The normal distribution of the data was tested using D'Agostino-Pearson tests. The data are given as means ± standard deviation (SD), as medians and 95% confidence interval (95% CI), or as absolute values (percentages based on the whole group or subgroup). Effects of transfusion on different variables were tested with paired samples T-test. Regression analysis was performed using Analysis of variance to test the effects of age of RBCs. ROC analysis and interactive dot diagram were used to find the age of RBCs, which predicted divergent response. MedCalc 13.0 software (MedCalc Software, Belgium) was used. $P < 0.05$ was considered as statistically significant.

Results

Before the RBC transfusions

Twenty-seven patients were initially included in the study. Two patients were excluded from further analysis due to technical difficulties while recording the NIRS, one because of received 3 units of RBCs. In remaining 24 patients, 11 (46%) were female. The mean age of the patients was 65 ± 12 years. Myelodysplastic syndrome was the cause of anaemia in 16 (67%) patients, plasmacytoma in 4 (17%), leukaemia in 3 (12%), amyloidosis in 1 (4%). The demographic data, laboratory values, haemodynamic variables and skeletal muscle NIRS data of the patients before transfusion are presented in Table 1.

TABLE 1. Demographics, laboratory, haemodynamic and skeletal muscle NIRS variables of the patients before the RBC transfusions

Characteristic	All patients (n = 28)
Demographics	
Female [n (%)]	13 (46)
Age (years)	65 ±12
Laboratory data	
Haemoglobin (g/L)	77.9 ± 12.4
Haematocrit (%)	0.23 ± 0.04
Haemodynamics	
Systolic blood pressure (mm Hg)	122 ± 19
Diastolic blood pressure (mm Hg)	68 ± 10
Heart rate (beats/min)	78 ± 17
Thenar skin temperature (°C)	35.6 ± 0.6
NIRS in resting conditions	
Basal StO_2, %	53 ± 7
Tissue haemoglobin (g/L)	1.13 ± 0.14
NIRS: during vascular occlusion test	
Downslope StO_2 (%/min)	-9.4 ± 4.6
Minimum StO_2 (%)	39 ± 5
Upslope StO_2 (%/min)	78 ± 51
Maximum StO_2 (%)	59 ± 10

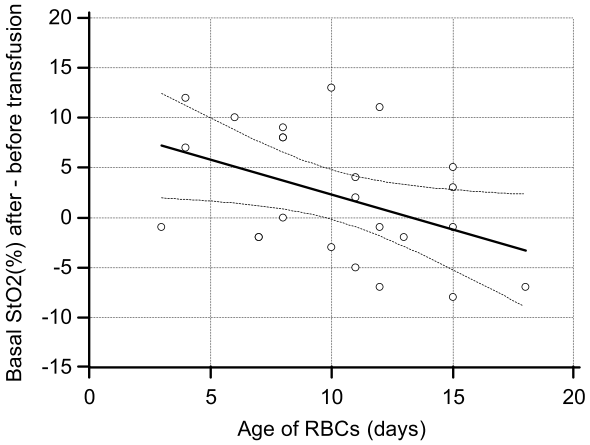
Data are means ± SD

In all patients received 2 units of RBCs. The average storage time of the RBCs was 10.5 ± 3.9days.

After the RBC transfusions

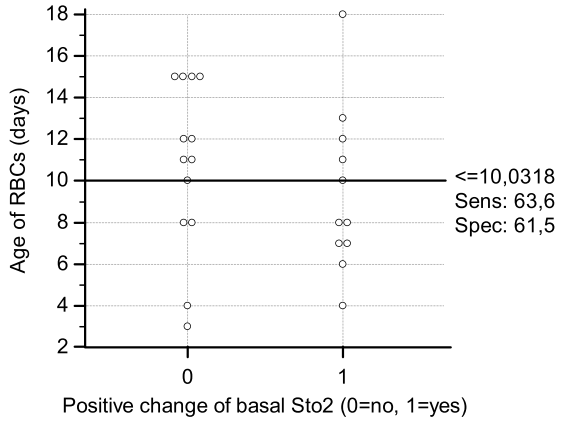
Compared to baseline Hb (77.9 ± 12.4 g/L *vs*. 94.4 ± 17.4 g/L, $p < 0.01$) and haematocrit (0.23 ± 0.04% *vs* 0.30 ± 0.03%, $p < 0.01$) increased after transfusion.

Systolic arterial pressure appeared not to be greatly affected by the RBC transfusions (122 ± 19 mm Hg *vs*. 124 ± 21 mm Hg, $p = 0.5$). Diastolic arterial pressure increased (68 ± 10 mm Hg *vs*. 77 ± 16 mm Hg, $p = 0.013$) and heart rate decreased after transfusion (78 ± 17 bpm *vs*. 73 ± 17 bpm, $p = 0.01$) after transfusion.



A

y = 9,3124 + -0,7016 x					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	9,3124	3,3712	2,3209 to 16,3039	2,7623	0,0114
Slope	-0,7016	0,3111	-1,3467 to -0,05654	-2,2556	0,0344
F-ratio	5,0878				
Significance level	P = 0,034				



B

FIGURE 2. Effects of the age of the RBCs for the transfusions on the basal StO_2. **(A)** Regression/analysis of variance. **(B)** ROC analysis, interactive dot diagram for optimal effect separation.

Prediction line (solid lines); 95% confidence line (dashed lines)

The thenar skin temperature remained unchanged, while THb increased after transfusion (1.13 ± 0.14 g/L *vs*. 1.22 ± 0.17 g/L, p = 0.006).

Basal StO_2 under resting conditions before the vascular occlusion did not differ significantly before and after RBC transfusion (53 ± 7% *vs*. 55 ± 7%, p = 0.10). As well there was no significant change of the downslope StO_2 (-9.4 ± 4.9%/min *vs*. -10.3 ± 8.4 min, p = 0.29), the minimum StO_2 (39 ± 5% *vs*. 40 ± 3%, p = 0.57) and the upslope StO_2 (78 ± 51%/min *vs*. 82 ± 59/min, p = 0.736). The maximum StO_2 values increased after RBCs transfusion compared to pre-transfusion measurements (64 ± 14% *vs*. 59 ± 10%, p = 0.049).

The age of RBCs transfused influenced the skeletal muscle StO_2 in resting conditions and during vascular occlusion testing. With increasing age the basal StO_2 increased less (basal StO_2 = 9.3124-0.7016 * age of RBCs in days; slope CI95%: -1.3467 to -0.0565, p = 0.0344) (Figure 2A). While receiving RBCs ≥ 10days old, the decrease of basal StO_2 was predicted with sensitivity 63.6% and specificity 61.5% (Figure 2B). Upslope StO_2 was also negatively related to the age of RBCs (upslope StO_2 = 64.3744 - 5.9782 * age of RBCs in days; slope CI95% : -11.5759 to -0.380, p = 0.0374); while receiving RBCs ≥ 10days old, the decrease of the upslope StO_2 was predicted with sensitivity 63.6% and specificity 61.5% (Figures 3A, 3B). The change of maximum StO_2 was negatively relate to RBCs age (Maximum StO_2 = 20.8870 + -1.4950* age of RBCs in days; slope CI95% : -2.7347 to -0.2553, p = 0.0203); while receiving RBCs ≥ 10days old, the decrease of the maximum StO_2 was predicted with sensitivity 70% and specificity 100%. There was no relationship between change of Hb, THb and downslope StO_2 with age of RBCs.

Discussion

Our findings confirm the low resting thenar skeletal muscle StO_2 in chronic anaemic haematology patients, and also the positive effects of RBCs on maximum StO_2 after vascular occlusion test- after reperfusion. Age of RBCs was negatively related to change of basal, upslope and maximum StO_2; age of RBCs ≥ 10days was found to predict divergent response of skeletal muscle StO_2 different responses of skeletal muscle StO_2.

In these patients, the resting StO_2 (53% ± 7%) was lower than that expected for normal healthy volunteers (83% ± 4%).[6] Studies that have including patients with acute anaemia have also reported low skeletal muscle StO_2. On the other hand, surprisingly, Yurku *et al.* did not detect such expected low StO_2 of thenar eminence in anaemic haematology outpatients[12], although their RBC transfusions were successful in improving these variables. The explanation for these contradictory data probably lies with the NIRS probe they used (length 15 mm; penetration, *ca*.7 mm). Their kind of probe mainly detects changes in skin and subdermal tissue, which is, however, not the main issue in clinical use of NIRS.[14] The thenar skin and subdermal tissue are 3–4 mm thick, and these layers are even

thicker for oedematous patients. By using probes with deeper penetration or devices that can filter out superficial layers and bones, the organ/skeletal muscle StO_2 can be better monitored, which is also more interesting for daily clinical practice (*i.e.*, the device used in the present study). The importance of the probe and site has been shown previously.[15,16] Superficial structures are more prone to changes in peripheral circulation and ambient temperature.[17]

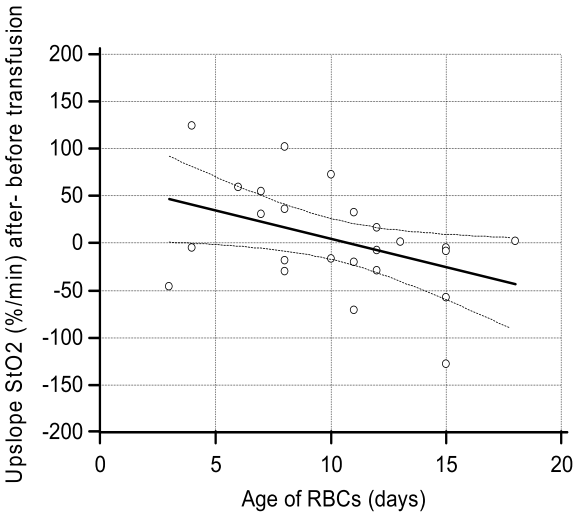
Our data that show here that skeletal muscle StO_2 in the resting period is influenced by age of the RBCs is supported by Leal-Noval *et al.*, who showed increases in cerebral oxygenation in patients with severe traumatic brain injury if the RBCs were stored for < 19 days. Their data suggested an inverse association between increments in brain oxygen tension and RBC storage time.[18]

Kiraly *et al.* using 25 mm NIRS showed probe, that transfusion of older RBCs (> 21 days) resulted in decreased skeletal muscle StO_2 in critically injured trauma patients. They reported a moderate correlation between increasing age of blood and decrease of oxygenation.[19]

Recent study also confirmed the deleterious effects of RBC storage on microvascular responses to transfusion in trauma patients.[13] The transfusion of relatively older RBC units was associated with a decline in both StO_2 and perfused capillary vascular density. They even predicted a mean decrease in StO_2 during the duration of the transfusion that was related to the RBC age (-0.1064 × age of transfusion in days).

Other studies carried out in septic patients did not confirm the present data that the age of the blood has an impact on the tissue saturation measured in the resting period.[20,21] Patients in sepsis/septic shock have imbalanced autoregulation of the blood flow in their peripheral tissues.[10] Volume resuscitated anaemic septic patients already have relatively high resting skeletal muscle StO_2. Roberson *et al.* did not find any differences in StO_2 of the brain and of the thenar muscle of healthy volunteers after transfusion of one unit of RBCs that was either 7 or 42 days old.[22]

In the present study, the divergent responses of resting thenar skeletal muscle StO_2 after receiving old blood could not be simply explained by an elevation of their blood Hb content only in the patients treated with fresh blood, which will lead to the increases in the oxygen delivery to the tissue, because there was no relationship between Hb or THb and age of RBCs. There is another explanation possible. In most tissues ratio of the arteriole to capillary to venous compartments is approximately 10:20:70.[23] In case of a ratio change due to benefits for the arterial or capillary parts in comparison to the venous part, we might expect an elevation of StO_2 in the resting period, without considering the equal increases in THb concentrations. The differences between these vascular compartments might be changed to the benefit of the arterial and capillary systems in the patients treated with fresh blood because of less disturbance of nitric oxide (NO) metabolism in these fresher RBCs, with fewer



y = 64,3744 + -5,9782 x					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	64,3744	29,2528	3,7077 to 125,0410	2,2006	0,0386
Slope	-5,9782	2,6991	-11,5759 to -0,3805	-2,2149	0,0374
F-ratio	4,9056				
Significance level	P = 0,037				

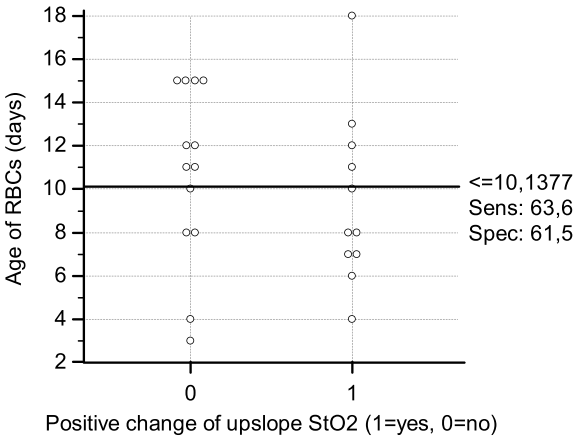
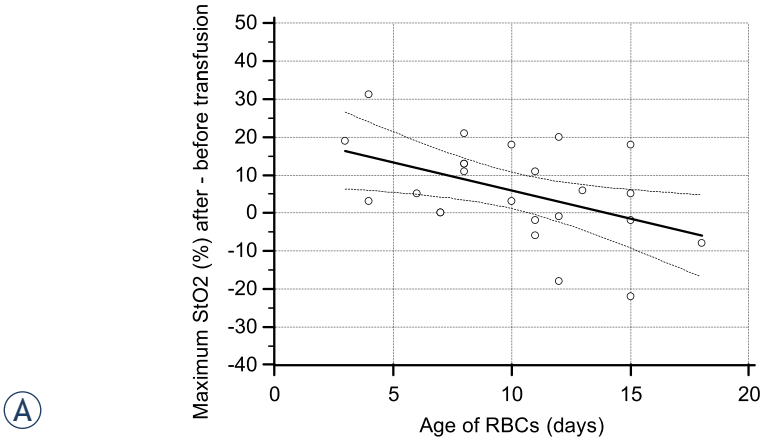


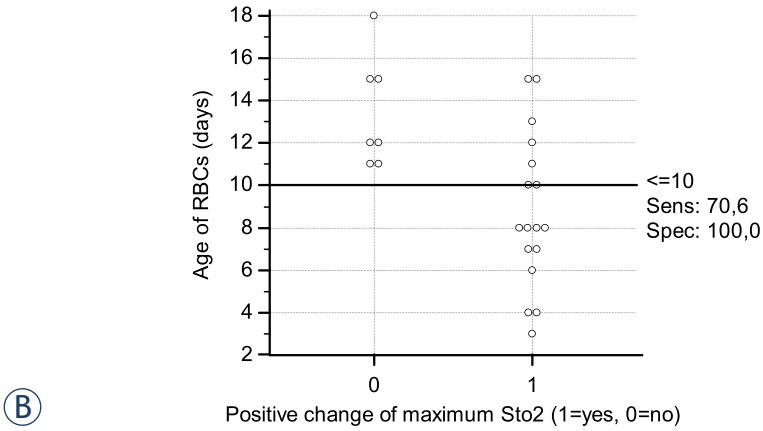
FIGURE 3. Effects of the age of the RBCs for the transfusions on the upslope StO_2. **(A)** Regression/analysis of variance. **(B)** ROC analysis, interactive dot diagram for optimal effect separation.

Prediction line (solid lines); 95% confidence line (dashed lines



A

y = 20,8870 + -1,4950 x					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	20,8870	6,4787	7,4511 to 34,3230	3,2240	0,0039
Slope	-1,4950	0,5978	-2,7347 to -0,2553	-2,5009	0,0203
F-ratio	6,2547				
Significance level	P = 0,020				



B

FIGURE 4. Effects of the age of the RBCs for the transfusions on the maximum StO_2. **(A)** Regression/analysis of variance. **(B)** ROC analysis, interactive dot diagram for optimal effect separation

Prediction line (solid lines); 95% confidence line (dashed lines)

degraded RBC products and a lower free Hb content, which are all NO-scavenging substances.[24]

It was shown that NO metabolism becomes disturbed in blood with storage duration > 14 days.[24] Using competition kinetics analysis, it was also recently demonstrated that compared with freshly prepared RBCs, the consumption rates of NO increase approximately 40-fold and NO-dependant vasodilatation is inhibited 2–4-fold in 42-day-old RBCs.[13]

This decreased vascular reactivity (upslope and maximum StO_2) found in patients treated with older blood can also be explained in terms of more disturbance of NO metabolism in older blood.[24] NO has an important effect on vascular homeostasis, which is known as NO-based vasodilatation.[24] Post-ischaemic hyperaemia, which develops with vascular occlusion, is one of the most important and reproducible indicators of microcirculatory responses.[25]

Bennett-Guerrero *et al.* studied effects of storage on the deformability of RBCs, on RBC-dependent vasoregulation, and on the changes in S-nitrosohaemoglobin (SNO-Hb) concentrations.[26] They observed significant drop in SNO-Hb concentrations in RBCs only 3h after blood donation, which might have been a reason for decreased vasodilatation after RBC transfusions. Reynolds *et al.* determined that there is possible regeneration of SNO-Hb in the received RBCs *in vivo*, which enables RBC-dependent vasodilatation and optimisation of blood perfusion through the peripheral tissues.[27]

The present study poses some new questions. Further studies should focus on the effects of NO scavenging in RBCs that are stored for longer; also, whether aged RBCs can also be used as a therapeutic option, such as for septic patients, who have excessively induced NO synthesis.

Conclusions

The resting skeletal muscle StO_2 in chronically anaemic haematology patients is low. The RBC storage time affects the skeletal muscle tissue oxygenation of these patients.

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Authors' contributions

MP contributed to the conception and design of the study, acquisition, analysis and interpretation of the data, statistical analysis, drafting and critically reviewing the manuscript for important intellec-

tual content, and submitting the manuscript; AUG, EP, and SS contributed to the conception and design of the study, acquisition of data, drafting the manuscript, and critical revision of the manuscript for important intellectual content; HM contributed to the conception and design of the study, statistical analysis, and critical revision of the manuscript for important intellectual content.

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Dinamična ultrazvočna preiskava s kontrastnim sredstvom. Kvantitativna ocena aktivnosti vnetja črevesne stene pri Crohnovi bolezni v otroškem obdobju

Ključevšek D, Vidmar D, Urlep D, Dežman R

Izhodišča. Ultrazvočna preiskava s kontrastnim sredstvom (UZ-KS) postaja uveljavljena neinvazivna in bolnikom prijazna slikovna metoda, ki bolje prikaže in opredeli patološke spremembe. Dinamična UZ-KS nam da dodatne informacije o prekrvljenosti preiskovanega organa. Pri bolnikih s Crohnovo boleznijo lahko z njo semi-kvantitativno in kvantitativno ocenjujemo prekrvljenost črevesne stene ter na ta način opredelimo aktivnost bolezni in odgovor na zdravljenje.

Zaključki. Crohnova bolezen je kronično vnetna črevesna bolezen. Značilna so pogosta poslabšanja. Ob vsakem poslabšanju moramo oceniti aktivnost bolezni, zato je pomembno, da je slikovna metoda čim manj invazivna, otrokom prijazna in ima visoko diagnostično zanesljivost. UZ je pomembna slikovna preiskava pri diagnosticiranju in spremljanju aktivnosti bolezni. Čeprav ultrazvočna kontrastna sredstva niso registrirana za otroke mlajše od 18 let (nenamenska uporába, *angl. off-label use*), jih velikokrat aplicirajo intravezikalno, manj pogosto intravenozno. V literaturi do sedaj nismo zasledili uporabe dinamičnih UZ-KS v otroškem obdobju za kvantitativno oceno aktivnosti Crohnove bolezni ozkega črevesa. Kljub temu da si rezultati različnih kinetičnih parametrov in zanesljivost metode pri oceni aktivnosti Crohovne bolezni v različnih raziskavah pri odraslih deloma celo nasprotujejo, menimo, da ima dinamična UZ-KS veliko vlogo. Postane lahko del celotne ultrazvočne preiskave ne samo pri odraslih, ampak tudi pri otrocih. Potrebne so nadaljnje kontrolirane in standardizirane klinične raziskave.

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Granulomatoza po avtologni presaditvi matičnih celic pri neHodgkinovem limfomu. Izkušnje posamične ustanove in pregled literature

Boltežar L, Žagar I, Jezeršek Novaković B

Izhodišča. Sarkoidoza pred in po zdravljenju raka je pomembna diferencialna diagnoza, ki jo je potrebno razlikovati od limfoma.

Bolniki in metode. Za oceno razširjenosti bolezni in učinkovitosti zdravljenja v skladu z novimi priporočili pri Hodgkinovem limfomu, difuznem velikoceličnem B limfomu in agresivnem folikularnem limfomu uporabljamo PET-CT. V članku opisujemo tri primere suma na ponovitev agresivnega limfoma (metabolno aktivne bezgavke na PET-CT) po visokodoznem zdravljenju in avtologni presaditvi krvotvornih matičnih celic. Pri vseh treh bolnikih smo histološko diagnosticirali sarkoidozo/granulomatozo. V literaturi smo zasledili, da so lažno pozitivne bezgavke pogostejše po alogenični kot pa po avtologni presaditvi matičnih celic.

Zaključki. Na PET-CT ugotovljene metabolno aktivne bezgavke po zdravljenju limfoma je potrebno vedno histološko opredeliti pred kakršno koli odločitvijo o nadaljnjem zdravljenju. Tako se izognemo morebitnim nepotrebnim postopkom, ki imajo lahko hude neželene učinke.

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Ocena napovedi poteka bolezni na osnovi metabolnih parametrov tumorja ob izhodiščnem FDG-PET/CT pri bolnikih s primarnim ekstranodalnim limfomom

Okuyucu K, Ozaydin S, Alagoz E, Ozgur G, Ince S, Oysul FG, Ozmen O, Tuncel M, Ozturk M, Arslan N

Izhodišča. Ne-Hodgkinove limfome, ki vzniknejo iz tkiv, ki niso primarni limfatični organi, imenujemo primarni ekstranodalni limfomi. Večina raziskav ocenjuje metabolne tumorske parametre v različnih organih in histopatoloških variantah te bolezni z namenom napovedati odgovor na zdravljenje. Namen pričujoče raziskave pa je bil oceniti napovedni pomen metabolnih parametrov tumorja na preživetje. Podatke smo pridobili iz izhodiščnega FDG-PET/CT pri bolnikih z različnimi primarnimi ekstranodalnimi limfomomi.

Bolniki in metode. Vključili smo 67 bolnikov s primarnim ekstranodalnim limfomom, pri katerih smo za izhodiščno oceno razširjenosti bolezni naredili FDG-PET/CT. Za oceno preživetja brez bolezni in celokupnega preživetja smo uporabili naslednje kvantitativne parametre FDG-PET/CT: vrednost maksimalnega standardiziranega privzema (*SUVmax*), povprečna vrednost standardiziranega privzema (*SUVmean*), metabolni volumen tumorja (*MTV*) in celokupna glikoliza lezije (*TLG*).

Rezultati. V multivariatni analizi so se kot statistično značilni parametri pokazali *SUVmean*, *MTV* in *TLG*. *SUVmean* je ostal statistično značilen tudi v analizi ROC. Pri izbrani razmejitveni vrednosti *SUVmean* 5,15 sta bili občutljivost in specifičnost 88 % in 64 %. Po analizi mesta prve prisotnosti patološkega kopičenja in histopatoloških variant v odnosu na ponovitev bolezni ni bilo razlik med variantami. Primarno mesto rasti ekstranodalnih limfomov je bilo statistično pomembno (p = 0,014): limfomi testisov in centralnega živčevja so imeli višjo stopnjo ponovitev (62,5 %, 73 %).

Zaključki. Visoke vrednosti *SUVmean, MTV* in *TLG*, pridobljene iz izhodiščnih FDG-PET/CT, ki so bili narejeni z namenom prve zamejitve bolezni, so potencialni napovedni dejavniki za preživetje brez bolezni in celokupno preživetje pri bolnikih s primarnim ekstranodalnim limfomom. Za napoved recidiva bolezni/metastaz je med navedenimi najpomembnejši *SUVmean*.

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Anatomske variante pankreatičnega voda in njihov pomen pri klasifikaciji pankreatitisa po sistemu Cambridge. Rezultati raziskave 1158 zaporednih magnetnoresonančnih holangiopankreatografij

Adibelli ZH, Adatepe M, Imamoglu C, Esen OS, Erkan N, Yildirim M

Izhodišča. Namen raziskave je bil ocena pojavnosti anatomskih variant pankreatičnega voda in njihove razporeditve med spoloma z metodo magnetnoresonančne holangiopankreatografije. Želeli smo tudi preveriti, ali obstoječe anatomske variante pankreatičnega voda pomembno vplivajo na opredelitev morfoloških sprememb kroničnega pankreatitisa po klasifikaciji sistema Cambridge.

Bolniki in metode. Retrospektivno smo pregledali rezultate 1312 magnetnoresonančnih holangiopankreatografij, ki smo jih naredili med januarjem 2013 in avgustom 2015. 154 preiskav smo izključili iz nadaljnje raziskave zaradi slabšega slikovnega zajema ali zaradi predhodno opravljenega kirurškega posega na pankreasu. Končno število preiskav, ki smo jih vključili v retrospektivno analizo, je bilo 1158.

Rezultati. Med 1158 preiskavami smo v 54 (4,6 %) primerih našli *pancreas divisum* in v 13 (1,2 %) primerih stanje *ansa pancreatica*. Prevalenca poteka pankreatičnega voda je bila 62,5 % za descendentni potek, 30 % za sigmoidni in 5,5 % za vertikalni potek, medtem ko je bila prevalenca poteka v obliki zanke 2 %. Najpogostejši obliki pankreatičnega voda sta bili tip 3 (528 primerov; 45,6 %) in tip 1 (521 primerov; 45 %).

Zaključki. Vertikalni potek (p = 0,004) pankreatičnega voda z obliko tip 2 (p = 0,03) smo pogosteje opazili pri ženskah kot pri moških. Med preostalimi variantami pankreatičnega voda nismo opazili statistično pomembnih razlik med spoloma. Med morfološkimi spremembami po klasifikaciji sistema Cambridge, *pancreas divisum* in ostalimi normalnimi anatomskimi variantami pankreatičnih vodov nismo našli povezave. *Ansa pancreatica* smo med opisanimi spremembami opazili kot redko anatomsko različico poteka pankreatičnega voda, ki pa lahko predstavlja predispozicijo za nastanek idiopatskega pankreatitisa.

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Znotrajžilno zdravljenje nepretrganih anevrizem kavernoznega in oftalmičnega segmenta internih karotidnih arterij s sistemom Pipeline

Jevšek M, Mounayer C, Šeruga T

Izhodišča. Znotraj žilno zdravljenje širokovratnih anevrizem s preusmerjanjem pretoka krvi je relativno nov način zdravljenja. Preusmeritev pretoka dosežemo z žilnimi opornicami, ki imajo znatno gosteje pleteno steno kot klasične opornice. Kri tako pretežno ostaja v lumnu žilne opornice in zmanjšuje hitrost pretoka znotraj same anevrizme. Zastajanje krvi v anevrizmatski vreči vodi do nastanka tromba in posledične izključitve anevrizme iz obtoka. V raziskavi smo želeli oceniti uspešnost uporabe gosto pletenih žilnih opornic Pipeline pri zdravljenju anevrizem s širokim vratom.

Bolniki in metode. V raziskavo smo zajeli 15 bolnikov, ki smo jih zdravili od novembra 2010 do februarja 2014. Pretežni del anevrizem je ležal na karotidni arteriji, intraduralno v oftalmičnem delu žile. Bolnike smo zdravili z gosto pleteno žilno opornico Pipeline (Ev3), ki smo jih postavili preko vratu anevrizme. Uspešnost zdravljenja smo ocenjevali angiografsko in klinično z nevrološkim pregledom.

Rezultati. Kontrolne angiografije, neposredno po postavitvi žilnih opornic, so pokazale upočasnitev pretoka znotraj anevrizmatske vreče. Pri nobenem izmed bolnikov ni prišlo do tehničnih ali kliničnih zapletov med posegom ali po njem. Kontrolne angiografije smo opravljali šest do dvanajst mesecev po posegu. V večini primerov so bile anevrizme v celoti izključene iz obtoka. Nevrološki status bolnikov ob kontrolnih pregledih je bil brez bolezenskih znakov.

Zaključki. Zdravljenje anevrizem z gosto pletenimi žilnimi opornicami Pipeline in preusmeritvijo obtoka je varna in časovno bistveno krajša metoda v primerjavi s standardno metodo z uporabo žilne opornice in elektolizno ločljivih platinastih zank. Nova metoda predstavlja velik napredek pri zdravljenju kompleksnih anevrizem karotidnih arteriji s širokim vratom in bo verjetno nadomestila dosedanji način zdravljenja.

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Somatske mutacije 1 in 2 izocitrat dehidrogenaze so napovedni označevalci bolnikov z akutno mieloično levkemijo z normalnim kariotipom

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Izhodišča. Mutacije genov 1 in 2 izocitrat dehidrogenaze (*IDH1* in *IDH2*) so pogoste molekularne spremembe pri akutni mieloični levkemiji z normalnim kariotipom (AML-NK). Mnogokrat so preučevali vpliv mutacij *IDH* na klinični potek bolezni in izid zdravljenja AML-NK, vendar samo v nekaj raziskavah so sledili spremembe po zdravljenju.

Bolniki in metode. V raziskavo smo vključili 110 odraslih bolnikov z AML-NK, ki so imeli mutacije *IDH1*. Sledili smo povezavo teh mutacij z ostalimi napovednimi označevalci in izidom bolezni. Preučili smo tudi stabilnost teh mutacij ob poteku bolezni, tako pri popolnih odgovorih na zdravljenje kot pri ponovitvah bolezni.

Rezultati. Mutacije *IDH* smo zaznali pri 25 (23 %) bolnikih. Bolniki IDH^+ so imeli nižjo stopnjo popolnih odgovorov kot bolniki IDH^- (44 % *vs* 62,2 %; p = 0,152) in nekoliko manjši interval brez ponovitve bolezni (12 mesecev *vs* 17 mesecev; p = 0,091). Prisotnost mutacij *IDH* je značilno zmanjšalo celokupno preživetje bolnikov (2 *vs* 7 mesecev; p = 0,039). Stabilnost mutacij *IDH* smo sledili pri 19 bolnikih IDH^+. Izgubo mutacij smo zabeležili pri bolnikih s popolnim odgovorom na zdravljenje, vendar smo enake mutacije zasledili tudi pri bolnikih s ponovitvijo bolezni.

Zaključki. Rezultati raziskave potrjujejo, da so mutacije *IDH* pri bolnikih z AML-NK napovedni dejavnik, ki skupaj z ostalimi molekularnimi označevalci lahko pripomorejo k stratifikaciji teh bolnikov in omogočijo boljši izbor zdravljenja. Mutacije *IDH* so zelo stabilne med zdravljenjem in bi zato lahko bile označevalec za minimalni preostanek bolezni.

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Dolgotrajno preživetje bolnikov z glioblastomom. Promoter metilacije metil gvanin metil transferaza (MGMT) je neodvisni ugodni napovedni dejavnik

Smrdel U, Popović M, Zwitter M, Bostjančič E, Zupan A, Kovač V, Glavač D, Bokal D, Jerebic J

Izhodišča. Po uvedbi kombiniranega zdravljenja se je preživetje bolnikov z glioblastomom pomembno izboljšalo, kljub temu pa je napoved poteka bolezni za večino bolnikov slaba. Klinični napovedni dejavniki (starost, spol, obseg operacije in splošno stanje zmogljivosti) ne najavljajo zanesljivo dolgotrajnega preživetja. Namen te raziskave je bil ovrednotiti imunohistokemične in genetske značilnosti tumorjev kot dodatnih napovednih dejavnikov pri bolnikih z glioblastomom.

Bolniki in metode. Iz skupine bolnikov z dolgotrajnim preživetjem smo izbrali 40 bolnikov, katerih preživetje je bilo daljše od 30 mesecev. Kontrolno skupino je sestavljalo 40 bolnikov z krajšim preživetjem, ki se glede na klinične napovedne dejavnike niso razlikovali od bolnikov iz skupine z dolgotrajnim preživetjem. Vse bolnike smo zdravili z operacijo, pooperativno radioterapijo ter temozolmidom med in po radiotrapiji. Bioptične vzorce smo testirali (1) glede metilacije promoterja gena za metil gvanin metil transferazo (MGMT) in pri tem uporabili metilacijskospecifično polimerazno verižno reakcijo; (2) ugotavljali smo tudi mutacije gena za encim izocitratedehidrogenazo 1 (IDH1) z imunohistokemično metodo in metodo od multipleksne povezave odvisne probatorne amplifikacije (angl. *multiplex ligation dependent probe amplification*, MLPA); (3) prav tako smo z metodo MLPA določevali mutacije IDH2 in mutacije za gen ciklin odvisnega kinaznega inhibitorja (angl. *cyclin-dependent kinase inhibitor* 2A, CDKN2A) in mutacije CDKN2B; (4) z metodo fluorescentne *in situ* hibridizacije pa smo ugotavljali preureditev 1p/19q.

Rezultati. Metilacijo promoterja za MGMT smo našli pri 95 % bolnikov v skupini z dolgotrajnim preživetjem in pri 36 % bolnikov v kontrolni skupini ($p < 0,001$). Bolniki z mutacijo IDH1 R132H so imeli neznačilno nižje tveganje za smrt zaradi glioblastoma ($p = 0.437$). Druge mutacije so bile redke in brez značilnih razlik med skupinama.

Zaključki. Z molekularnim in genetskim testiranjem lahko ugotovimo dodatne napovedne dejavnike za preživetje in odgovor na zdravljenje pri bolnikih z glioblastomom. Najpomembnejša ugotovitev naše raziskave je, da je dolgotrajno preživetje brez promotorja metilacije MGMT zelo redko. Pri bolnikih, ki imajo glioblastom brez teh mutacij, bo potrebno raziskati drugačen način zdravljenja.

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Mobilizacija s ciklofosfamidom zmanjša limfocitne populacije v koncentratu krvotvornih matičnih celic in zavre njihovo ponovno repopulacijo po avtologni presaditvi krvotvornih matičnih celic pri bolnikih z diseminiranim plazmocitomom

Škerget M, Skopec B, Žontar D, Černelč P

Izhodišča. Za mobilizacijo krvotvornih matičnih celic uporabljamo rastne dejavnike same in v kombinaciji s ciklofosfamidom. Namen raziskave je bil ugotoviti razlike med tremi različnimi načini mobilizacije na limfocitne populacije med mobilizacijo, v koncentratu krvotvornih matičnih celic in 15. dan po avtologni presaditvi krvotvornih matičnih celic.

Bolniki in metode. 48 bolnikov smo razdelili med tri različne načine mobilizacije: (i) filgrastim (20), (ii) pegfilgrastim (19) in (iii) ciklofosfamid + filgrastim (9). Limfocite, CD16+/56+ naravne celice ubijalke (NK) in CD4+/CD25^{high} T regulatorne celice smo določili s pretočnim citometrom.

Rezultati. Med različnimi mobilizacijami smo ugotovili statistično pomembne razlike. Število limfocitov in celic NK je bilo znižano na dan zbiranja pri mobilizaciji s ciklofosfamidom (limfociti 1.08 x 10^9/L; celice NK 0.07 x 10^9/L) v primerjavi z filgrastimom (limfociti 3.08 x 10^9/L; celice NK 0.52 x 10^9/L) in pegfilgrastimom (limfociti 3 x 10^9/L; celice NK 0.42 x 10^9/L). Koncentrat krvotvornih matičnih celic je po mobilizacijo s ciklofosfamidom vseboval manj limfocitovov in celic NK (limfociti 50.1 x 10^9/L; celice NK 4.18 x 10^9/L) kot po filgrastimu (limfociti 112 x 10^9/L; celice NK 17.5 x 10^9/L) in pegfilgrastimu (limfociti 112 x 10^9/L; celice NK 14.3 x 10^9/L). Med postopki mobilizacije nismo ugotovili razlike v številu T regulatornih celic. Pri vseh treh mobilizacijah smo beležili podvojitev števila T regulatornih celic na dan zbiranja.

Zaključki. Mobilizacija s ciklofosfamidom zmanjša število limfocitov in celic NK na dan zbiranja in v koncentratu krvotvornih matičnih celic v primerjavi z mobilizacijo s filgrastimom in pegfilgrastimom, kar vodi do njihove zakasnele repopulacije po avtologni presaditvi krvotvornih matičnih celic. Med mobilizacijo s filgrastimom in pegfilgrastimom ni bilo razlik.

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Rak sapnika – rezultati zdravljenja, napovedni dejavniki in incidence drugih rakov

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Izhodišča. Rak sapnika sodi med redke rake in rezultati zdravljenja, o katerih so poročali do sedaj, niso zadovoljivi. Namen raziskave je bil oceniti rezultate zdravljenja bolnikov z rakom sapnika, prepoznati morebitne dodatne bolnike, ki so primerni za kirurško zdravljenje, ovrednotiti napovedne dejavnike in oceniti pojav drugih rakov.

Bolniki in metode. Pregledali smo Regionalno zbirko podatkov raka in Bolnišnično podatkovno zbirko, da smo poiskali bolnike z novotvorbami sapnika. 58 od 418 bolnikov je izpolnjevalo pogoje za vključitev (primarni rak sapnika s potrjenim histološkim izvidom in popolno razvidnost zdravljenja). Uporabili smo standardne statistične teste.

Rezultati. Ploščatocelični karcinom (SCC; 63,8 %) in adenoidnocistični karcinom (ACC, 15,5 %) sta bila najpogosteje diagnosticirana histološka vrsta raka sapnika. Z radioterapijo smo zdravili 48 bolnikov, kirurško ali endoskopsko 20 in s kemoterapijo 14 bolnikov. Rak sapnika smo diagnosticirali kot drugi rak pri 10 bolnikih, med temi pri 1 bolniku pred rakom na pljučih in pri enem sočasno. Po srednjem času spremljanja 12,7 mesecev je 85,5 % bolnikov umrlo zaradi bolezni. Lokalno ponovitev bolezni smo odkrili pri 17 % bolnikov. V univariatni analizi so imeli bolniki z ACC statistično boljše 5-letno celokupno preživetje (77,8 %) kot tisti z diagnozo SCC (8,4 %) (p = 0,0001). V multivariatni analizi so radioterapija, stanje zmogljivosti in hemoptize bistveno vplivali na celokupno preživetje. Med bolniki, ki niso bili zdravljeni kirurško, smo našli 15–26 % bolnikov, ki bi lahko bili primerni za kirurško zdravljenje, odvisno od meril za izbor.

Zaključki. Diagnostika mora biti osredotočena na prepoznavanje bolnikov z rakom sapnika, ki so primerni za invazivno zdravljenje in za radioterapijo. Bolniki, ki so preživeli raka dihalnega sistema, sodijo v skupino z večjim tveganja za rak sapnika. Radioterapija je pomemben del zdravljenja bolnikov z rakom sapnika.

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Preživetje bolnikov z jetrnoceličnim karcinomom v srednjem stadiju bolezni, zdravljenih s transarterijsko kemoembolizacijo z doksorubicinom, vezanim na DC Bead delce in pod kontrolo Cone-beam CT

Popović P, Štabuc B, Janša R, Garbajs M

Izhodišče. Z našo retrospektivno raziskavo smo ocenjevali odgovor na zdravljenje, stranske učinke in preživetje bolnikov z jetrnoceličnim karcinomom (HCC) v srednjem stadiju bolezni, zdravljenih s transarterijsko kemoembolizacijo z doksorubicinom, vezanim na DC Bead delce (DEBDOX TACE) in pod kontrolo računalniške tomografije s stožčastim snopom (CBCT).

Bolniki in metode. Med oktobrom 2010 in junijem 2012 je bilo z DEBDOX TACE zdravljenih 35 bolnikov s HCC v srednjem stadiju bolezni (32 moških, 3 ženske; povprečna starost, 67,5 ± 7,8 let; 22 bolnikov stopnje A po Child-Pugh, 8 stopnje B; 5 bolnikov brez ciroze). Tromboza vene porte je bila prisotna pri 6 (17.1%) bolnikih. DEBDOX TACE je bil opravljen s superselektivno kateterizacjo arterije, ki prehranjuje tumor, čemur je sledila embolizacija s 100-300 mikronskimi mikrosferami, na katere je bilo vezanega 50-100 mg doksorubicina. Vse kemoembolizacije so bile opravljene pod kontrolo CBCT. Odgovor tumorja na zdravljenje je bil določen glede na mRECIST kriterije.

Rezultati. Skupno je bilo opravljenih 120 posegov (povprečno 3,2 na bolnika). Zdravili smo 97 lezij s povprečnim premerom 4,9 ± 1,9 cm. Po ali med posegom je prišlo do 32 manjših in dveh (1,6%) večjih zapletov (en jetrni absces in en cerebrovaskularni inzult). Po povprečnem obdobju sledenja 27,7 ± 10,5 mesecev je 94,3% bolnikov doseglo objektivni odgovor na zdravljenje (42,4% popolni in 57,6% delni odgovor). Povprečni čas do progresa bolezni je bil 10,9 ± 5,3 mesece. Povprečno preživetje bolnikov je bilo 33,9 mesecev (95% CI; 28,9 – 38,9 mesecev), enoletno preživetje je bilo 97,1%, dvoletno pa 65,7%.

Zaključek. Superselektivna DEBDOX TACE pod kontrolo CBCT je varna in učinkovita metoda z velikim deležem objektivnega odgovora tumorjev na zdravljenje in visokim celokupnim preživetjem.

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Razmerje med izgubo telesne teže in planirnim tarčnim volumnom pomembno vpliva na nastaviteveno napako pri bolnikih z rakom nosnega žrela, ki smo jih obsevali s helično tomoterapijo in kontrolirali z dnevno megavoltno računalniško tomografijo

Hou WH, Wang CW, Tsai CL, Hsu FM, Cheng JCH

Izhodišča. Pri bolnikih z rakom nosnega žrela lahko spremembe v anatomiji področja glave in vratu med radioterapijo povzročijo nastavitvene napake. V retrospektivni raziskavi smo analizirali podatke slikovno vodenega obsevanja, da bi poiskali napovedne kazalce za nastavitvene napake.

Bolniki in metode. Analizirali smo podatke 217 bolnikov z rakom nosnega žrela, ki smo jih obsevali s helično tomoterapijo. Stadij tumorja, indeks telesne mase, izgubo telesne teže in planirni tarčni volumen (PTV) smo ocenili kot kazalce za napoved dnevnih nastavitvenih odstopanj, zabeleženih avtomatično z megavoltno računalniško tomografijo in programskim paketom.

Rezultati. Povprečna dnevna nastavitvena odstopanja v mediolateralni smeri, superioinferiorni in anterioposteriorni smeri so bila (v mm) 1,2 ± 0,6; 1,8 ± 0,8 in 3,4 ± 1,4. Povprečna izguba telesne teže je bila 4,6 ± 3,3 kg (6,8 ± 4,9 %). Bolniki z izgubo telesne teže > 5 % so imeli pomembno večje nastavitveno odstopanje v anterioposteriorni smeri (3,6 ± 1,5 proti 2,9 ± 1,1 mm; $p < 0,001$) in superioinferiorni smeri (1,6 ± 0,7 proti 1,9 ± 0,9 mm; $p = 0.01$), ne pa tudi v mediolateralni smeri ($p = 0,279$). Anterioposteriorna nastavitvena napaka se je povečala za 0,06 mm ($y = 0,055 x + 2,927$; x pomeni odstotni delež izgube telesne teže/PTV; y pomeni anterioposteriorno odstopanje) za vsak procent znižanja telesne teže, normalizirano na PTV.

Zaključki. Pri bolnikih z izgubo telesne teže > 5 % in manjšimi PTV-ji je bila, verjetno zaradi majhnega obsega telesa ali vratu, povečana verjetnost za večje nastavitvene napake v anterioposteriorni smeri.

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Računalniško modeliranje prototipov aplikatorjev za brahiterapijo raka vratu maternice

Petrič P, Hudej R, Al-Hammadi N, Šegedin B

Izhodišča. Standardni aplikatorji za brahiterapijo raka vratu maternice ne omogočajo vedno zadovoljivega razmerja med obsevanostjo tarčnega volumna in okolnih zdravih tkiv. Naš cilj je bil razviti metodologijo za razvoj prototipov aplikatorjev z optimalnimi dozimetričnimi lastnostmi.

Bolniki in metode. Prednostna naloga je bila razvoj računalniškega orodja za slikovni prikaz porazdelitve tarčnih volumnov (*ang. Target-volume Density Map*; TDM). Uvodoma smo opredelili zahteve glede funkcionalnosti načrtovanega orodja, na podlagi katerih smo programirali. Z uporabo objektov znanih oblik in velikosti smo preverili natančnost razvitega programa. Končno smo opredelili postopek nadaljnje obdelave TDM-ja, na katerem je temeljil razvoj novih aplikatorjev.

Rezultati. Izdelali smo programsko orodje za analizo kontur 2 (*ang. Contour Analysis Tool 2*; CAT 2). TDM smo naredili z združevanjem obrisov tarčnih področij iz vzorca slik DICOM (ang. Digital Imaging and Communications in Medicine) bolnic z vstavljenim aplikatorjem. Pri združevanju tarčnih obrisov smo s CAT-2 poravnali tarčne obrise na referenčni aplikator. Vsak voksel (*angl. voxel*, *volumetric pixel*) TDM-ja je opredelil vrednost, ki je predstavljala število obrisov, ki so ta voksel obdajali. Številčne vrednosti smo pretvorili v področja sivine in shranil TDM v obliki slike DICOM. Nadaljnjo obdelavo TDM-ja smo naredili v računalniškem planirnem sistemu. Z uporabo orodja za avtomatsko vrisovanje smo ustvarili obrise področij z enako sivino (*ang. Iso Density Contours*; IDC). Območja, ki so obdajala posamezen IDC, smo opredelili kot prostor, v katerem bi lahko bila okolna zdrava tkiva (*angl. Residual Volume at Risk;* RVR). Po namestitvi standardnih in prototipnih aplikatorjev na TDM in optimizaciji doznih porazdelitev smo zabeležili dozno-volumske parametre in ustvarili karakteristične krivulje aplikatorjev. Slednje, ob upoštevanju predvidene zahtevnosti klinične uporabe in prostorske porazdelitve doze, so služile za opredelitev prototipov z najboljšimi dozimetričnimi lastnostmi.

Zaključki. Razvita metoda predstavlja osnovo za razvoj prototipov brahiterapevtskih aplikatorjev, ki omogočajo optimalno dozno porazdelitev v katerikoli klinični situaciji. Pred morebitno rutinsko uporabo novih aplikatorjev je potrebno testiranje, da bi lahko ugotovili njihovo varnost in klinično učinkovitost.

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Preživetje pri nevtropeničnih bolnikih z rakom in hudo okužbo brez vročine

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Izhodišča. Vročina pri nevtropeničnih bolnikih z rakom je lahko odsotna kljub mikrobiološko in/ali klinično potrjeni okužbi. Postavili smo hipotezo, da imajo afebrilni nevtropenični bolniki z rakom in hudo okužbo slabši potek bolezni, kot tisti s febrilno nevtropenijo.

Metode. V retrospektivno raziskavo smo vključili vse odrasle bolnike z rakom, zdravljene s kemoterapijo, ki so zaradi hude nevtropenične okužbe potrebovali intenzivno zdravljenje na Oddelku za intenzivno terapijo Onkološkega inštituta Ljubljana med leti 2000–2011. Naš primarni namen je bil ugotoviti celokupno 30-dnevno bolnišnično umrljivost. Povezavo med febrilnim statusom in 30-dnevno bolnišnično umrljivostjo smo ugotavljali s Fisherjevim eksaktnim testom.

Rezultati. Ugotovili smo 69 epizod hudih nevtropeničnih okužb pri 65 bolnikih z rakom. Med temi je bilo 9 (13 %) epizod afebrilnih. Tisti z afebrilnimi nevtropeničnimi okužbami so imeli naslednje klinične znake oz. simptome okužbe: hipotenzijo, hudo utrujenost z inapetenco, mrzlico, spremenjeno mentalno stanje ter kašelj; pri vseh je okužba napredovala v hudo sepso ali septični šok. Celokupna 30-dnevna bolnišnična umrljivost je bila 55,1 %. Bolniki z afebrilno nevtropenično okužbo so imeli težnjo višje umrljivosti v primerjavi z bolniki s febrilno nevtropenijo (78 % vs. 52 %, p = 0,17).

Zaključki. Izsledki raziskave kažejo težnjo višje umrljivosti pri rakavih bolnikih z afebrilno nevtropenično okužbo v primerjavi s tistimi s febrilno nevtropenijo. Bolnike z rakom, ki se zdravijo s kemoterapijo, bi bilo potrebno opozoriti, da lahko hude nevtropenične okužbe potekajo tudi brez povišane telesne temperature.

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Transfuzija eritrocitov in oksigenacija skeletnega mišičnega tkiva pri ambulantnih hematoloških bolnikih z anemijo

Podbregar M, Gavrić AU, Podbregar E, Možina H, Stefanović S

Izhodišča. V shranjenih eritrocitih pride s staranjem do biokemičnih in biofizikalnih sprememb, ki jih imenujemo poškodbe shranjevanja. Namen raziskave je bil ponovno preveriti rezultate dosedanjih raziskav, da anemija pri kronično anemičnih hematoloških bolnikih ni povezana z znižano tkivno oksigenacijo skeletnih mišic (StO_2) in da dolžina shranjevanja eritrocitov ne vpliva na odgovor tkiv po ishemični provokaciji; kar smo proučevali s pomočjo spektroskopije blizu rdečega spektra.

Bolniki in metode. V raziskavo smo vključili 24 kronično anemičnih hematoloških bolnikov. StO_2 skeletnih mišic smo merili na tenarju v mirovanju (bazalna StO_2) ter po arterijskem zažemu nadlahti (StO_2 pri naklonu navzgor in maksimalna StO_2), pred in po transfuziji.

Rezultati. Bazalna StO_2 pred transfuzijo je bila nizka (53 % ± 7 %). Povprečni čas shranjevanja eritrocitov je znašal 10,5 ± 3,9 dni. Ugotovili smo sledeče učinke transfuzije eritrocitov: bazalna StO_2 in StO_2 pri naklonu navzgor po arterijskem zažemu se nista pomembno spremenila; maksimalna StO_2 se je po transfuziji povišala (64 ± 14 % vs. 59 ± 10 %, p = 0.049). Spremembe bazalne StO_2, StO_2 pri naklonu navzgor in maksimalne StO_2 so bili negativno povezane s trajanjem shranjevanja eritrocitov. Zmanjšanje maksimalne StO_2 po prejemu eritrocitov starih ≥ 10 dni smo napovedali s senzitivnostjo 70 % in specifičnostjo 100 %.

Zaključki. Tkivna oksigenacija skeletnih mišic pri kronično anemičnih hematoloških bolnikih je nizka. Dolžina shranjevanja RBC vpliva na tkivno oksigenacijo skeletnih mišic v mirovanju in po ishemični provokaciji.

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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the final quarter of 2016

Doc. dr. Josip Cholewa Foundation for cancer research and education continues with its planned activities in the second quarter of 2016 and is commencing to prepare for the activities next year. Its primary focus remains the provision of grants and scholarships and other forms of financial assistance for basic, clinical and public health research in the field of oncology. An analysis of the still ongoing activities in 2016 is planned in order to make an assessment of the impact of Foundation's activities, thus providing a basis for developing new strategies and approaches in its scope of fight against cancer.

The Foundation continues to provide support for »Radiology and Oncology«, a quarterly scientific magazine with a respectable impact factor, that publishes research and review articles about all aspects of cancer. The magazine is edited and published in Ljubljana, Slovenia. »Radiology and Oncology« is an open access journal available to everyone free of charge. Its long tradition represents a guarantee for the continuity of international exchange of ideas and research results in the field of oncology for all in Slovenia that are interested and involved in helping people affected by many different aspects of cancer.

The Foundation makes great efforts to provide financial and other kinds of support for the organisation of various forms of meetings to extend and broaden the knowledge about prevention of cancer, early detection of various types of cancer, its treatment and rehabilitation of cancer patients. The advances in knowledge of all aspects of dealing with cancer should be in Foundation's opinion available to all the professionals that treat cancer patients, to the patients themselves and their closest relatives and friends, and finally also to the general public.

The problems associated with cancer affect more and more people and their relatives in Slovenia and elsewhere. The Foundation will therefore continue with its activities in the years to come. Treatment of cancer is ever more successful with many patients surviving decades after the start of their treatment and many new problems and challenges have thus come into place. Longer survival of an increasing number of patients with previously incurable cancer conditions adds many new dimensions to their life and to the life of their families. It also confronts cancer specialists, all the other experts and lay public dealing with cancer with new challenges and new goals to achieve.

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Edini dolgodelujoči epoetin, ki omogoča priročno in bolniku prilagojeno zdravljenje[1,2]

Aranesp® je indiciran za zdravljenje simptomatske anemije pri odraslih onkoloških bolnikih z nemieloičnimi malignomi, ki dobivajo kemoterapijo.

ARANESP® 150, 300 in 500 mikrogramov raztopina za injiciranje v napolnjeni injekcijski brizgi (darbepoetin alfa) – SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA
Samo za strokovno javnost. Pred predpisovanjem preberite celoten Povzetek glavnih značilnosti zdravila (SmPC).
SESTAVA ZDRAVILA: Ena napolnjena injekcijska brizga vsebuje 150, 300 ali 500 mikrogramov darbepoetina alfa v 0,3 ml (500 µg/ml), 0,6 ml (500 µg/ml) ali 1 ml (500 µg/ml). **TERAPEVTSKE INDIKACIJE:** Zdravljenje simptomatske anemije pri odraslih onkoloških bolnikih z nemieloičnimi malignomi, ki dobivajo kemoterapijo. **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje z zdravilom Aranesp® mora uvesti zdravnik, ki ima izkušnje z navedeno indikacijo. Bolniki z anemijo (npr. koncentracijo hemoglobina ≤ 100 g/l (6,2 mmol/l)) morajo zdravilo Aranesp® dobiti subkutano s ciljem, da se hemoglobin zviša na ne več kot 120 g/l (7,5 mmol/l). Simptomi in posledice anemije se lahko razlikujejo glede na starost, spol in celotno breme bolezni; zdravnik mora oceniti klinični potek in stanje pri posameznem bolniku. Zaradi variabilnosti med bolniki se lahko občasno pri posameznem bolniku pojavijo vrednosti hemoglobina, ki so višje ali nižje od želene koncentracije. Variabilnosti hemoglobina se je treba prilagoditi z uravnavanjem odmerka, pri čemer je treba upoštevati ciljno območje koncentracije hemoglobina od 100 g/l (6,2 mmol/l) do 120 g/l (7,5 mmol/l). Izogniti se je treba temu, da bi bila koncentracija hemoglobina stalno večja od 120 g/l (7,5 mmol/l). Priporočeni začetni odmerek je 500 µg (6,75 µg/kg) enkrat na 3 tedne, uporabiti pa je mogoče tudi odmerjanje 2,25 µg/kg telesne mase enkrat na teden. Če bolnikov klinični odziv (utrujenost, odziv hemoglobina) po 9 tednih ni zadosten, nadaljnje zdravljenje morda ne bo učinkovito. Zdravljenje z zdravilom Aranesp® je treba končati približno 4 tedne po koncu kemoterapije. Ko je terapevtski cilj pri posameznem bolniku dosežen, je treba odmerek zmanjšati za 25 do 50% in tako zagotoviti uporabo najmanjšega odobrenega odmerka zdravila Aranesp®, potrebnega za vzdrževanje takšne koncentracije hemoglobina, ki obvladuje simptome anemije. V poštev pride ustrezno titriranje odmerka med 500 µg, 300 µg in 150 µg. Bolnike morate natančno kontrolirati. Če hemoglobin preseže 120 g/l (7,5 mmol/l), morate odmerek zmanjšati za približno 25 do 50%. Če koncentracija hemoglobina preseže 130 g/l (8,1 mmol/l), morate uporabo zdravila Aranesp® začasno prekiniti. Potem, ko se hemoglobin zniža na 120 g/l (7,5 mmol/l) ali manj, je treba terapijo znova uvesti z odmerkom, približno 25% manjšim od prejšnjega. Če se koncentracija hemoglobina v 4 tednih zviša za več kot 20 g/l (1,25 mmol/l), je odmerek treba zmanjšati za 25 do 50%. Mesto injiciranja krožno menjajte, zdravilo pa injicirajte počasi, da se boste izognili neprijetnemu občutku na mestu injiciranja. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. Slabo urejena hipertenzija. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Za izboljšanje sledljivosti zdravil za stimulacijo eritropoeze (ESA) je treba v bolnikovi dokumentaciji jasno zabeležiti trgovsko ime uporabljenega ESA. Vsem bolnikom morate kontrolirati krvni tlak, zlasti med začetkom zdravljenja z zdravilom Aranesp®. Če je krvni tlak po uvedbi ustreznih ukrepov težko obvladovati, lahko hemoglobin znižate z zmanjšanjem ali zadržanjem odmerka zdravila Aranesp®. Za zagotovitev učinkovite eritropoeze je treba vsem bolnikom pred začetkom zdravljenja in med zdravljenjem kontrolirati stanje železa; potrebna utegne biti terapija z dodatki železa. Če se bolnik na terapijo z zdravilom Aranesp® ne odzove, je treba raziskati vzročne dejavnike za to. Pomanjkanje železa, folne kisline ali vitamina B12 zmanjša učinkovitost ESA in jih je zato treba korigirati. Eritropoetski odziv lahko poslabšajo tudi sočasne okužbe, vnetja ali poškodbe, prikrito izgubljanje krvi, hemoliza, huda toksičnost aluminija, osnovne hematološke bolezni ali fibroza kostnega mozga. Določanje števila retikulocitov mora biti del ocenjevanja. Če ste izključili tipične vzroke neodzivnosti, bolnik pa ima retikulocitopenijo, pride v poštev pregled kostnega mozga. Če se izvid kostnega mozga sklada s čisto aplazijo rdečih celic, je potrebno testiranje za antieritropoetinska protitelesa. Ugotovljeno je, da ta protitelesa navzkrižno reagirajo z vsemi eritropoetičnimi beljakovinami; bolnikov, pri katerih obstaja sum ali pri katerih je potrjeno, da imajo nevtralizirajoča protitelesa proti eritropoetinu, ne smete prevesti na darbepoetin alfa. V primeru paradoksnega znižanja hemoglobina in nastanka hude anemije, ki jo spremlja majhno število retikulocitov, je treba zdravljenje z epoetinom prekiniti in opraviti testiranje antieritropoetinskih protiteles. Epoetini niso odobreni za obvladovanje anemije, povezane s hepatitisom C. Pri bolnikih z okvarjenim delovanjem jeter ni podatkov, vendar jetra veljajo za glavno pot eliminacije darbepoetina alfa in r-HuEPO, zato morate zdravilo Aranesp® pri bolnikih z boleznimi jeter uporabljati previdno. Zdravilo Aranesp® morate uporabljati previdno pri bolnikih z anemijo srpastih celic. Zloraba zdravila Aranesp® pri zdravih osebah lahko povzroči čezmerno zvečanje hematokrita. To je lahko povezano s smrtno nevarnimi zapleti na srčno-žilnem sistemu. Pokrovček igle na napolnjeni injekcijski brizgi vsebuje suho naravno gumo (derivat lateksa), ki lahko povzroči alergijske reakcije. Pri bolnikih z epilepsijo morate zdravilo Aranesp® uporabljati previdno. To zdravilo vsebuje manj kot 1 mmol (23 mg) natrija na odmerek, kar v bistvu pomeni 'brez natrija'. Vpliv na rast tumorja: Tako kot pri vseh rastnih dejavnikih tudi pri epoetinih obstaja skrb, da bi lahko spodbudili rast tumorjev. V nekaterih kliničnih primerih je treba anemijo pri bolnikih z rakom prednostno zdraviti s transfuzijo krvi. Odločitev za uporabo rekombinantnih eritropoetinov mora temeljiti na oceni koristi in tveganja in mora biti sprejeta v sodelovanju z bolnikom, upoštevaje posameznikove specifične klinične okoliščine. Med dejavniki, ki jih je treba upoštevati pri takšni oceni, so vrsta in stadij tumorja, stopnja anemije, pričakovana življenjska doba, okolje, v katerem poteka bolnikovo zdravljenje, in bolnikove želje. Če hemoglobin pri bolnikih s čvrstimi tumorji ali limfoproliferativnimi malignomi preseže 120 g/l (7,5 mmol/l), natančno upoštevajte prilagoditev odmerka, da boste zmanjšali morebitno tveganje za trombembolične incidente. V rednih presledkih je treba kontrolirati tudi število trombocitov in koncentracijo hemoglobina. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Če darbepoetin alfa dajete sočasno s snovmi, ki so v veliki meri vezane na rdeče krvne celice, npr. s ciklosporinom ali takrolimusom, je treba koncentracijo teh snovi v krvi kontrolirati in odmerke prilagajati, ko se koncentracija hemoglobina zvišuje. **NEŽELENI UČINKI:** *Povzetek varnostnega profila:* Med ugotovljenimi neželenimi učinki, povezanimi z zdravilom Aranesp®, so hipertenzija, možganska kap, trombembolični dogodki, konvulzije, alergijske reakcije, izpuščaj/eritem in čista aplazija rdečih celic (PRCA). V študijah, v katerih so zdravilo Aranesp® uporabljali subkutano, so kot o posledici zdravljenja poročali o bolečini na mestu injiciranja. Nelagodje na mestu injiciranja je bilo praviloma blago in prehodno ter se je pojavilo predvsem po prvi injekciji. *Neželeni učinki, zabeleženi pri onkoloških bolnikih v kontroliranih kliničnih študijah in postmarketinških izkušnjah, so:* zelo pogosti (≥ 1/10): preobčutljivost, edemi; pogosti (≥ 1/100 do < 1/10): hipertenzija, trombembolični dogodki, vključno s pljučno embolijo, izpuščaj/eritem, bolečina na mestu injiciranja; občasni (≥ 1/1.000 do < 1/100): konvulzije. **FARMACEVTSKI PODATKI:** Zaradi pomanjkanja študij kompatibilnosti tega zdravila ne smete mešati ali aplicirati v infuziji z drugimi zdravili. Shranjujte v hladilniku (2–8ºC). Ne zamrzujte. Napolnjeno injekcijsko brizgo shranjujte v zunanji ovojnini za zagotovitev zaščite pred svetlobo. Za ambulantno rabo je zdravilo Aranesp® mogoče vzeti iz prostora za shranjevanje enkrat za največ eno 7-dnevno obdobje pri sobni temperaturi (do 25ºC). Ko brizgo vzamete iz hladilnika in doseže sobno temperaturo (do 25ºC), jo morate uporabiti v 7 dneh, sicer jo morate zavreči. **NAČIN IN REŽIM PREDPISOVANJA TER IZDAJE ZDRAVILA:** Predpisovanje in izdaja zdravila je le na recept s posebnim režimom – H/Rp. **IMETNIK DOVOLJENJA ZA PROMET:** Amgen Europe B.V., Minervum 7061, 4817 ZK Breda, Nizozemska. **Dodatna pojasnila lahko dobite v lokalni pisarni:** Amgen zdravila d.o.o., Šmartinska 140, SI-1000 Ljubljana. **DATUM ZADNJE REVIZIJE BESEDILA:** September 2015. **DATUM PRIPRAVE INFORMACIJE:** Oktober 2016. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu/.
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AMGEN®

Aranesp®
(darbepoetin alfa)
Energija, usklajena z življenjem

Iclusig® ▼ (ponatinib)

Ključ do učinkovitega zdravljenja bolnikov s KML in Ph + ALL

Zdravilo Iclusig® je peroralni zaviralec tirozin-kinaze (TKI) za doziranje enkrat dnevno z učinkovitim delovanjem pri odraslih bolnikih s KML in Ph+ ALL[1]

ICLUSIG®
(ponatinib) tablete

Za bolnike s kronično mieloidno levkemijo (KML) v kronični, pospešeni ali blastni fazi, ki:

- so odporni na dasatinib ali nilotinib **ali**
- ne prenašajo dasatiniba ali nilotiniba in pri katerih nadaljnje zdravljenje z imatinibom ni klinično ustrezno **ali**
- imajo mutacijo T315I

Za bolnike z akutno limfoblastno levkemijo s prisotnim kromosomom Philadelphia (Ph+ ALL), ki:

- so odporni na dasatinib **ali**
- ne prenašajo dasatiniba in pri katerih nadaljnje zdravljenje z imatinibom ni klinično ustrezno **ali**
- imajo mutacijo T315I

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA
Iclusig 15 mg, 30 mg in 45 mg filmsko obložene tablete

Pred predpisovanjem natančno preberite celoten Povzetek glavnih značilnosti zdravila.
Samo za strokovno javnost.
▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

Sestava: Ena filmsko obložena tableta vsebuje 15mg, 30mg ali 45 mg ponatiniba (v obliki ponatinibijevega klorida). **Indikacije:** Zdravilo Iclusig je indicirano pri odraslih bolnikih s kronično mieloidno levkemijo (KML) v kronični fazi, pospešeni fazi ali blastni fazi, ki so odporni na dasatinib ali nilotinib; ki ne prenašajo dasatiniba ali nilotiniba in pri katerih nadaljnje zdravljenje z imatinibom ni klinično ustrezno; ali ki imajo mutacijo T315I ter pri odraslih bolnikih z akutno limfoblastno levkemijo s prisotnim kromosomom Philadelphia (Ph+ ALL), ki so odporni na dasatinib; ki ne prenašajo dasatiniba in pri katerih nadaljnje zdravljenje z imatinibom ni klinično ustrezno; ali ki imajo mutacijo T315I. **Odmerjanje in način uporabe:** Terapijo mora uvesti zdravnik z izkušnjami v diagnosticiranju in zdravljenju bolnikov z levkemijo. Med zdravljenjem se lahko bolniku nudi hematološka podpora, če je to klinično indicirano. Pred začetkom zdravljenja s ponatinibom je treba oceniti kardiovaskularni status bolnika, vključno z anamnezo in telesnim pregledom, in aktivno obravnavati kardiovaskularne dejavnike tveganja. Kardiovaskularni status je treba še naprej spremljati in med zdravljenjem s ponatinibom optimizirati zdravljenje z zdravili in podporno zdravljenje stanj, ki prispevajo h kardiovaskularnim tveganjem.
Odmerjanje: Priporočeni začetni odmerek ponatiniba je 45 mg enkrat na dan. Potrebno je razmisliti o ukinitvi ponatiniba, če v 3 mesecih ni celovitega hematološkega odgovora. Z zdravljenjem je treba prenehati, če se pojavijo znaki napredovanja bolezni ali v primeru hudih neželenih učinkov. Prilagoditev odmerjanja: tveganje za žilni okluzivni dogodek je verjetno povezano z odmerkom. Zdravljenje z zdravilom Iclusig je treba pri sumu, da se je pri bolniku razvil arterijski ali venski okluzivni dogodek, takoj prekiniti. Ko se dogodek razreši, je treba pri odločitvi o ponovni uvedbi zdravljenja upoštevati oceno koristi in tveganj. Pri obravnavi hematoloških in nehematoloških toksičnosti je treba razmisliti o prilagoditvi ali prekinitvi odmerjanja. V primeru hudih neželenih učinkov je treba z zdravljenjem prekiniti. Prilagajanje odmerka je priporočljivo v primeru nevtropenije ali trombocitopenije, ki nista povezani z levkemijo, pri pankreatitisu in zvišani ravni lipaze/amilaze. Način uporabe: tablete je treba pogoltniti cele, ne sme se jih drobiti ali raztapljati, lahko pa se jih jemlje s hrano ali brez nje. **Kontraindikacije:** Preobčutljivost na ponatinib ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** *Mielosupresija* – Zdravilo Iclusig je povezano s hudo trombocitopenijo, nevtropenijo in anemijo. Prve 3 mesece je treba vsaka 2 tedna opraviti pregled celotne krvne slike, nato pa mesečno ali kot je klinično indicirano. *Žilna okluzija* – Pojavili so se arterijska in venska tromboza in okluzija, vključno s smrtnim miokardnim infarktom, možgansko kapjo, retinalna žilna okluzija, v nekaterih primerih povezana s trajno okvaro vida ali slepoto, stenozo velikih arterijskih žil v možganih, hudo periferno žilno boleznijo in potrebo po nujnem postopku revaskularizacije.

Zdravila Iclusig se ne sme uporabljati pri bolnikih z miokardnim infarktom, predhodno revaskularizacijo ali možgansko kapjo v anamnezi, razen če so možne koristi zdravljenja večje od možnih tveganj. Med zdravljenjem s ponatinibom je treba spremljati znake trombembolije in žilne okluzije in zdravljenje je treba takoj prekiniti, če se pojavi žilna okluzija. V primeru, da se pojavi poslabšanje vida ali zamegljen vid, je treba opraviti oftalmološki pregled (vključno s fundoskopijo). *Hipertenzija* - Pri zdravljenju z zdravilom Iclusig, se je pojavila z zdravljenjem povezana hipertenzija (vključno s hipertenzivno krizo), ki lahko prispeva k tveganju arterijskih trombotičnih dogodkov. Zato je treba ob vsakem obisku zdravnika spremljati krvni tlak. Zdravljenje z zdravilom Iclusig je treba prekiniti, če hipertenzija ni pod zdravniškim nadzorom. *Kongestivno srčno popuščanje* – Pojavilo se je smrtno in resno srčno popuščanje ter dogodki, povezani s predhodnimi vaskularnimi okluzivnimi dogodki. Bolnike je treba spremljati in jih zdraviti, kot je klinično ustrezno, vključno s prekinitvijo zdravljenja z zdravilom Iclusig. Pri bolnikih, pri katerih se razvije resno srčno popuščanje, je treba razmisliti o ukinitvi ponatiniba. *Pankreatitis in serumska lipaza* – Pogostnost pojava pankreatitisa je večja prva 2 meseca uporabe. Prva 2 meseca vsaka 2 tedna preverjajte serumsko lipazo, nato pa periodično. Morda bo treba odmerek prekiniti ali zmanjšati. Če zvišanje ravni lipaz spremljajo abdominalni simptomi, je treba z uporabo zdravila Iclusig prenehati in preveriti, ali ima bolnik pankreatitis. Pri bolnikih s pankreatitisom ali zlorabo alkohola v anamnezi se priporoča previdnost. Bolnike s hudo ali zelo hudo hipertrigliceridemijo je treba ustrezno obravnavati. *Laktoza* - Zdravilo Iclusig vsebuje laktozo monohidrat. Bolniki z redkimi dednimi težavami neprenašanja galaktoze, laponsko obliko zmanjšane aktivnosti laktaze ali slabo absorpcijo glukoze-galaktoze ne smejo jemati tega zdravila. *Podaljšanje intervala QT* - Klinično pomembnih učinkov na interval QT ni mogoče izključiti. *Hepatotoksičnost* – Lahko se zvišajo ravni ALT, AST, bilirubina in alkalne fosfataze. Opazili so jetrno odpoved (vključno s smrtnim izidom). Teste delovanja jeter je treba opraviti pred uvedbo zdravljenja in nato periodično, kot je klinično indicirano. *Krvavitev* - Pojavili so se smrtni ter resni hemoragični dogodki. Pri resni ali hudi krvavitvi je treba zdravljenje z zdravilom Iclusig prekiniti. *Okvara jeter* - Pri bolnikih s hudo okvaro jeter se priporoča previdnost. *Okvara ledvic* - Pri bolnikih z ocenjenim očistkom kreatinina < 50 ml/min ali ledvično boleznijo v zadnjem stadiju se priporoča previdnost. *Starejši bolniki* - Verjetnost neželenih učinkov je večja. *Pediatrična populacija* - Varnost in učinkovitost zdravila Iclusig pri bolnikih, starih do 18 let, še nista bili dokazani. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Sočasni uporabi zdravila Iclusig z močnimi induktorji CYP3A4 se je treba izogniti; pri sočasni uporabi močnih zaviralcev CYP3A je potrebna previdnost, razmisliti pa je treba tudi o uporabi zdravila Iclusig z začetnim odmerkom 30 mg; potrebna je previdnost pri sočasno uporabljenih substratih P-glikoproteina (P-gp) ali beljakovine rezistence za raka dojke (BCRP). Pri sočasni uporabi ponatiniba z zdravili proti strjevanju krvi pri bolnikih, pri katerih obstaja tveganje za krvavitev, je potrebna previdnost. **Plodnost, nosečnost in dojenje:** Ženskam v rodni dobi je treba svetovati, da naj v času zdravljenja z zdravilom Iclusig ne zanosijo, moškim pa, da naj v času zdravljenja ne zaplodijo otroka. Med zdravljenjem je treba uporabljati alternativno ali dodatno metodo kontracepcije. Ni zadostnih podatkov o uporabi zdravila Iclusig pri nosečnicah. Študije na živalih so pokazale vpliv na sposobnost razmnoževanja. Če se zdravilo uporablja med nosečnostjo, je treba bolnico obvestiti o možnem tveganju za plod. Z dojenjem je treba med zdravljenjem z zdravilom Iclusig prenehati. **Vpliv na sposobnost vožnje in upravljanja s stroji:** Pri vožnji ali upravljanju strojev je potrebna previdnost. **Neželeni učinki:** Zelo pogosti *(≥ 1/10)*: okužba zgornjih dihal, nespečnost, anemija, zmanjšanje števila trombocitov, zmanjšanje števila nevtrofilcev, zmanjšan apetit, glavobol, omotica, hipertenzija, dispneja, kašelj, bolečine v trebuhu, driska, bruhanje, zaprtje, navzea, zvišanje ravni lipaz, zvišanje ravni alanin aminotransferaze, zvišanje ravni aspartat-aminotransferaze, izpuščaj, suha koža, bolečine v kosteh, artralgija, mialgija, bolečine v okončinah, bolečine v hrbtu, mišični krči, utrujenost, astenija, periferni edem, pireksija, bolečine. Pogosti *(≥ 1/100 do < 1/10)*: pljučnica, sepsa, folikulitis, pancitopenija, febrilna nevtropenija, zmanjšanje števila levkocitov, dehidracija, zastajanje tekočine, hipokalciemija, hiperglikemija, hiperurikemija, hipofosfatemija, hipertrigliceridemija, hipokaliemija, zmanjšanje telesne mase, cerebrovaskularni dogodek, cerebralni infarkt, periferna nevropatija, letargija, migrena, hiperestezija, hipoestezija, parestezija, prehodni ishemični napad, zamegljen vid, suhe oči, periorbitalni edem, edem veke, srčno popuščanje, miokardni infarkt, kongestivno srčno popuščanje, bolezen koronarnih arterij, angina pektoris, perikardni izliv, atrijska fibrilacija, zmanjšanje iztisnega deleža, periferna arterijska okluzivna bolezen, periferna ishemija, stenoza periferne arterije, intermitentna klavdikacija, globoka venska tromboza, vročinski oblivi, zariplost, pljučna embolija, plevralni izliv, epistaksa, disfonija, pljučna hipertenzija, pankreatitis, zvišanje amilaz v krvi, gastroezofagealna refluksna bolezen, stomatitis, dispepsija, trebušna distenzija, nelagodje v trebuhu, suha usta, zvišanje ravni bilirubina v krvi, zvišanje ravni alkalne fosfataze v krvi, zvišanje ravni gama-glutamiltransferaze, pruritični izpuščaj, eksfolativni izpuščaj, eritem, alopecija, pruritis, eksfoliacija kože, nočno potenje, hiperhidroza, petehija, ekhimoza, boleča koža, eksfoliativni dermatitis, mišično-skeletne bolečine, bolečine v vratu, mišično-skeletne bolečine v prsnem košu, erektilna disfunkcija, mrzlica, gripi podobna bolezen, nekardiogena bolečina v prsnem košu, tipljiv vozlič, obrazni edem. Občasni *(≥ 1/1000 do < 1/100)*: sindrom tumorske lize, cerebralna arterijska stenoza, tromboza mrežnične vene, okluzija mrežnične vene, okluzija mrežnične arterije, okvara vida, miokardna ishemija, akutni koronarni sindrom, kardialno nelagodje, ishemična kardiomiopatija, spazem koronarnih arterij, disfunkcija levega prekata, atrijska undulacija, slaba periferna cirkulacija, vranični infarkt, venska embolija, venska tromboza, hipertenzivna kriza, krvavitev v želodcu, hepatotoksičnost, odpoved jeter, zlatenica. **Režim izdaje zdravila:** Predpisovanje in izdaja zdravila je le na recept. **Imetnik dovoljenja za promet z zdravilom:** ARIAD Pharma Ltd., Riverbridge House, Guildford Road, Leatherhead, Surrey KT22 9AD, Velika Britanija. **Zadnja revizija besedila:** marec 2016. **Informacija pripravljena:** april 2016.
Podrobnejše informacije o zdravilu Iclusig so na voljo pri predstavniku imetnika dovoljenja za promet z zdravilom: Angelini Pharma d.o.o., Koprska ulica 108A, 1000 Ljubljana, Tel.: +386 1 544 65 79, E-pošta: info@angelini.si

Iclusig- oglas- 01/jun- 2016

ANGELINI

Predstavnik:
Angelini Pharma d.o.o.
Koprska ulica 108 A, Ljubljana

PRVA REGISTRIRANA TERAPIJA V 2. LINIJI ZA ZDRAVLJENJE ADENOKARCINOMA ŽELODCA ALI GASTRO-EZOFAGEALNEGA PREHODA[1]

CYRAMZA™ (ramucirumab)

UKREPAJTE ZDAJ

USPOSOBLJENI ZA SPREMEMBE, ZA NEPRIMERLJIVE IZKUŠNJE

Skrajšan povzetek glavnih značilnosti zdravila

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila.

Cyramza 10 mg/ml koncentrat za raztopino za infundiranje

En mililiter koncentrata za raztopino za infundiranje vsebuje 10 mg ramucirumaba. Ena 10-mililitrska viala vsebuje 100 mg ramucirumaba. **Terapevtske indikacije** Zdravilo Cyramza je v kombinaciji s paklitakselom indicirano za zdravljenje odraslih bolnikov z napredovalim rakom želodca ali adenokarcinomom gastro-ezofagealnega prehoda z napredovalo boleznijo po predhodni kemoterapiji, ki je vključevala platino in fluoropirimidin. Monoterapija z zdravilom Cyramza je indicirana za zdravljenje odraslih bolnikov z napredovalim rakom želodca ali adenokarcinomom gastro-ezofagealnega prehoda z napredovalo boleznijo po predhodni kemoterapiji s platino ali fluoropirimidinom, za katere zdravljenje v kombinaciji s paklitakselom ni primerno. Zdravilo Cyramza je v kombinaciji s shemo FOLFIRI indicirano za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mCRC), z napredovanjem bolezni ob ali po predhodnem zdravljenju z bevacizumabom, oksaliplatinom in fluoropirimidinom. Zdravilo Cyramza je v kombinaciji z docetakselom indicirano za zdravljenje odraslih bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim rakom, z napredovanjem bolezni po kemoterapiji na osnovi platine. **Odmerjanje in način uporabe** Zdravljenje z ramucirumabom morajo uvesti in nadzirati zdravniki z izkušnjami v onkologiji. Odmerjanje *Rak želodca in adenokarcinom gastro-ezofagealnega prehoda* Priporočeni odmerek ramucirumaba je 8 mg/kg 1. in 15. dan 28-dnevnega cikla, pred infuzijo paklitaksela. Priporočeni odmerek paklitaksela je 80 mg/m^2 in se daje z intravenskim infundiranjem, ki traja približno 60 minut, 1., 8. in 15. dan 28-dnevnega cikla. Pred vsakim infundiranjem paklitaksela je treba pri bolnikih pregledati celotno krvno sliko in izvide kemičnih preiskav krvi, da se oceni delovanje jeter. Priporočeni odmerek ramucirumaba kot monoterapije je 8 mg/kg vsaka 2 tedna. *Kolorektalni rak* Priporočeni odmerek ramucirumaba je 8 mg/kg vsaka 2 tedna, dan z intravensko infuzijo pred dajanjem sheme FOLFIRI. Pred kemoterapijo je treba bolnikom odvzeti kri za popolno krvno sliko. *Nedrobnocelični pljučni rak (NSCLC)* Priporočeni odmerek ramucirumaba je 10 mg/kg na 1. dan 21-dnevnega cikla, pred infuzijo docetaksela. Priporočeni odmerek docetaksela je 75 mg/m^2, dan z intravensko infuzijo v približno 60 minutah na 1. dan 21-dnevnega cikla. *Premedikacija* Pred infundiranjem ramucirumaba je priporočljiva premedikacija z antagonistom histaminskih receptorjev H1. Način uporabe Po redčenju se zdravilo Cyramza daje kot intravenska infuzija v približno 60 minutah. Zdravila ne dajajte v obliki intravenskega bolusa ali hitre intravenske injekcije. Da boste dosegli zahtevano trajanje infundiranja približno 60 minut, največja hitrost infundiranja ne sme preseči 25 mg/minuto, saj morate sicer podaljšati trajanje infundiranja. Bolnika je med infundiranjem treba spremljati glede znakov reakcij, povezanih z infuzijo, zagotoviti pa je treba tudi razpoložljivost ustrezne opreme za oživljanje. **Kontraindikacije** Pri bolnikih z NSCLC je ramucirumab kontraindiciran, kjer gre za kavitacijo tumorja ali prepletenost tumorja z glavnimi žilami. **Posebna opozorila in previdnostni ukrepi** Trajno prekinite zdravljenje z ramucirumabom pri bolnikih, pri katerih se pojavijo resni arterijski trombembolični dogodki, gastrointestinalne perforacije, krvavitev stopnje 3 ali 4, če zdravstveno pomembne hipertenzije ni mogoče nadzirati z antihipertenzivnim zdravljenjem ali če se pojavi fistula, raven beljakovin v urinu > 3 g/24 ur ali v primeru nefrotskega sindroma. Pri bolnikih z neuravnano hipertenzijo zdravljenja z ramucirumabom ne smete uvesti, dokler oziroma v kolikor obstoječa hipertenzija ni uravnana. Pri bolnikih s ploščatocelično histologijo obstaja večje tveganje za razvoj resnih pljučnih krvavitev. Če se pri bolniku med zdravljenjem razvijejo zapleti v zvezi s celjenjem rane, prekinite zdravljenje z ramucirumabom, dokler rana ni povsem zaceljena. V primeru pojava stomatitisa je treba takoj uvesti simptomatsko zdravljenje. Pri bolnikih, ki so prejemali ramucirumab in docetaksel za zdravljenje napredovalega NSCLC z napredovanjem bolezni po kemoterapiji na osnovi platine, so opazili trend manjše učinkovitosti z naraščajočo starostjo. **Plodnost, nosečnost in dojenje** Ženskam v rodni dobi je treba svetovati, naj se izogneje zanositvi med zdravljenjem z zdravilom Cyramza in jih je treba seznaniti z možnim tveganjem za nosečnost in plod. Ni znano, ali se ramucirumab izloča v materino mleko. **Neželeni učinki** *Zelo pogosti (≥ 1/10)* nevtropenija, levkopenija, trombocitopenija, hipoalbuminemija, hipertenzija, epistaksa, gastrointestinalne krvavitve, stomatitis, driska, proteinurija, utrujenost/astenija, periferni edem, bolečina v trebuhu. *Pogosti (≥ 1/100 do < 1/10)* hipokaliemija, hiponatriemija, glavobol. **Rok uporabnosti** 3 leta **Posebna navodila za shranjevanje** Shranjujte v hladilniku (2 °C–8 °C). Ne zamrzujte. Vialo shranjujte v zunanji ovojnini, da zagotovite zaščito pred svetlobo. **Pakiranje** 2 viali z 10 ml **IMETNIK DOVOLJENJA ZA PROMET Z ZDRAVILOM** Eli Lilly Nederland B.V., Papendorpseweg 83, 3528 BJ Utrecht, Nizozemska **DATUM ZADNJE REVIZIJE BESEDILA** 25.01.2016

Režim izdaje: Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah.

Pomembno obvestilo:

Pričujoče gradivo je namenjeno **samo za strokovno javnost**. Zdravilo Cyramza se izdaja le na recept. Pred predpisovanjem zdravila Cyramza vas vljudno prosimo, da preberete celotni Povzetek glavnih značilnosti zdravila Cyramza. Podrobnejše informacije o zdravilu Cyramza in o zadnji reviziji besedila Povzetka glavnih značilnosti zdravila so na voljo na sedežu podjetja Eli Lilly (naslov podjetja in kontaktni podatki spodaj) in na spletni strani European Medicines Agency (EMA): www.ema.europa.eu. in na spletni strani European Commission http://ec.europa.eu/health/documents/community-register/html/alfregister.htm.

Eli Lilly farmacevtska družba, d.o.o., Dunajska cesta 167, 1000 Ljubljana, telefon: (01) 5800 010, faks: (01) 5691 705

Referenca: 1. Cyramza, Povzetek glavnih značilnosti zdravila, zadnja odobrena verzija.

EERAM00010a, 12.02.2016.

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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 cetuksimaba 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 RAS 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 zdravljenje na osnovi irinotekana ni bilo uspešno in 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 najmanj 1 uro pred uporabo cetuksimaba. 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 RAS ali kadar status RAS ni znan. **Posebna opozorila in previdnostni ukrepi:** Pojav hude reakcije, povezane z infundiranjem, zahteva takojšnjo in stalno ukinitev terapije s cetuksimabom. 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 kožna reakcija, ki je ne more prenašati, ali huda kožna reakcija (≥ 3. stopnje po kriterijih CTCAE), morate prekiniti terapijo s cetuksimabom. Z zdravljenjem smete nadaljevati le, če se je reakcija izboljšala do 2. stopnje. Če ugotovite intersticijsko bolezen pljuč, morate zdravljenje s cetuksimabom prekiniti, in bolnika ustrezno zdraviti. Zaradi možnosti pojava znižanja nivoja elektrolitov v serumu se pred in periodično med zdravljenjem s cetuksimabom priporoča določanje koncentracije elektrolitov v serumu. Pri bolnikih, ki prejemajo cetuksimab v kombinaciji s kemoterapijo na osnovi platine, obstaja večje tveganje za pojav hude nevtropenije. Takšne bolnike je potrebno skrbno nadzorovati. Pri predpisovanju cetuksimaba je treba upoštevati kardiovaskularno stanje in indeks zmogljivosti bolnika in sočasno dajanje kardiotoksičnih učinkovin kot so fluoropirimidini. Če je diagnoza ulcerativnega keratitisa potrjena, je treba zdravljenje s cetuksimabom prekiniti ali ukiniti. Cetuksimab je treba uporabljati previdno pri bolnikih z anamnezo keratitisa, ulcerativnega keratitisa ali zelo suhih oči. Cetuksimaba ne uporabljajte za zdravljenje bolnikov s kolorektalnim rakom, če imajo tumorje z mutacijo RAS ali pri katerih je tumorski status RAS neznan. **Interakcije:** Pri kombinaciji s fluoropirimidini se je v primerjavi z uporabo fluoropirimidinov, kot monoterapije, 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. V kombinaciji s kapecitabinom in oksaliplatinom (XELOX) se lahko poveča pogostnost hude driske. **Neželeni učinki:** Zelo pogosti (≥ 1/10): hipomagneziemija, povečanje ravni jetrnih encimov, kožne reakcije, blage ali zmerne reakcije povezane z infundiranjem, mukozitis, v nekaterih primerih resen. Pogosti (≥ 1/100 do < 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:** Izdaja zdravila je le na recept-H. **Imetnik dovoljenja za promet:** Merck KGaA, 64271 Darmstadt, Nemčija.

Datum zadnje revizije besedila: november 2014.

Pred predpisovanjem zdravila natančno preberite celoten Povzetek glavnih značilnosti zdravila.

Samo za strokovno javnost.

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

www.merckserono.net
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Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila. Kako poročati o neželenih učinkih, si poglejte skrajšani povzetek glavnih značilnosti zdravila pod "Poročanje o domnevnih neželenih učinkih".

Ime zdravila: Cotellic 20 mg filmsko obložene tablete. **Kakovostna in količinska sestava:** Ena filmsko obložena tableta vsebuje kobimetinibijev hemifumarat, kolikor ga ustreza 20 mg kobimetiniba. Pomožna snov z znanim učinkom: Ena filmsko obložena tableta vsebuje 36 mg laktoze monohidrata. **Terapevtske indikacije:** Zdravilo Cotellic je v kombinaciji z vemurafenibom indicirano za zdravljenje odraslih bolnikov z neoperabilnim ali metastatskim melanomom, ki ima mutacijo BRAF V600. **Odmerjanje in način uporabe:** Zdravljenje z zdravilom Cotellic v kombinaciji z vemurafenibom sme uvesti in nadzorovati le usposobljen zdravnik, ki ima izkušnje z uporabo zdravil proti raku. Pred začetkom zdravljenja je treba z validirano preiskavo potrditi, da ima bolnik melanom z mutacijo BRAF V600. Odmerjanje: Vsak odmerek obsega tri 20-mg tablete (60 mg) in ga je treba vzeti enkrat na dan 21 dni zapored, temu sledi 7-dnevni premor. Zdravljenje z zdravilom Cotellic je treba nadaljevati, dokler bolniku ne koristi več oziroma do pojava nesprejemljive toksičnosti. Če bolnik izpusti odmerek, ga lahko vzame do 12 ur pred naslednjim odmerkom, da ohrani shemo enkrat na dan. Če bolnik po uporabi zdravila Cotellic bruha, tisti dan ne sme vzeti dodatnega odmerka, temveč mora zdravljenje nadaljevati naslednji dan, kot je predpisano. *Splošne prilagoditve odmerka:* Odločitev za zmanjšanje odmerka zdravila Cotellic ali vemurafeniba mora temeljiti na zdravnikovi oceni varnosti in prenašanja pri posameznem bolniku. Ko je bil odmerek enkrat zmanjšan, se ga kasneje ne sme več povečati. *Nasvet za prilagoditev odmerka v primeru disfunkcije levega prekata:* Če so srčni simptomi posledica zdravila Cotellic in se po prehodni prekinitvi njegove uporabe ne izboljšajo, je treba razmisliti o trajnem prenehanju zdravljenja z zdravilom Cotellic. *Nasvet za prilagoditev odmerka zdravila Cotellic med uporabo z vemurafenibom: Jetrna laboratorijska odstopanja:* Stopnja 3: Zdravilo Cotellic je treba nadaljevati v predpisanem odmerku. Odmerek vemurafeniba je mogoče zmanjšati, kot je klinično primerno. Stopnja 4: Zdravljenje z zdravilom Cotellic in zdravljenje z vemurafenibom je treba prekiniti. Za dodatna navodila glejte celotni povzetek glavnih značilnosti zdravila Cotellic. *Zvišanja kreatin-fosfokinaze (CPK):* Za obvladovanje nesimptomatskih zvišanj CPK odmerka zdravila Cotellic ni treba prilagoditi ali prekiniti. *Fotosenzibilnost:* Fotosenzibilnost stopnje ≤ 2 je treba obvladovati s podpornim zdravljenjem. Fotosenzibilnost stopnje 2 ali stopnje ≥ 3: zdravili Cotellic in vemurafenib je treba prekiniti, dokler se ne zmanjša na stopnjo ≤ 1. Zdravljenje je mogoče znova začeti brez spremembe odmerka zdravila Cotellic. Odmerjanje vemurafeniba je treba zmanjšati, kot je klinično primerno; za dodatne informacije glejte povzetek glavnih značilnosti vemurafeniba. *Izpuščaj:* Izpuščaj se lahko pojavi tako med zdravljenjem z zdravilom Cotellic kot med zdravljenjem z vemurafenibom. Odmerek zdravila Cotellic in/ali vemurafeniba je mogoče začasno prekiniti in/ali zmanjšati, kot je klinično primerno. Za dodatna navodila glejte celotni povzetek glavnih značilnosti zdravila Cotellic. *Podaljšanje intervala QT:* Če interval QTc med zdravljenjem preseže 500 ms, prosimo, glejte povzetek glavnih značilnosti vemurafeniba. Spremenba odmerkov zdravila Cotellic ni potrebna, kadar se ta uporablja v kombinaciji z vemurafenibom. Posebne populacije bolnikov: Bolnikom, starim ≥ 65 let, odmerka ni treba prilagoditi. Pri bolnikih s hudo okvaro ledvic je treba zdravilo Cotellic uporabljati previdno. Bolnikom z okvaro jeter odmerka ni treba prilagoditi. Bolniki s hudo okvaro jeter imajo lahko v primerjavi z bolniki z normalnim delovanjem jeter zvišane koncentracije prostega kobimetiniba v plazmi. Pri zdravljenju z zdravilom Cotellic se lahko pojavijo z jetri povezane laboratorijske nepravilnosti, zato je treba pri bolnikih z okvaro jeter katere koli stopnje zdravilo uporabljati previdno.Varnost in učinkovitost zdravila Cotellic pri otrocih in mladostnikih, mlajših od 18 let, nista ugotovljeni. Način uporabe: Zdravilo Cotellic je za peroralno uporabo. Tablete je treba zaužiti cele, z vodo. Lahko se jemljejo skupaj s hrano ali brez nje. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Zdravilo Cotellic v kombinaciji z vemurafenibom pri bolnikih, katerih bolezen je napredovala med zdravljenjem z zaviralcem BRAF: Podatkov pri teh bolnikih je malo. Ti kažejo, da je učinkovitost kombinacije pri teh bolnikih manjša. Zato je treba razmisliti o drugih možnostih zdravljenja pred uvedbo kombinacije. Zdravilo Cotellic v kombinaciji z vemurafenibom pri bolnikih z zastavki v možganih: Varnost in učinkovitost kombinacije zdravila Cotellic in vemurafeniba pri teh bolnikih ni bila ocenjena. Intrakranialna aktivnost kobimetiniba trenutno ni poznana. Serozna retinopatija: Pri bolnikih, zdravljenih z zaviralci MEK, vključno z zdravilom Cotellic, so opažali serozno retinopatijo. Večina primerov je bila opisana kot hiporetinopatija ali odstop mrežnice. Večina dogodkov, opaženih v kliničnih preskušanjih, je po prekinitvi ali zmanjšanju odmerka izzvenela ali se izboljšala na nesimptomatsko stopnjo 1. Bolnike je treba na vsakem pregledu oceniti glede simptomov novih motenj vida ali poslabšanja obstoječih motenj vida. V primeru simptomov novih motenj vida ali poslabšanju obstoječih je priporočljiv oftalmološki pregled. Serozno retinopatijo je mogoče obvladati s prekinitvijo zdravljenja, zmanjšanjem odmerka ali prenehanjem zdravljenja. Disfunkcija levega prekata: Pri bolnikih, ki so prejemali zdravilo Cotellic, so poročali o zmanjšanju iztisnega deleža levega prekata (LVEF) v primerjavi z izhodiščem. LVEF je treba izmeriti pred začetkom zdravljenja za določitev izhodiščne vrednosti, nato pa ga kontrolirati po prvem mesecu zdravljenja ter vsaj na 3 mesece oziroma kot je klinično indicirano, do prenehanja zdravljenja. Zmanjšanje LVEF od izhodišča je mogoče obvladati s prekinitvijo zdravljenja, zmanjšanjem odmerka ali prenehanjem zdravljenja. Bolnikov z izhodiščnim LVEF pod spodnjo mejo normalne vrednosti (SMN) za ustanovo ali pod 50 % niso proučevali. Jetrna laboratorijska odstopanja: Jetrna laboratorijska odstopanja se lahko pojavijo tako med uporabo zdravila Cotellic v kombinaciji z vemurafenibom kot med samostojnim zdravljenjem z vemurafenibom. Pri bolnikih, zdravljenih s kombinacijo zdravila Cotellic in vemurafeniba, so opažali jetrna laboratorijska odstopanja, zlasti zvišanje ALT, AST in AF. Jetrna laboratorijska odstopanja je treba kontrolirati z laboratorijskimi preiskavami jeter pred začetkom kombiniranega zdravljenja in vsak mesec med zdravljenjem, lahko pa tudi pogosteje, če je klinično indicirano. Laboratorijska odstopanja stopnje 3 je treba obvladati s prekinitvijo uporabe vemurafeniba ali zmanjšanjem odmerka. Jetrna laboratorijska odstopanja stopnje 4 se obvlada s prekinitvijo zdravljenja, zmanjšanjem odmerka ali prenehanjem zdravljenja z zdravilom Cotellic in z vemurafenibom. Driska: Pri bolnikih, zdravljenih z zdravilom Cotellic, so poročali o primerih driske stopnje ≥ 3 in resni driski. Drisko je treba obvladati z antidiaroiki in podpornim zdravljenjem. V primeru driske stopnje ≥ 3, ki se pojavi kljub podpornemu zdravljenju, je treba obe zdravili, Cotellic in vemurafenib, prekiniti, dokler se driska ne izboljša na stopnjo ≤ 1. Če se driska stopnje ≥ 3 ponovi, je treba odmerek zdravila Cotellic in vemurafeniba zmanjšati. Intoleranca za laktozo: Zdravilo vsebuje laktozo. Bolniki z redko dedno intoleranco za galaktozo, laponsko obliko pomanjkanja laktaze ali malabsorpcijo glukoze/galaktoze se morajo posvetovati z zdravnikom in se z njim pogovoriti, ali zanje koristi zdravljenja odtehtajo tveganje. Podaljšanje intervala QT: Če interval QTc med zdravljenjem preseže 500 ms, prosimo, glejte povzetka glavnih značilnosti vemurafeniba. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Učinki drugih zdravil na kobimetinib: *Zaviralci CYP3A:* Med zdravljenjem s kobimetinibom se izogibajte sočasni uporabi močnih zaviralcev CYP3A. Če se sočasni uporabi močnega zaviralca CYP3A ni mogoče izogniti, je treba bolnike skrbno nadzirati glede varnosti. Previdnost je potrebna, če se kobimetinib uporablja sočasno z zmernimi zaviralci CYP3A. Če se kobimetinib uporablja sočasno z zmernim zaviralcem CYP3A, je treba bolnike skrbno nadzirati glede varnosti. *Induktorji CYP3A:* Treba se je izogibati sočasni uporabi z zmernimi in močnimi induktorji CYP3A. Razmisliti je treba o uporabi drugih zdravil, ki CYP3A inducirajo le malo ali sploh ne. Ker se med sočasno uporabo zmernih do močnih induktorjev CYP3A koncentracija kobimetiniba verjetno bistveno zniža, se lahko njegova učinkovitost pri bolniku poslabša. *Zaviralci P-glikoproteina:* Sočasna uporaba zaviralcev P-gp, npr. ciklosporina in verapamila, lahko zviša koncentracijo kobimetiniba v plazmi. Učinki kobimetiniba na druga zdravila: *Substrati CYP3A in CYP2D6:* Klinična študija medsebojnega delovanja zdravil pri onkološkib bolnikih je pokazala, da se koncentracij midazolama in dekstrometorfana v plazmi v prisotnosti kobimetiniba nista spremenili. *Substrati CYP1A2: In vitro* je kobimetinib potencialen induktor CYP1A2 in lahko zato zmanjša izpostavljenost substratom tega encima, npr. teofilinu. *Substrati BCRP: In vitro* kobimetinib zmerno zavira BCRP. Klinično pomembnega zavrtja BCRP na ravni črevesja ni mogoče izključiti. Druga zdravila proti raku: *vemurafenib:* Pri bolnikih z neoperabilnim ali metastatskim melanomom ni dokazov o klinično pomembnem medsebojnem delovanju med kobimetinibom in vemurafenibom, zato prilagoditve odmerkov niso potrebne. Vpliv kobimetiniba na transportne sisteme zdravil: Študije *in vitro* kažejo, da kobimetinib ni substrat jetrnih privzemnih prenašalcev OATP1B1, OATP1B3 in OCT1, vendar pa jih rahlo zavira. Klinični pomen teh izsledkov ni raziskan. Pediatrična populacija: Študije medsebojnega delovanja so izvedli le pri odraslih. *Neželeni učinki:* Neželeni učinki pri bolnikih, zdravljenih z zdravilom Cotellic v kombinaciji z vemurafenibom: *Zelo pogosti:* anemija, serozna retinopatija, zamegljen vid, hipertenzija, krvavitev, driska, navzea, bruhanje, fotosenzibilnost, izpuščaj, makulopapulozen izpuščaj, akneiformni dermatitis, hiperkeratoza, zvišana telesna temperatura, mrzlica, zvišanja CPK, ALT, AST, gama-glutamiltransferaze (GGT) in AF v krvi. *Pogosti:* bazalnocelični karcinom, ploščatocelični karcinom kože, keratoakantom, dehidracija, hipofosfatemija, hiponatriemija, hiperglikemija, okvara vida, pnevmonitis, zmanjšan iztisni delež in zvišanje bilirubina v krvi. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** Rp/Spec **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija **Verzija: 2.0/16 Informacija pripravljena:** september 2016

Skrajšan povzetek glavnih značilnosti zdravila ZELBORAF:

Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila. Kako poročati o neželenih učinkih, si poglejte skrajšani povzetek glavnih značilnosti zdravila pod "Poročanje o domnevnih neželenih učinkih".

Ime zdravila: Zelboraf 240 mg filmsko obložene tablete. **Kakovostna in količinska sestava:** Ena tableta vsebuje 240 mg vemurafeniba (v obliki precipitata vemurafeniba in hipromeloze acetat sukcinata). **Terapevtske indikacije:** Vemurafenib je indiciran za samostojno zdravljenje odraslih bolnikov z neresektabilnim ali metastatskim melanomom, s pozitivno mutacijo BRAF V600. **Odmerjanje in način uporabe:** Zdravljenje z vemurafenibom mora uvesti in nadzorovati usposobljen zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje raka. Odmerjanje: Priporočeni odmerek vemurafeniba je 960 mg (4 tablete po 240 mg) dvakrat na dan (to ustreza celotnemu dnevnemu odmerku 1920 mg). Vemurafenib lahko vzamemo s hrano ali brez nje, izogibati pa se moramo stalnemu jemanju obeh dnevnih odmerkov na prazen želodec. Zdravljenje z vemurafenibom moramo nadaljevati do napredovanja bolezni ali pojava nesprejemljive toksičnosti. Če bolnik izpusti odmerek, ga lahko vzame do 4 ure pred naslednjim odmerkom za ohranitev sheme dvakrat na dan. Obeh odmerkov pa ne sme vzeti hkrati. Če bolnik po zaužitju vemurafeniba bruha, ne sme vzeti dodatnega odmerka zdravila, ampak mora z zdravljenjem normalno nadaljevati. Prilagoditve odmerjanja: Za obvladovanje neželenih učinkov ali ob podaljšanju intervala QTc je potrebno zmanjšanje odmerka, začasna prekinitev in/ali dokončno prenehanje zdravljenja (za podrobnosti o prilagoditvi odmerka, prosimo glejte SmPC zdravila). Zmanjšanje odmerka pod 480 mg dvakrat na dan ni priporočljivo. Če se pri bolniku pojavi ploščatocelični karcinom kože, priporočamo nadaljevanje zdravljenja brez zmanjšanja odmerka vemurafeniba. *Posebne populacije:* Za bolnike, starejše od 65 let, prilagajanje odmerka ni potrebno. O bolnikih z okvaro ledvic ali jeter je na voljo malo podatkov. Bolnike s hudo okvaro ledvic ali z zmerno do hudo okvaro jeter je treba pozorno spremljati. Varnost in učinkovitost vemurafeniba pri otrocih in mladostnikih, mlajših od 18 let, še nista bili dokazani. Podatkov ni na voljo. Način uporabe: Tablete vemurafeniba je treba zaužiti cele, z vodo. Ne sme se jih žvečiti ali zdrobiti. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Pred uporabo vemurafeniba je treba z validirano preiskavo potrditi, da ima bolnik tumor s pozitivno mutacijo BRAF V600. Dokazi o učinkovitosti in varnosti vemurafeniba pri bolnikih s tumorji z izraženo redko BRAF V600 mutacijo, ki ni V600E ali V600K, niso prepričljivi. Vemurafeniba se ne sme uporabljati pri bolnikih z malignim melanomom, ki ima divji tip BRAF. *Preobčutljivostne reakcije:* V povezavi z vemurafenibom so bile opisane resne preobčutljivostne reakcije, vključno z anafilaksijo. Hude preobčutljivostne reakcije lahko vključujejo Stevens-Johnsonov sindrom, generaliziran izpuščaj, eritem ali hipotenzijo. Pri bolnikih, pri katerih se pojavijo resne preobčutljivostne reakcije, je treba zdravljenje z vemurafenibom dokončno opustiti. *Kožne reakcije:* Pri bolnikih, ki so prejemali vemurafenib, so v ključnem kliničnem preskušanju poročali o hudih kožnih reakcijah, vključno z redkim Stevens-Johnsonovim sindromom in toksično epidermalno nekrolizo. Po prihodu vemurafeniba na trg so v povezavi z njim poročali o reakciji na zdravilo z eozinofilijo in sistemskimi simptomi. Pri bolnikih, pri katerih se pojavi huda kožna reakcija, je treba zdravljenje z vemurafenibom dokončno opustiti. *Povečanje toksičnosti obsevanja:* Pri bolnikih, ki so se pred, med ali po zdravljenju z vemurafenibom zdravili z obsevanjem, so poročali o primerih vnetnih reakcij na mestu obsevanja (t.i. *radiation recall*) in povečane občutljivosti na obsevanje. Večina primerov je bila po naravi kožnih, a nekaj primerov, ki je vključevalo visceralne organe, je imelo smrtni izid. Pri sočasni ali zaporedni uporabi vemurafeniba in obsevanja je potrebna previdnost. *Podaljšanje intervala QT:* V nekontrolirani, odprti študiji faze II pri predhodno zdravljenih bolnikih z metastatskim melanomom, so opazili podaljšanje intervala QT, odvisnega od izpostavljenosti vemurafenibu. Podaljšanje intervala QT lahko poveča tveganje za ventrikularne aritmije, vključno s t. i. *Torsade de Pointes*. Z vemurafenibom ni priporočljivo zdraviti bolnikov z elektrolitskimi motnjami (vključno z magnezijem), ki jih ni mogoče odpraviti, bolnikov s sindromom dolgega intervala QT in bolnikov, zdravljenih z zdravili, ki podaljšajo interval QT. Pred zdravljenjem z vemurafenibom, en mesec po zdravljenju in po spremembi odmerka je treba pri vseh bolnikih posneti EKG in kontrolirati elektrolite (vključno z magnezijem). Nadaljnje kontrole so priporočljive predvsem pri bolnikih z zmerno do hudo jetrno okvaro, in sicer mesečno prve 3 mesece zdravljenja, potem pa na 3 mesece oziroma pogosteje, če je to klinično indicirano. Zdravljenja z vemurafenibom ni priporočljivo uvesti pri bolnikih, ki imajo interval QTc > 500 ms. *Bolezni oči:* Poročali so o resnih neželenih učinkih na očeh, vključno z uveitisom, iritisom in zaporo mrežnične vene. Bolnikom je treba oči redno kontrolirati glede morebitnih neželenih učinkov na očeh. *Ploščatocelični karcinom kože:* Pri bolnikih, zdravljenih z vemurafenibom, so bili opisani primeri ploščatoceličnega karcinoma kože, vključno s ploščatoceličnim karcinomom, opredeljenim kot keratoakantom ali mešani keratoakantom. Priporočljivo je, da vsi bolniki pred uvedbo zdravljenja opravijo dermatološki pregled in da so med zdravljenjem deležni rednih kontrol. Vsako sumljivo spremembo je treba izrezati, poslati na histopatološko oceno in jo zdraviti v skladu z lokalnimi smernicami. Med zdravljenjem in do šest mesecev po zdravljenju ploščatoceličnega karcinoma mora zdravnik enkrat mesečno pregledati bolnika. Pri bolnikih, ki se jim pojavi ploščatocelični karcinom kože, je priporočljivo nadaljevati zdravljenje brez zmanjšanja odmerka. Nadzor se mora nadaljevati še 6 mesecev po prenehanju zdravljenja z vemurafenibom ali do uvedbe drugega antineoplastičnega zdravljenja. Bolnikom je treba naročiti, naj svojega zdravnika obvestijo o pojavu kakršnih koli sprememb na koži. *Ploščatocelični karcinom, ki se ne nahaja na koži:* Pri bolnikih, ki so prejemali vemurafenib v kliničnih preskušanjih, so poročali o primerih ploščatoceličnega karcinoma, ki se ne nahaja na koži. Bolnikom je treba pred uvedbo zdravljenja in na 3 mesece med zdravljenjem pregledati glavo in vrat (pregled mora obsegati vsaj ogled ustne sluznice in palpacijo bezgavk). Poleg tega morajo bolniki pred zdravljenjem in na 6 mesecev med zdravljenjem opraviti računalniško tomografijo prsnega koša. Pred in po končanem zdravljenju ali kadar je klinično indicirano, je priporočljivo opraviti pregled zadnjika in ginekološki pregled. Po prenehanju zdravljenja z vemurafenibom se mora nadzor glede ploščatoceličnega karcinoma, ki se ne nahaja na koži, nadaljevati še 6 mesecev ali do uvedbe drugega antineoplastičnega zdravljenja. Nenormalne spremembe je treba obravnavati v skladu s klinično prakso. *Novi primarni melanom:* V kliničnih preskušanjih so poročali o novih primarnih melanomih. Bolnike s takšnimi primeri so zdravili z ekscizijo, bolniki pa so nadaljevali z zdravljenjem brez prilagoditve odmerka. Nadzor nad pojavom kožnih lezij je treba izvajati, kot je navedeno zgoraj pri ploščatoceličnem karcinomu kože. *Druge malignosti:* Glede na mehanizem delovanja lahko vemurafenib povzroči napredovanje rakov, povezanih z mutacijo RAS. Pred dajanjem vemurafeniba bolnikom, ki so imeli ali imajo raka, povezanega z mutacijo RAS, skrbno razmislite o koristih in tveganjih. *Pankreatitis:* Pri bolnikih, zdravljenih z zdravilom Zelboraf, so poročali o pankreatitisu. Nepojasnjeno bolečino v trebuhu je treba nemudoma preiskati. Bolnike je treba skrbno spremljati, ko po epizodi pankreatitisa ponovno uvedemo vemurafenib. *Poškodbe jeter:* Med uporabo vemurafeniba so poročali o poškodbah jeter, vključno s primeri hudih poškodb. Pred uvedbo zdravljenja je treba izmeriti jetrne encime (transaminaze in alkalno fosfatazo) ter bilirubin in jih spremljati mesečno med zdravljenjem oz. kot je klinično indicirano. Laboratorijske nepravilnosti je treba obvladati z zmanjšanjem odmerka, prekinitvijo zdravljenja ali prenehanjem zdravljenja (za podrobnosti o prilagoditvi odmerka, prosimo glejte SmPC zdravila). *Ledvična toksičnost:* Med uporabo vemurafeniba so poročali o ledvični toksičnosti v razponu od zvišanega serumskega kreatinina do akutnega intersticijskega nefritisa in akutne tubulne nekroze. Serumski kreatinin je treba izmeriti pred začetkom zdravljenja in ga med zdravljenjem spremljati, kot je klinično indicirano. *Jetrna okvara:* Bolnikom z jetrno okvaro začetnih odmerkov ni treba prilagajati. Bolnike, ki imajo zaradi metastaz v jetrih blago jetrno okvaro in nimajo hiperbilirubinemije, se lahko nadzoruje v skladu s splošnimi priporočili. Podatkov o bolnikih z zmerno do hudo jetrno okvaro je le malo; pri takih bolnikih je izpostavljenost lahko večja. Tako je posebej po prvih tednih zdravljenja potreben skrben nadzor, saj lahko po daljšem obdobju (več tednih) pride do kopičenja. *Ledvična okvara:* Bolnikom z blago ali zmerno ledvično okvaro začetnih odmerkov ni treba prilagajati. Pri bolnikih z hudo ledvično okvaro je treba vemurafenib uporabljati previdno ter jih pozorno spremljati. *Fotosenzibilnost:* Pri bolnikih, ki so v kliničnih študijah prejemali vemurafenib, je bila opisana blaga do huda fotosenzibilnost. Vsem bolnikom je treba naročiti, naj se med jemanjem vemurafeniba ne izpostavljajo soncu. V primeru fotosenzibilnosti stopnje 2 ali več so priporočljive prilagoditve odmerka. *Ženske v rodni dobi* morajo med zdravljenjem in vsaj še 6 mesecev po zdravljenju uporabljati učinkovito kontracepcijsko zaščito. Vemurafenib lahko zmanjša učinkovitost hormonskih kontraceptivov. *Sočasna uporaba ipilimumaba* in vemurafeniba ni priporočljiva. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** *Vplivi vemurafeniba na substrate CYP* Vemurafenib lahko poveča izpostavljenost v plazmi tistim snovem, ki se presnavljajo pretežno s CYP1A2 (npr. agomelatinu, duloksetinu, melatoninu, tizanidinu, teofilinu); v takem primeru se lahko razmisli o prilagoditvi odmerka, če je klinično indicirano. Vemurafenib lahko zmanjša plazemsko izpostavljenost zdravilom, ki se presnavljajo pretežno s CYP3A4. Tako je lahko učinkovitost kontracepcijskih tablet, ki se presnavljajo s CYP3A4 in se uporabljajo sočasno z vemurafenibom, zmanjšana. Pri substratih CYP3A4, ki imajo ozko terapevtsko okno, se lahko razmisli o prilagoditvi odmerka, če je klinično indicirano. Zaenkrat še ni znano ali lahko vemurafenib pri koncentraciji v plazmi, ki je bila opažena pri bolnikih v stanju dinamičnega ravnovesja, zmanjša plazemske koncentracije sočasno dajanih substratov CYP2B6, kot je bupropion. Kadar se vemurafenib pri bolnikih z melanonom uporabi hkrati z varfarinom (CYP2C9), je potrebna previdnost. Tveganja za klinično pomemben učinek na sočasno uporabljene učinkovine, ki so substrati CYP2C8, ni mogoče izključiti. Zaradi dolge razpolovne dobe vemurafeniba je mogoče, da popolnega inhibitornega učinka vemurafeniba na sočasno dajano zdravilo ne opazimo, dokler ne mine 8 dni zdravljenja z vemurafenibom. Po končanem zdravljenju z vemurafenibom bo morda potreben 8-dnevni premor, da se izognemo interakcijam z nadaljnjim zdravljenjem. *Zdravljenje z obsevanjem:* Pri bolnikih, zdravljenih z vemurafenibom, so poročali o povečanju toksičnosti obsevanja. V večini primerov so bolniki prejeli protokole obsevanja z 2 Gy/dan ali več (hipofrakcionirane protokole). *Medsebojno delovanje vemurafeniba s transportnimi sistemi zdravil:* Ob sočasni uporabi vemurafeniba in substrata P-gp je potrebna previdnost. Pri uporabi zdravil, ki so substrati P-gp in imajo ozko terapevtsko okno (npr. digoksina, dabigatran eteksilata, aliskirena), je treba razmisliti o dodatnem spremljanju koncentracije zdravila. Možnosti, da vemurafenib morda poveča izpostavljenost zdravil, ki se prenašajo z BCRP, ni mogoče izključiti. *Vplivi sočasno uporabljenih zdravil na vemurafenib:* Vemurafenib je treba uporabljati previdno v kombinaciji z močnimi inhibitorji CYP3A4, glukuronidacije in/ali prenašalnih beljakovin (npr. ritonavirjem, sakvinavirjem, telitromicinom, ketokonazolom, itrakonazolom, vorikonazolom, posakonazolom, nefazodonom, atazanavirjem). Sočasna uporaba močnih induktorjev P-gp, glukuronidacije, in/ali CYP3A4 (npr. rifampicina, rifabutina, karbamazepina, fenitoina ali šentjanževke) lahko vodi v suboptimalno izpostavljenost vemurafenibu in se ji je treba izogibati. Vplivi induktorjev in inhibitorjev P-gp in BCRP na izpostavljenost vemurafenibu niso znani. Ne moremo pa izključiti možnosti, da imajo lahko zdravila, ki vplivajo na P-gp (npr. verapamil, ciklosporin, ritonavir, kinidin, itrakonazol) ali BCRP (npr. ciklosporin, gefitinib), vpliv na farmakokinetiko vemurafeniba. **Neželeni učinki:** Med najpogostejšimi neželenimi učinki (> 30 %), o katerih so poročali v zvezi z vemurafenibom, so artralgija, utrujenost, kožni izpuščaj, fotosenzibilnostna reakcija, navzea, alopecija in srbenje. Zelo pogosto je bil opisan ploščatocelični karcinom kože. Sledijo drugi zelo pogosti in pogosti neželeni učinki. *Zelo pogosti:* seboroična keratoza, kožni papilom, zmanjšanje teka, glavobol, disgevzija, kašelj, driska, bruhanje, zaprtost, aktinična keratoza, makulo-papulozen izpuščaj, papulozen izpuščaj, hiperkeratoza, eritem, suha koža, sončne opekline, mialgija, bolečina v okončini, mišično-skeletne bolečine, bolečine v hrbtu, pireksija, periferni edem, astenija, zvišanje GGT. *Pogosti:* folikulitis, bazalnocelični karcinom, novi primarni melanom, ohromelost sedmega živca, omotica, uveitis, sindrom palmarno-plantarne eritrodisestezije, panikulitis, pilarna keratoza, artritis, zvišanje ALT, alkalne fosfataze, bilirubina in izguba telesne mase, podaljšanje QT in zvišanje kreatinina v krvi. *Posebne populacije:* Pri starejših bolnikih (≥ 65 let) je možna večja verjetnost neželenih učinkov, vključno s ploščatoceličnim karcinomom kože, zmanjšanjem teka in motnjami srčnega ritma. Med neželene učinke stopnje 3, ki so bili med kliničnimi preskušanji vemurafeniba pri ženskah opisani pogosteje kot pri moških, spadajo kožni izpuščaj, artralgija in fotosenzibilnost. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** Rp/Spec **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija **Verzija: 1.0/16 Informacija pripravljena:** september 2016

Samo za strokovno javnost

DODATNE INFORMACIJE SO NA VOLJO PRI: Roche farmacevtska družba d.o.o., Vodovodna cesta 109, 1000 Ljubljana.

PG-047-0016-ME2

COTELLIC® kobimetinib | Zelboraf® vemurafenib

DVOJNO ZAVIRANJE. VEČJA UČINKOVITOST.

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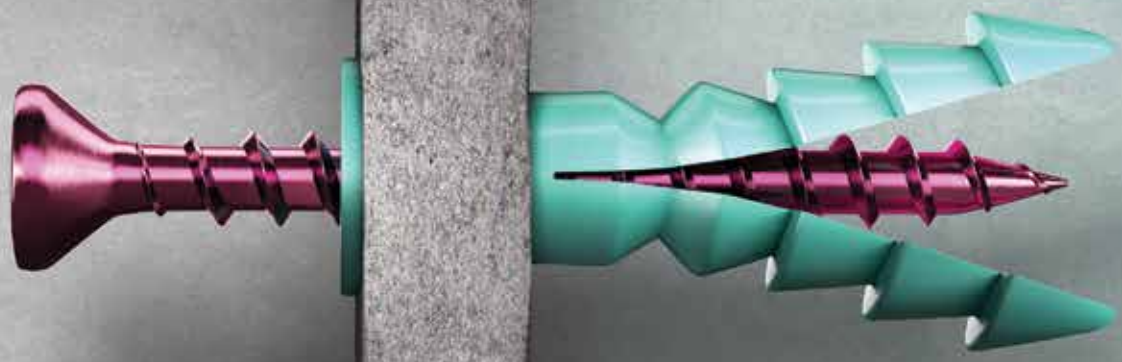
SKUPAJ MOČNEJŠA



Kombinacija zdravil Cotellic®▼ in Zelboraf®▼ za zdravljenje odraslih bolnikov z neoperabilnim ali metastatskim melanomom, ki ima mutacijo BRAF V600.[1,2]

1 Povzetek glavnih značilnosti zdravila Cotellic. Dostopano september 2016 na: http://www.ema.europa.eu/docs/sl_SI/document_library/EPAR_-_Product_Information/human/003960/WC500198563.pdf

2 Povzetek glavnih značilnosti zdravila Zelboraf. Dostopano september 2016 na: http://www.ema.europa.eu/docs/sl_SI/document_library/EPAR_-_Product_Information/human/002409/WC500124317.pdf

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA:

Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila, ki ga dobite pri naših strokovnih sodelavcih ali na sedežu družbe!

KEYTRUDA 50 mg prašek za koncentrat za raztopino za infundiranje vsebuje pembrolizumab, humanizirano monoklonsko protitelo proti receptorjem programirane celične smrti 1 (PD-1). Zdravilo KEYTRUDA je **v obliki monoterapije indicirano za zdravljenje napredovalega (neoperabilnega ali metastatskega) melanoma pri odraslih. Zdravilo je indicirano tudi za zdravljenje lokalno napredovalega ali metastatskega nedrobnoceličnega pljučnega raka (NSCLC - non-small cell lung carcinoma) pri odraslih, ki imajo tumorje z ekspresijo PD-L1 in so bili predhodno zdravljeni z vsaj eno shemo kemoterapije.** Bolniki s pozitivnimi mutacijami EGFR ali ALK so pred prejemom zdravila KEYTRUDA morali prejeti tudi zdravljenje, odobreno v primeru teh mutacij.PRIPOROČENI ODMEREK ZDRAVILA JE **2 mg/kg i.v. vsake tri tedne** do napredovanja bolezni ali nesprejemljivih tksičnih učinkov. Odmerka ni potrebno prilagajati pri starejših bolnikih ali bolnikih z blago do zmerno okvaro ledvic ali blago okvaro jeter. Zdravljenje je **kontrainidicirano** ob preobčutljivosti na zdravilno učinkovino ali katerokoli od pomožnih snovi.

Povzetek posebnih opozoril previdnostnih ukrepov, interakcij in neželenih učinkov: Pri ocenjevanju statusa PD-L1 tumorja je pomembno izbrati dobro validirano in robustno metodlogijo, da bi čim bol zmanjšali možnost lažno negativnih ali lažno pozitivnih določitev. Ob zdravljenju z zdravilom KEYTRUDA so bili poročani imunsko pogojeni neželeni učinki (pnevmonitis, kolitis, nefritis, endokronopatije, hepatitis) od katerih je večina bila reverzibilna in so jih obvladali s prekinitvami uporabe pembrolizumaba, uporabo kortikosteroidov in/ali podporno oskrbo. V primeru suma na imunsko pogojene neželene učinke je treba poskrbeti za ustrezno oceno za potrditev etiologije, ter glede na izrazitost neželenega učinka zadržati uporabo pembrolizumaba oz uporabiti kortikosteroide. Pembrolizumab se lahko začne znova uporabljati v 12 tednih po zadnjem odmerku zdravila KEYTRUDA, če neželeni učinek ostane na ≤ 1. stopnji in je bil odmerek kortikosteroida znižan na ≤ 10 mg prednizona ali ekvivalenta na dan, drugače je treba zdravljenje trajno ukiniti. Zdravljenje je treba ukiniti tudi, če se kateri koli imunsko pogojeni neželeni učinek 3. stopnje znova pojavi ali se pojavi toksičen imunsko pogojeni neželeni učinke 4 stopnje razen v primeru endokrinopatij, ki so obvladljive z nadomeščanjem hormonov. Pembrolizumab se iz obtoka odstrani s katabolizmom, zato presnovnih medsebojnih delovanj zdravil ni pričakovati. Uporabi sistemskih kortikosteroidov ali imunosupresivov pred uvedbo pembrolizumaba se je treba izogibati, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost pembrolizumaba. Vendar pa je kortikosteroide ali druge imunosupresive mogoče uporabiti po uvedbi zdravljenja s pembrolizumabom za zdravljenje imunsko pogojenih neželenih učinkov. Zaradi mehanizma delovanja je možno pričakovati okvaro ploda in več splavov ali mrtvorojenosti zato uporebo med nosečnostjo odsvetujemo razen ko klinično stanje ženske zahteva.Ženske v rodni dobi morajo med zdravljenjem in vsaj še 4 mesece po zadnjem odmerku pembrolizumaba uporabljati učinkovito kontracepcijo. Ker se protitelesa lahko izločajo v materino mleko, tveganja za novorojence/otroke ne moremo izključiti . Varnost pembrolizumaba so v kliničnih študijah ocenili pri 2.799 bolnikih s tremi odmerki (2 mg/kg na 3 tedne in 10 mg/kg na 2 ali 3 tedne) kjer so najpogosteje poročali o naslednjih neželenih učinkih: utrujenosti, izpuščajih , pruritusus, diareji, navzei in arthralgijah. Večina poročanih neželenih učinkov je bila po izrazitosti 1. ali 2. stopnje. Najresnejši neželeni učinki so bili imunsko pogojeni neželeni učinki in hude z infuzijo povezane reakcije. Večina, vključno s hudimi učinki, je po uvedbi ustreznega zdravljenja ali ukinitvi zdravljenja s pembrolizumabom izzvenela. Za popolniseznam neželenih učinkov prosimo preberite Povzetek glavnih značilnosti zdravila.

Način in režim izdaje zdravila: Zdravilo se izdaja le v bolnišnicah.

Imetnik dovoljenja za promet z zdravilom: Merck Sharp & Dohme Limited Hertford Road, Hoddesdon, Hertfordshire EN11 9BU Velika Britanija

Datum zadnje revizije besedila: 22.8.2016

KEYTRUDA®

(pembrolizumab, MSD)

KEYTRUDA®

(pembrolizumab, MSD)

Zdravilo KEYTRUDA®:

MOČ ZAVIRALCA PD-1

za podaljšano preživetje[1]

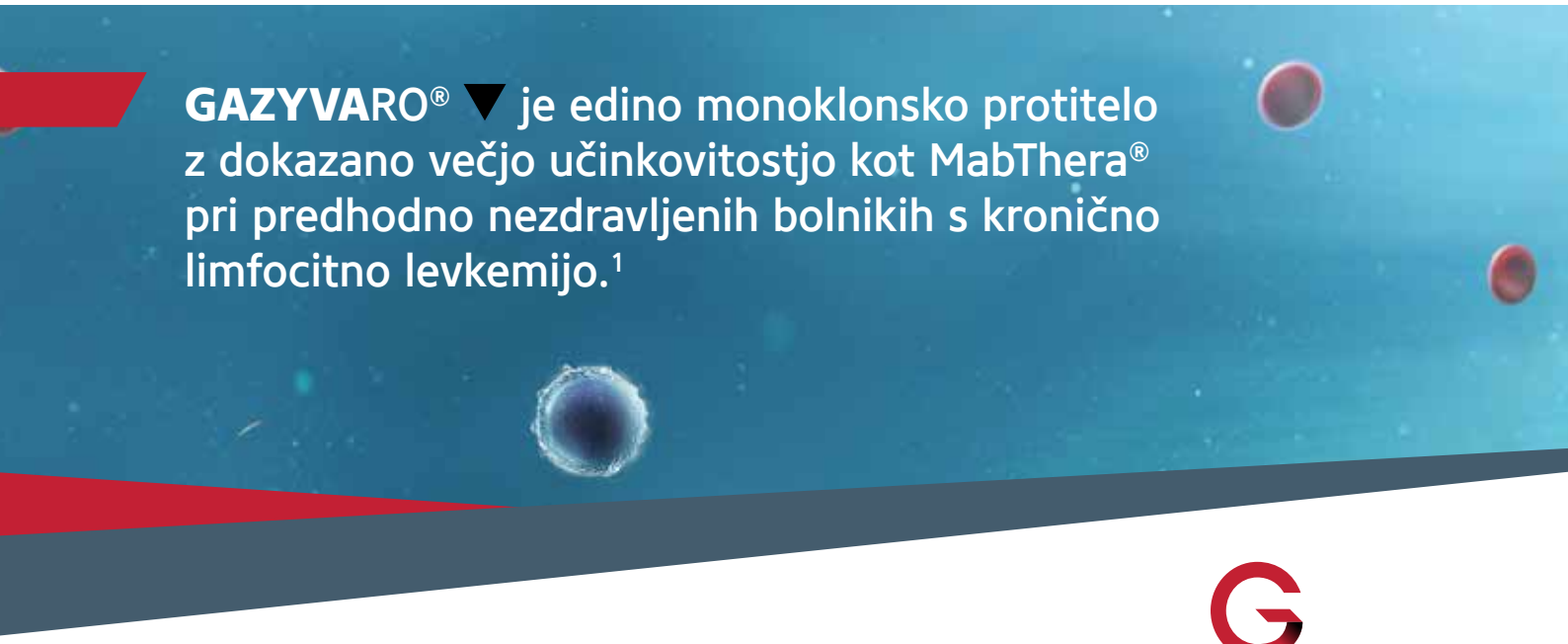
Literatura: 1. Povzetek glavnih značilnosti zdravila Keytruda, julij 2016

MSD

Merck Sharp & Dohme, inovativna zdravila d.o.o.
Šmartinska cesta 140, 1000 Ljubljana, telefon: 01/ 520 42 01, faks: 01/ 520 43 49/50,
Pripravljeno v Sloveniji, avgust 2016, ONCO-1160070-0001 EXP: 08/2017

Samo za strokovno javnost.
H - Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah.
Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila Keytruda, ki je na voljo pri naših strokovnih sodelavcih ali na lokalnem sedežu družbe.

Za ogled celotnega Povzetka glavnih značilnosti zdravila skenirajte QR kodo.



GAZYVARO®
obinutuzumab

Referenca: 1. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med 2014;370(12):1101-1110.

▼ **Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila. Kako poročati o neželenih učinkih, si poglejte skrajšani povzetek glavnih značilnosti zdravila pod "Poročanje o domnevnih neželenih učinkih".**

Skrajšan povzetek glavnih značilnosti zdravila GAZYVARO® (obinutuzumab) 1000 mg koncentrat za raztopino za infundiranje

Ime zdravila: Gazyvaro 1000 mg koncentrat za raztopino za infundiranje
Kakovostna in količinska sestava: Ena viala s 40 ml koncentrata vsebuje 1000 mg obinutuzumaba; to ustreza koncentraciji 25 mg/ml pred razredčenjem. Obinutuzumab je humanizirano anti-CD20 monoklonsko protitelo tipa II podrazreda IgG1, pridobljeno s humanizacijo parentalnega mišjega protitelesa B-Ly1 in proizvedeno v celični liniji jajčnika kitajskega hrčka s tehnologijo rekombinantne DNA. **Terapevtske indikacije: Kronična limfocitna levkemija:** Zdravilo Gazyvaro je v kombinaciji s klorambucilom indicirano za zdravljenje odraslih bolnikov s predhodno nezdravljeno kronično limfocitno levkemijo (KLL), pri katerih zdravljenje s polnim odmerkom fludarabina zaradi pridruženih bolezni ni primerno. **Folikularni limfom:** Zdravilo Gazyvaro je v kombinaciji z bendamustinom ter s poznejšo vzdrževalno uporabo zdravila Gazyvaro indicirano za zdravljenje bolnikov s folikularnim limfomom (FL), ki se na zdravljenje z rituksimabom ali shemo, ki je vključevala rituksimab, niso odzvali ali jim je bolezen med ali v 6 mesecih po takšnem zdravljenju napredovala. **Odmerjanje in način uporabe:** Dajanje zdravila Gazyvaro mora potekati pod natančnim nadzorom izkušenega zdravnika in v okolju, kjer je takoj na voljo vse potrebno za oživljanje. Odmerjanje: *Profilaksa in premedikacija sindroma razpada tumorja:* Bolniki z velikim tumorskim bremenom in/ali velikim številom limfocitov v krvnem obtoku in/ali ledvično okvaro imajo večje tveganje za sindrom razpada tumorja in morajo prejeti profilakso. To naj sestavljata ustrezna hidracija in dajanje urikostatikov ali primerno alternativno zdravljenje, kot je uratna oksidaza. *Profilaksa reakcij, povezanih z infuzijo, in premedikacija zanje:* Premedikacija s kortikosteroidom je v prvem ciklusu za bolnike s FL priporočljiva, za bolnike s KLL pa obvezna. Med intravenskim infundiranjem zdravila Gazyvaro se lahko kot simptom reakcij, povezanih z infuzijo, pojavi hipotenzija. Zato je treba uporabo antihipertenzivnih zdravil zadržati v obdobju 12 ur pred infundiranjem zdravila Gazyvaro, med celotnim infundiranjem in še prvo uro po koncu dajanja zdravila. *Odmerek:* **KLL:** 1. ciklus: Priporočeni odmerek zdravila Gazyvaro v kombinaciji s klorambucilom je 1000 mg, ki ga dajemo na 1. in 2. dan (ali v nadaljevanju 1. dne) ter 8. in 15. dan prvega 28-dnevnega ciklusa zdravljenja. Za infundiranje 1. in 2. dan je treba pripraviti dve infuzijski vrečki (100 mg za 1. dan in 900 mg za 2. dan). Če med dajanjem prve vrečke ni bilo treba prilagoditi hitrosti infundiranja ali infundiranja prekiniti, je mogoče drugo vrečko uporabiti isti dan. Če je med dajanjem prvih 100 mg treba spremeniti hitrost infundiranja ali infundiranje prekiniti, je treba drugo vrečko dati naslednji dan. 2. do 6. ciklus: Priporočeni odmerek zdravila Gazyvaro v kombinaciji s klorambucilom je 1000 mg, uporabljen 1. dan vsakega ciklusa. *Trajanje zdravljenja:* Šest ciklusov zdravljenja, od katerih vsak traja 28 dni. **FL:** 1. ciklus: Priporočeni odmerek zdravila Gazyvaro v kombinaciji z bendamustinom je 1000 mg, uporabljen 1. dan, 8. dan in 15. dan prvega 28-dnevnega ciklusa zdravljenja. 2. do 6. ciklus: Priporočeni odmerek zdravila Gazyvaro v kombinaciji z bendamustinom je 1000 mg 1. dan vsakega 28-dnevnega ciklusa zdravljenja. Vzdrževanje: Bolniki, ki se odzovejo na indukcijsko zdravljenje z zdravilom Gazyvaro v kombinaciji z bendamustinom ali imajo stabilno bolezen, morajo še naprej prejemati monoterapijo z zdravilom Gazyvaro 1000 mg kot vzdrževalno zdravljenje enkrat na 2 meseca v obdobju dveh let ali do napredovanja bolezni. Trajanje zdravljenja: Šest 28-dnevnih ciklusov zdravljenja, ki jim sledi vzdrževalno zdravljenje enkrat na dva meseca v obdobju dveh let ali do napredovanja bolezni. *Prilagoditev odmerka med zdravljenjem:* Zmanjšanja odmerka zdravila Gazyvaro niso priporočljiva. Posebne skupine bolnikov: Starejšim bolnikom in bolnikom z blago do zmerno ledvično okvaro odmerka ni treba prilagoditi. Varnost in učinkovitost zdravila Gazyvaro pri bolnikih s hudo ledvično okvaro, okvarjenim delovanjem jeter ter pri otrocih in mladostnikih, mlajših od 18 let, nista ugotovljeni. Način uporabe: za intravensko uporabo. Po razredčenju ga je treba dati kot intravensko infuzijo po posebni liniji. Infuzij zdravila Gazyvaro se ne sme dati v obliki hitre intravenske infuzije ali kot bolus. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Za izboljšanje sledljivosti bioloških zdravil je treba zaščiteno ime in številko serije uporabljenega zdravila jasno zabeležiti v bolnikovi dokumentaciji. Reakcije, povezane z infuzijo: Najpogosteje opaženi neželeni učinki pri bolnikih, ki so prejemali zdravilo Gazyvaro, so bile reakcije, povezane z infuzijo, ki so se pretežno pojavile med infundiranjem prvih 1000 mg. Reakcije, povezane z infuzijo, so lahko povezane s sindromom sproščanja citokinov. Za zmanjšanje reakcij, povezanih z infuzijo, je treba upoštevati ukrepe za ublažitev. Incidenca in izrazitost simptomov reakcij, povezanih z infuzijo, se je bistveno zmanjšala po infundiranju prvih 1000 mg in večina bolnikov med nadaljnjo uporabo zdravila Gazyvaro reakcij, povezanih z infuzijo, ni imela. Pri večini bolnikov ne glede na indikacijo so bile reakcije, povezane z infuzijo, blage do zmerne in jih je bilo mogoče obvladati z upočasnitvijo ali začasno prekinitvijo prvega infundiranja. Vendar pa so poročali tudi o hudih in življenje ogrožajočih reakcijah, povezanih z infuzijo, ki so zahtevale simptomatsko zdravljenje. Reakcije, povezane z infuzijo, so lahko klinično nerazločljive od alergijskih reakcij. Če se pri bolniku pojavi reakcija, povezana z infuzijo, je treba v zvezi z infundiranjem ukrepati glede na stopnjo reakcije. Bolnike z obstoječo boleznijo srca ali pljuč je treba skrbno nadzorovati ves čas infundiranja in v obdobju po njem. Uporabo antihipertenzivnih zdravil je treba začasno prekiniti. Preobčutljivostne reakcije, vključno z anafilakso: Pri bolnikih, zdravljenih z zdravilom Gazyvaro, so poročali o anafilaktičnih reakcijah. Pri sumu na preobčutljivostno reakcijo med infundiranjem je treba infundiranje prekiniti in zdravljenje trajno končati. Sindrom razpada tumorja: Med uporabo zdravila Gazyvaro so poročali o sindromu razpada tumorja. Bolniki z velikim tveganjem za sindrom razpada tumorja morajo prejeti profilakso. Vse bolnike s tveganjem je treba pozorno spremljati med prvimi dnevi zdravljenja s posebno pozornostjo na ledvično delovanje, vrednosti kalija in sečne kisline. Nevtropenija: Med zdravljenjem z zdravilom Gazyvaro so poročali o hudi in življenje ogrožajoči nevtropeniji, vključno s febrilno nevtropenijo. Bolnike, pri katerih se razvije nevtropenija, je treba skrbno nadzirati z rednimi laboratorijskimi kontrolami krvne slike, dokler ne mine. Če je potrebno zdravljenje, ga je treba uporabiti v skladu z lokalnimi smernicami in razmisliti o uporabi granulocitne kolonije stimulirajočih faktorjev. Pri hudi ali življenje ogrožajoči nevtropeniji je treba razmisliti o preložitvi odmerka na kasnejši čas. Poročali so tudi o primerih zapoznele nevtropenije ali dolgotrajne nevtropenije. Bolniki z ledvično okvaro imajo večje tveganje za nevtropenijo. Trombocitopenija: Med zdravljenjem z zdravilom Gazyvaro so opažali hudo in življenje ogrožajočo trombocitopenijo, vključno z akutno trombocitopenijo. Bolniki z ledvično okvaro imajo večje tveganje za trombocitopenijo. Pri bolnikih, zdravljenih z zdravilom Gazyvaro, so bile v 1. ciklusu opisane tudi krvavitve s smrtnim izidom. Jasne povezanosti med trombocitopenijo in krvavitvami niso ugotovili. Bolnike je treba natančno spremljati glede trombocitopenije, zlasti v prvem ciklusu; izvajati je treba redne laboratorijske preiskave krvne slike, dokler dogodek ne mine, pri hudi ali življenje ogrožajoči trombocitopeniji pa je treba razmisliti o preložitvi odmerka na kasnejši čas. Upoštevati je treba tudi sočasno uporabo katerih koli zdravil, ki lahko poslabšajo s trombocitopenijo povezane dogodke, na primer zaviralcev trombocitov in antikoagulantov; to je treba še zlasti upoštevati v prvem ciklusu. Poslabšanje obstoječih srčnih bolezni: Pri bolnikih z obstoječo srčno boleznijo so se med zdravljenjem z zdravilom Gazyvaro pojavile motnje srčnega ritma, angina pektoris, akutni koronarni sindrom, akutni miokardni infarkt in srčno popuščanje. Ti dogodki se lahko pojavijo kot del reakcij, povezanih z infuzijo, in so lahko smrtni. Zato je treba bolnike z anamnezo srčne bolezni natančno nadzirati. Poleg tega je treba pri teh bolnikih hidracijo izvajati previdno, da bi preprečili možno preobremenitev s tekočino. Okužbe: Zdravila Gazyvaro se ne sme uporabiti pri bolnikih z aktivno okužbo, pri bolnikih z anamnezo ponavljajočih se ali kroničnih okužb pa je pri razmisleku o uporabi zdravila Gazyvaro potrebna previdnost. Med zdravljenjem z zdravilom Gazyvaro in po njem se lahko pojavijo resne bakterijske, glivične in nove ali reaktivirane virusne okužbe. Poročali so o okužbah s smrtnim izidom. Bolniki, ki imajo hkrati CIRS > 6 in očistek kreatinina < 70 ml/min, imajo večje tveganje za okužbe, vključno s hudimi okužbami. Reaktivacija hepatitisa B: Bolnikom, zdravljenim s protitelesi proti CD20 (vključno z zdravilom Gazyvaro), se lahko pojavi reaktivacija virusa hepatitisa B, v nekaterih primerih s posledičnim fulminantnim hepatitisom, jetrno odpovedjo in smrtjo. Pred začetkom zdravljenja z zdravilom Gazyvaro je treba pri vseh bolnikih opraviti presejalno testiranje za virus hepatitisa B. Bolnikov z aktivnim hepatitisom B se ne sme zdraviti z zdravilom Gazyvaro. Bolniki s pozitivnimi serološkimi izvidi za hepatitis B se morajo pred začetkom zdravljenja posvetovati s specialistom za jetrne bolezni; treba jih je nadzorovati in voditi v skladu z lokalnimi zdravstvenimi standardi, da bi preprečili reaktivacijo hepatitisa. Progresivna multifokalna levkoencefalopatija (PML): Pri bolnikih, zdravljenih z zdravilom Gazyvaro, je bila opisana PML. Na diagnozo PML je treba pomisliti pri vsakem bolniku z novonastalimi nevrološkimi spremembami ali spremembi že obstoječih nevroloških stanj. Simptomi PML so nespecifični in se lahko razlikujejo glede na prizadet možganski predel. Ovrednotenje PML vključuje posvet z nevrologom, magnetnoresonančno slikanje možganov in lumbalno punkcijo. Med preiskovanjem suma na PML je treba zdravljenje z zdravilom Gazyvaro prekiniti, če je PML potrjena, pa ga je treba trajno končati. V poštev pride tudi prenehanje ali zmanjšanje morebitne sočasne kemoterapije ali imunosupresivnega zdravljenja. Imunizacija: Varnost imunizacije z živimi ali oslabljenimi virusnimi cepivi po zdravljenju z zdravilom Gazyvaro ni raziskana in cepljenje z živimi virusnimi cepivi med zdravljenjem in do okrevanja celic B ni priporočljivo. *Izpostavljenost obinutuzumabu in utero in cepljenje dojenčkov z živimi virusnimi cepivi:* Zaradi možnega zmanjšanega števila celic B pri dojenčkih mater, ki so bile med nosečnostjo izpostavljene zdravilu Gazyvaro, je treba pri dojenčkih kontrolirati njihovo število. Cepljenja z živimi virusnimi cepivi je treba odložiti, dokler se število celic B pri dojenčku ne popravi. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Farmakokinetično medsebojno delovanje: Obinutuzumab ni substrat, zaviralec ali induktor CYP450, encimov UGT ali prenašalcev, kakršen je P-glikoprotein. Zato ni pričakovati farmakokinetičnih medsebojnih delovanj z zdravili, ki se presnavljajo s temi encimskimi sistemi. Sočasno dajanje zdravila Gazyvaro ni imelo vpliva na farmakokinetiko bendamustina, FC, klorambucila ali posameznih učinkovin v CHOP. Dodatno ni bilo vidnih učinkov bendamustina, FC, klorambucila ali CHOP na farmakokinetiko zdravila Gazyvaro. Farmakodinamično medsebojno delovanje: Zaradi imunosupresivnega učinka obinutuzumaba cepljenje z živimi virusnimi cepivi ni priporočljivo med zdravljenjem in do okrevanja celic B. Obinutuzumab v kombinaciji s klorambucilom ali bendamustinom lahko poveča delež nevtropenije. **Neželeni učinki:** Povzetek neželenih učinkov z večjo incidenco (razlika ≥ 2 %) pri bolnikih s KLL, ki so v klinični študiji prejemali kombinacijo zdravila Gazyvaro in klorambucila, v primerjavi s klorambucilom samim ali rituksimabom in klorambucilom, in pri bolnikih z iNHL, ki so v klinični študiji prejemali kombinacijo zdravila Gazyvaro in bendamustina, čemur je pri nekaterih bolnikih sledilo vzdrževalno zdravljenje z zdravilom Gazyvaro, v primerjavi z bendamustinom samim: *Zelo pogosti:* okužba zgornjih dihal, sinuzitis, nevtropenija, trombocitopenija, anemija, kašelj, diareja, zaprtost, artralgija, zvišana telesna temperatura, astenija in reakcije, povezane z infuzijo. *Pogosti:* okužba sečil, nazofaringitis, herpes labialis, rinitis, faringitis, okužba pljuč, gripa, ploščatocelični karcinom kože, levkopenija, bolečina v bezgavki, sindrom razpada tumorja, hiperurikemija, depresija, okularna hiperemija, atrijska fibrilacija, srčno popuščanje, hipertenzija, nazalna kongestija, rinoreja, dispepsija, kolitis, hemoroidi, alopecija, srbečica, nočno potenje, ekcem, bolečina v hrbtu, mišično-skeletna bolečina v prsnem košu, bolečina v okončinah, bolečina v kosteh, dizurija, urinarna inkontinenca, bolečina v prsnem košu, zmanjšanje števila belih krvnih celic, zmanjšanje števila nevtrofilcev in povečanje telesne mase. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** H **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija
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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

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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/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:* Varnost in učinkovitost sunitiniba pri bolnikih, mlajših od 18 let, še nista bili dokazani. *Starejši (≥ 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 katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** *Bolezni kože in tkiv:* obarvanje kože, gangrenozna pioderma (običajno izgine po prekinitvi zdravljenja), hude kožne reakcije (multiformni 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 diareje, navzee/bruhanja, bolečine v trebuhu, dispepsije, stomatitisa/bolečine v ustih in ezofagitisa tudi hudi zapleti (včasih s smrtnim izidom), vključno z gastrointestinalno perforacijo. *Hipertenzija:* 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, miokardno ishemijo in miokardnim infarktom, v nekaterih primerih s smrtnim izidom; sunitinib povečuje tveganje za pojav kardiomiopatije; previdna uporaba pri bolnikih s tveganjem za te dogodke, ali ki so te dogodke imeli v preteklosti. *Podaljšanje intervala QT:* previdna uporaba pri bolnikih z znano anamnezo podaljšanja intervala QT, tistih, ki jemljejo antiaritmike ali zdravila, ki lahko podaljšajo interval QT, in tistih z relevantno, že obstoječo srčno boleznijo, bradikardijo ali elektrolitskimi motnjami. *Venski in arterijski trombembolični dogodki*; arterijski včasih s smrtnim izidom. *Trombotična mikroangiopatija (TMA)*: TMA, vključno s trombotično trombocitopenično purpuro in hemolitično-uremičnim sindromom, v nekaterih primerih z odpovedjo ledvic ali 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. *Holecistitis*, vključno z akalkuloznim in emfizemskim holecistitisom. *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 Šutent in intravenskih bisfosfonatov je potrebna previdnost; invazivni zobozdravstveni posegi predstavljajo dodatni dejavnik tveganja. *Preobčutljivost/angioedem. Motnje okušanja. Konvulzije:* obstajajo poročila, nekatera s smrtnim izidom, o preiskovancih s konvulzijami in radiološkimi znaki sindroma reverzibilne posteriorne levkoencefalopatije. *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; redki primeri nekrotizitajočega fasciitisa, vključno s prizadetostjo presredka, ki so bili včasih smrtni. *Hipoglikemija*: če se pojavi simptomatska hipoglikemija, je treba zdravljenje s sunitinibom začasno prekiniti. Pri sladkornih bolnikih je treba redno preverjati raven glukoze v krvi in, če je treba, prilagoditi odmerek antidiabetika. **Medsebojno delovanje z drugimi zdravili:** (Študije so izvedli le pri odraslih.) Zdravila, ki lahko zvečajo koncentracijo sunitiniba v plazmi (ketokonazol, ritonavir, itrakonazol, eritromicin, klaritromicin ali sok grenivke). Zdravila, ki lahko zmanjšajo 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 (nekateri s smrtnim izidom) so: odpoved ledvic, srčno popuščanje, pljučna embolija, gastrointestinalna perforacija in krvavitve (npr. v dihalih, prebavilih, tumorju, sečilih in možganih). Najpogostejši neželeni učinki (ki so se pojavili v registracijskih preskušanjih) so: zmanjšan tek, motnje okušanja, hipertenzija, utrujenost, prebavne motnje (npr. diareja, navzea, stomatitis, dispepsija in bruhanje), sprememba barve kože in sindrom palmarno-plantarne eritrodisestezije. Med najbolj pogostimi neželenimi učinki so tudi hematološke motnje (nevtropenija, trombocitopenija, anemija in levkopenija). Ostali zelo pogosti (≥ 1/10) neželeni učinki so: hipotiroidizem, nespečnost, omotica, glavobol, dispneja, epistaksa, kašelj, bolečina v trebuhu, zaprtje, izpuščaj, spremembe barve las, suha koža, bolečine v udih, artralgija, bolečine v hrbtu, vnetje sluznice, edem, pireksija. **Način in režim izdaje:** Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Izjemoma se lahko uporablja pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. **Imetnik dovoljenja za promet:** Pfizer Limited, Ramsgate Road, Sandwich, Kent, CT13 9NJ, Velika Britanija. **Datum zadnje revizije besedila:** 25.02.2016

Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.

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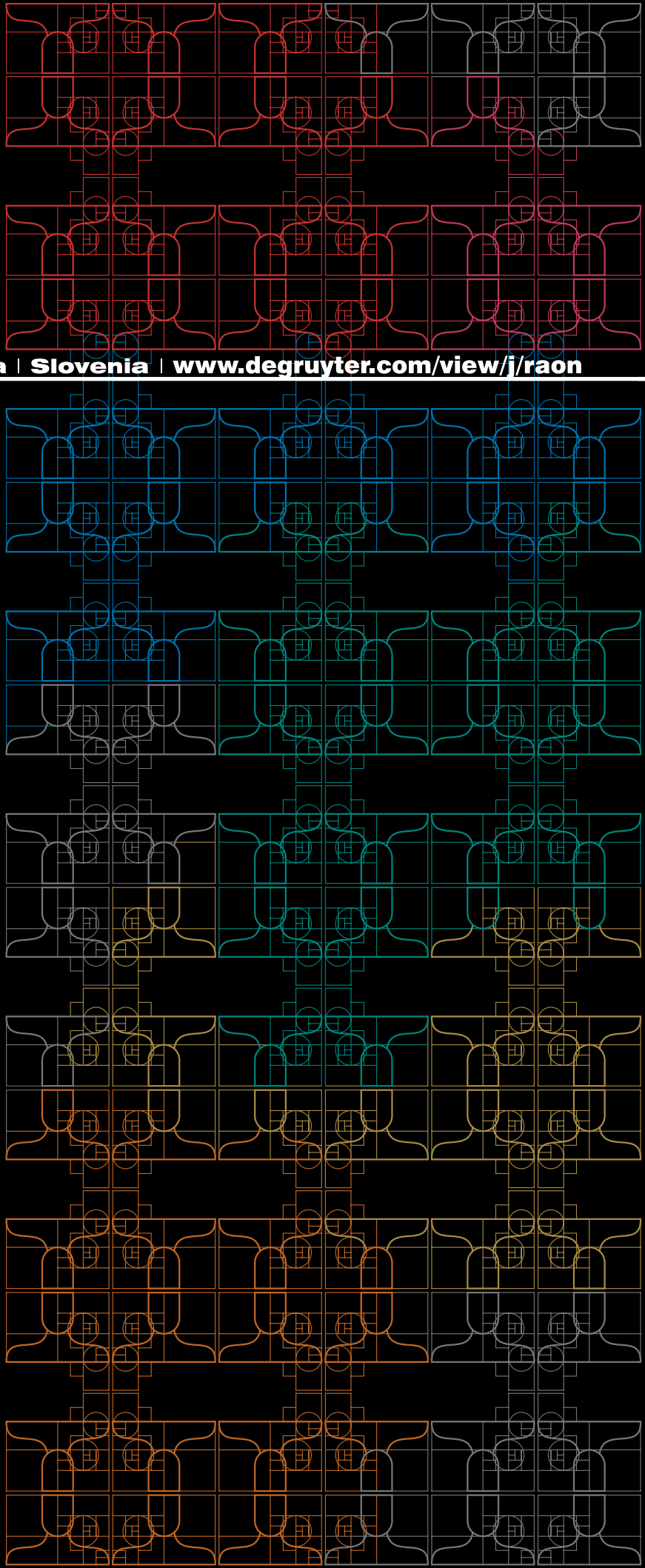

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