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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]

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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.
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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 article

^{18}F-fluorodeoxyglucose and ^{18}F-flumazenil positron emission tomography in patients with refractory epilepsy

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Background. Epilepsy is a neurological disorder characterized by epileptic seizures as a result of excessive neuronal activity in the brain. Approximately 65 million people worldwide suffer from epilepsy; 20–40% of them are refractory to medication therapy. Early detection of disease is crucial in the management of patients with epilepsy. Correct localization of the ictal onset zone is associated with a better surgical outcome. The modern non-invasive techniques used for structural-functional localization of the seizure focus includes electroencephalography (EEG) monitoring, magnetic resonance imaging (MRI), single photon emission tomography/computed tomography (SPECT/CT) and positron emission tomography/computed tomography (PET/CT). PET/CT can predict surgical outcome in patients with refractory epilepsy. The aim of the article is to review the current role of routinely used tracer 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) as well as non routinely used ^{18}F-Flumazenil (^{18}F-FMZ) tracers PET/CT in patients with refractory epilepsy.

Conclusions. Functional information delivered by PET and the morphologic information delivered by CT or MRI are essential in presurgical evaluation of epilepsy. Nowadays ^{18}F-FDG PET/CT is a routinely performed imaging modality in localization of the ictal onset zone in patients with refractory epilepsy who are unresponsive to medication therapy. Unfortunately, ^{18}F-FDG is not an ideal PET tracer regarding the management of patients with epilepsy: areas of glucose hypometabolism do not correlate precisely with the proven degree of change within hippocampal sclerosis, as observed by histopathology or MRI. Benzodiazepine-receptor imaging is a promising alternative in nuclear medicine imaging of epileptogenic focus. The use of ^{11}C-FMZ in clinical practice has been limited by its short half-life and necessitating an on-site cyclotron for production. Therefore, ^{18}F-FMZ might be established as one of the tracers of choice for patients with refractory epilepsy because of better sensitivity and anatomical resolution.

Key words: epilepsy; nuclear medicine; PET/CT; ^{18}F-FDG; ^{18}F-Flumazenil

Introduction

Epilepsy is a neurological disorder characterized by epileptic seizures as a result of excessive neuronal activity in the brain (the word "epilepsy" is derived from the Greek word meaning to be attacked or seized). For diagnosis of epilepsy at least two unprovoked seizures are required. Metabolic, genetic or structural conditions can be recognized as causes of epilepsy, but in 60% of patients, the cause remains unknown.[1] Approximately 65 million people worldwide suffer from epilepsy[2], 20–40% of them are refractory to medication therapy.[3] Depending on the epilepsy syndrome, dietary changes, neurostimulation or surgery may be considered as treatment options in patients whose seizures do not

respond to medication therapy. With the advancement of surgical techniques and devices, surgical treatment has become the treatment of choice in patients who are unresponsive to medication therapy. In selected patients with focal structural lesions such as cortical dysplasia, mesial temporal sclerosis, vascular malformations and in some paediatric epilepsy syndromes, surgery may substantially reduce the frequency of epileptic seizures and improve the patient´s quality of life. Several studies over the last decades support the statement that surgical treatment significantly improved long-term outcomes of seizure control.[4-8]

Early detection of disease is crucial in the management of patients with epilepsy. Correct localization of the ictal onset zone is associated with a better surgical outcome. Epilepsy surgery may lead to seizure freedom. When "surgical cure" is impossible, epilepsy surgery may help achieve palliative goals such as minimizing the frequency and severity of seizures. Following successful epilepsy surgery, quality of life, cognition and behaviour may improve substantially.[9] However, careful patient selection and weighing of risks and benefits are of paramount importance, as surgery may not only fail in terms of improving seizure control, but come with serious adverse events such as intracranial bleeding. Despite international guidelines recommending early and systematic assessment of patient´s eligibility, epilepsy surgery is still being underused and referral of patients with drug refractory epilepsy is often delayed with deleterious consequences on outcome and quality of life.[10]

The scope of this paper on PET imaging in refractory epilepsy patients cannot cover all aspects of patient management including the range of indications, specific issues in children and adults, surgical techniques or predictors of outcome. For this, the interested reader is referred to a recent review of Ryvlin *et al*.[11]

Different modalities in diagnosis of epilepsy

Non-invasive focus localisation of seizures precedes invasive intracranial electrodes procedures. The modern non-invasive techniques used for structural-functional localization of the seizure focus includes electroencephalography (EEG) monitoring, magnetic resonance imaging (MRI), single photon emission tomography/computed tomography (SPECT/CT) and positron emission tomography/computed tomography (PET/CT).

EEG has low sensitivity in the diagnosis of seizure disorders (25–56%).

MRI is a sensitive and specific imaging modality for identifying hippocampal sclerosis as well as other lesions responsible for epilepsy. However 1–1.5 T MRI still fails to reveal approximately 20% of abnormalities in patients with medically refractory epilepsy[12]; MRI may miss mild changes and subtle lesions. In patients with intractable extratemporal epilepsy, the most common underlying pathology is microscopic cortical dysplasia which sometimes cannot be detected by MRI and hence may pass unnoticed by ^{18}F-FDG PET/CT as well. A recent study of 194 adult patients with medically refractory focal epilepsy showed that ^{18}F-FDG PET/CT is helpful for decision making in 53% of presurgical patients with normal or discordant MRI.[13] Advanced MRI technologies (MR spectroscopy, MR volumetry, MR perfusion) may provide additional and more precise information.

In recent years brain perfusion SPECT imaging has been widely used for detection of epileptic focus. In their meta-analysis, Devous *et al*. reported a 44% (interictal), 75% (postictal) and 97% (ictal) sensitivity of SPECT in patients with temporal lobe epilepsy.[14] Extratemporal lobe epilepsy showed SPECT sensitivity in 66% (ictal) and in 40% (interictal) of patients.[15,16]

PET procedures in patients with epilepsy

In recent years, multiple studies[17-22] have demonstrated that ^{18}F-FDG PET/CT can predict surgical outcome in patients with refractory epilepsy. Actually ^{18}F-FDG PET/CT has proven to be the most sensitive imaging technique for presurgical localization of epileptogenic foci in patients with medically refractory partial epilepsy who have non-contributory MRI and EEG. In comparison to SPECT, PET technology provides much better resolution and allows quantitative measurement.[23-26]

There is a variety of PET tracers used for imaging of epileptic focus: tracers that measure glucose metabolism, serotonine receptors and transport, oxygen metabolism, cerebral blood flow and other receptor binding.[27] PET scan findings at the area of a seizure focus are different according to the PET tracer used (Table 1). Multiple PET radiotracers for neurotransmitter and neuromodulator systems still form part of preclinical trials.

In this review article we focus on the routinely used tracer ^{18}F-FDG as well as on ^{18}F-labelled

flumazenil tracers which are less available but becoming popular.

^{18}F-FDG PET/CT in patients with epilepsy

The first application of ^{18}F-FDG PET/CT in patients with epilepsy dates back to the early 1980s.[28,29] As glucose is the main energy source for the brain, a radioactive glucose analogue has been the most widely used tracer for PET imaging in patients with refractory epilepsy. There is a good match of glucose metabolism and neuronal activity. Glucose transporters (predominantly GLUT1) transfer ^{18}F-FDG from the blood into cells. Once in the cell, FDG is phosphorylated by hexokinase and forms FDG-6-phosphate. Further metabolism of FDG-6-phosphate is stopped and FDG-6-phosphate is essentially trapped in the cell.

In 2009 the European Association of Nuclear Medicine established an imaging protocol for ^{18}F-FDG PET/CT imaging of the brain.[30] According to this protocol patients should fast at least 4 hours before scanning. Psychotropic pharmaceuticals may influence reagional metabolism of the glucose. Serum glucose levels must be checked before ^{18}F-FDG PET/CT examination. If the value is greater than 160 mg/dl, the patient must be rescheduled. Diabetic patients should undergo scanning in euglycemic state. At least 30 minutes before examination as well as 30 minutes after tracer injection patients must rest in a quiet dimly-lighted room. They should be instructed not to talk, read or to be otherwise active. ^{18}F-FDG dose for adults patients is 300–600 MBq in 2-D mode and 125–250 MBq in 3-D mode. For children, dose is calculated by body wight (EANM dosage card). Continuous EEG recording is required 2 hours before tracer injection (to exclude that tracer is not injected postictal state) and at least until 20 minutes after tracer injection. Static PET scan acquisition starts 30 up to 60 minutes after tracer injection and lasts for 15–30 minutes. There are small variations in the protocols depending on quality of imaging technology. In PET scans interpretation, combination of visual inspection and semiquantitive analysis is crucial. Semiquantitative analysis helps to detect abnormalities which are not present on visual inspection.

Physiological^{18}F-FDG distribution is as follows: high in cerebral and cerebellar cortices and subcortical grey matter and mild in the white matter. In children and with normal aging there is decrease in cerebral metabolic rates (particularly in lateral, medial frontal cortex and anterior cingulate cortex).[31,32] Regions involving epileptic foci may present increased, reduced, or, absent metabolic activity.

^{18}F-FDG PET/CT scans in patients with epilepsy are usually obtained in the interictal phase. Ictal ^{18}F-FDG PET/CT appears to be highly sensitive (Figure 1), but is difficult to obtain because of unpredictability and very rapid onset of major seizures. In addition, medical personnel and the radiotracer must be available at the patient´s bedside at the time of seizure onset. Postictal ^{18}F-FDG PET/CT scans can be very complex, and it represents a mishmash of increased and/or decreased metabolism, depending on the time of ^{18}F-FDG injection after the onset of seizure.

TABLE 1. Some PET tracers used for imaging of epileptic focus and PET scan findings

PET scan	Findings on PET scan
^{18}F-FDG *interictal*	Decreased metabolism
^{18}F-FDG *ictal*	Increased and decreased metabolism (complex pattern)
^{18}F-FDG *postictal*	Increased and decreased metabolism (complex pattern)
Serotonin receptor (e.g. ^{18}F-Mefway)	Reduced binding
Dopamine receptor (e.g. ^{18}F-Fallypride)	Reduced binding
^{18}F-Flumazenil (GABA receptor)	Decreased binding
^{15}O-H_20 *interictal*	Reduced perfusion
^{15}O-H_20 *ictal*	Increased perfusion

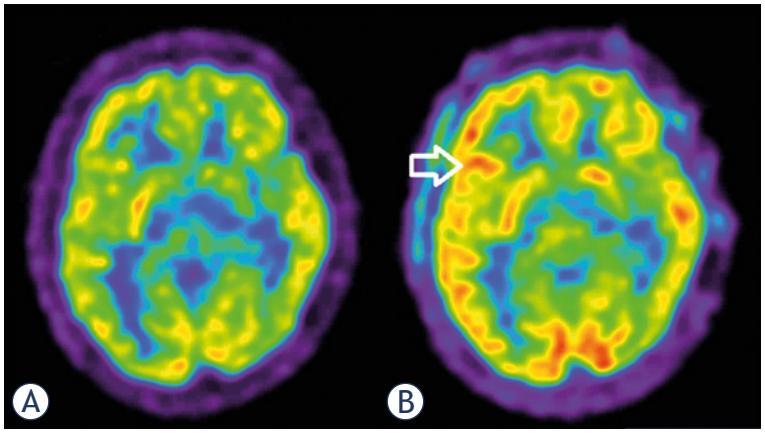

FIGURE 1. Focal epilepsy in 17 year old male patient. Interictal ^{18}F-FDG PET **(A)**: physiological distribution of ^{18}F-FDG in the brain. Ictal ^{18}F-FDG PET **(B)**: hypermetabolism frontolateral in the right hemisphere (arrow).

Interictal ^{18}F-FDG PET/CT in patients with epilepsy

The goal of implementing ^{18}F-FDG PET/CT in the management of patients with drug refractory epilepsy was to obtain functional imaging of interictal brain glucose metabolism. Multiple studies have shown that interictal ^{18}F-FDG PET/CT is more sensitive than interictal perfusion SPECT[33-34,15], because in the interictal period, reduction of regional glucose cerebral metabolism is more pronounced than reduction of cerebral perfusion.[35-37]

Neuronal loss due to chronic seizure activity, reduction in density of synapses, inhibitory processes in the interictal period and diaschisis also influence glucose hypometabolism, the characteristic sign of epilepsy on interictal ^{18}F-FDG PET/CT. Interictal ^{18}F-FDG PET/CT cannot precisely define the surgical margins, because some areas of hypometabolism extend beyond epileptic zones. Drzezga *et al.* showed that automated analysis of ^{18}F-FDG PET/CT scans in patients with epilepsy is more sensitive than visual analysis in patients with temporal lobe epilepsy and extra temporal lobe epilepsy.[36] The combination of MRI and interictal ^{18}F-FDG PET/CT will probably evolve as the future modality of choice to get the best results for presurgical evaluation.

Ictal ^{18}F-FDG PET/CT in patients with epilepsy

Ictal ^{18}F-FDG PET in the presurgical workup of refractory epilepsy is rarely performed; it is performed either in status epilepticus or in a status of induced epileptic seizures. As status epilepticus is defined as seizure longer than 30 minutes or more than one seizure within thirty minutes without the person returning to normal, ^{18}F-FDG PET is difficult to be obtained in those moments. Despite technical difficulties ictal ^{18}F-FDG PET has the advantage of a high spatial resolution. Ictal ^{18}F-FDG PET/CT shows hypermetabolism, although an ictal scan reveals a complex combination of hyper and hypometabolism and for that reason it is important to continuously monitor the patient with scalp EEG. A study from 2013 showed that in patients with incompatible EEG, MRI and clinical features, ictal ^{18}F-FDG PET/CT helped to localize the origin of status epilepticus.[38] Definition of an ictal onset zone is usually made visually with the support of semiquantitative analysis. A difference above 15% between the affected and the contralateral side suggests significant physiological asymmetry.

Unfortunately, ^{18}F-FDG is not an ideal PET tracer regarding the management of patients with epilepsy. Areas of glucose hypometabolism do not correlate with the proven degree of change within hippocampal sclerosis, as observed by histopathology or MRI; the area of hypometabolism may be larger than the pathological seizure focus.[39] So, the presurgical definition of epileptogenic foci cannot be based on ^{18}F-FDG PET/CT imaging alone, because ^{18}F-FDG changes may appear larger than the real ictal onset zone. The reason for this anatomical-functional discrepancy has not been explained yet. However, ^{18}F-FDG PET/CT can reveal and confirm surgical targets and therefore represents a valuable tool in the management of patients with intractable epilepsy.

^{18}F- Flumazenil tracers for PET/CT of patients with epilepsy

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the central nervous system.[40-43] Its main role consists of reducing neuronal excitability and regulating muscle tone. There are two types of GABA receptors: $GABA_A$ and $GABA_B$; benzodiazepine receptors are modulatory sites on $GABA_A$ receptors. Benzodiazepine distribution in the human brain includes the occipital cortex, temporal cortex, cerebellum, thalamus, and the pons. The majority of $GABA_A$ receptors are benzodiazepine-sensitive, but there are also $GABA_A$ receptors which are insensitive to classical benzodiazepines. Benzodiazepines enhance the action of GABA on its receptors, thus having anticonvulsant, sedative, hypnotic, anxiolytic and muscle-relaxant effects.

Flumazenil blocks the benzodiazepine sites on $GABA_A$ receptors and thus antagonizes the action benzodiazepines have on the central nervous system. Flumazenil was introduced in 1987 and over time it was used as an antidote for the treatment of benzodiazepine overdoses. In epileptogenic regions a reduced level of benzodiazepine receptors is found[44]; also, irreversible ischemic cortical damage after stroke[45], Alzheimer disease[46,47] chronic alcoholism[48] and schizophrenia[49] may influence benzodiazepine complex density.

In the 1980s, Flumazenil was proposed as a promising new marker for imaging of benzodiazepine receptors by PET. The substance was ini-

tially labelled with Carbon-11 (^{11}C-FMZ)[50] and used in some studies: the majority of patients with seizures refractory to medical treatment had hippocampal sclerosis characterized by neuronal loss and gliosis, which was detected by this positron-labelled $GABA_A$ receptor antagonist.[51] Six years later a SPECT agent, ^{123}I-iomazenil, was synthetized.[52,53] Both compounds showed high benzodiazepine binding potential in the brain. However, use of ^{11}C-FMZ in clinical practice has been limited by its short half-life, necessitating an on-site cyclotron for production, and multiple syntheses if several subjects have to be examined. The first paper on a fluorinated analogue of flumazenil was published in 1992.[54] ^{18}F-labelled PET radiotracers are more suited for clinical use. Also, the shorter positron range of ^{18}F provides better image resolution, which may enhance the detection of small pathological complexes such as epileptic foci. Following this, several ^{18}F-labelled FMZ tracers, including 5-(2′-[18F]fluoroethyl) flumazenil (^{18}F-FEFMZ), 3-(2′-[^{18}F]fluoroflumazenil (^{18}F-FFMZ), 5-(2′-[18F]fluoroethyl)flumazenil (^{18}F-FEF) and [18F]flumazenil (^{18}F–FMZ) have been developed.[55-60] Compared to ^{11}C-FMZ, ^{18}F-FEFMZ has lower receptor affinity, and higher nonspecific binding due to faster metabolism and faster kinetics. ^{18}F-FFMZ also has faster kinetics than ^{11}C-FMZ. Compared to ^{11}C-FMZ, ^{18}F-FEF has lower affinity to benzodiazepine receptors, rapid kinetics in the brain and faster metabolism.[55-60] ^{18}F-FMZ, with an identical structure to ^{11}C-FMZ, has been shown to have similar pharmacokinetics and peripheral metabolism and PET imaging characteristics as ^{11}C-FMZ.

^{18}F-FMZ has been established as the tracer of choice for patients with refractory epilepsy in some neurological centres in Europe. PET/CT imaging using flumazenil tracers identify a more restricted region of abnormality in the epileptogenic zone than ^{18}F-FDG PET/CT.[61] In comparison to ^{18}F-FDG PET/CT scans benzodiazepine-receptor scans appears "sharper" (Figure 2, 3).

Imaging protocol for PET/CT with ^{18}F-labelled-flumazenil radiotracers is almost similar to protocol for ^{18}F-FDG PET/CT, except no serum glucose level measurement is required. All flumazenil tracers bind non-selectively to all benzodiazepine receptor subtypes, so pre-treatment with unlabelled flumazenil can result in reduced tracer uptake on benzodiazepine receptors. As a young modality, benzodiazepine-receptor PET imaging is still strengthening its place in neurology.

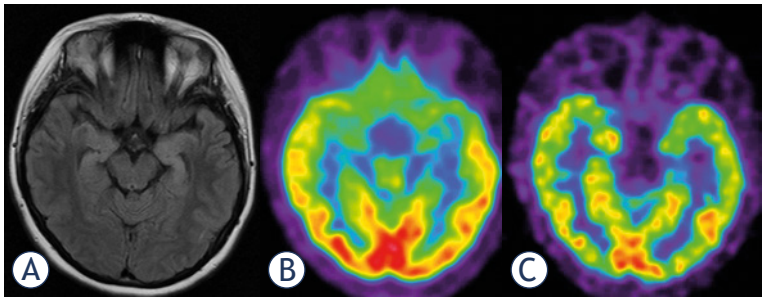

FIGURE 2. ^{18}F-FDG PET **(B)** scan vs. ^{18}F-Flumazenil PET **(C)** in a 19 year old female patient with bilateral hippocampal sclerosis as shown by MRI **(A)**. Both PET modalities present low temporomesial uptake being larger on the left side, but benzodiazepine-receptor imaging appears sharper and presents a focal defect.

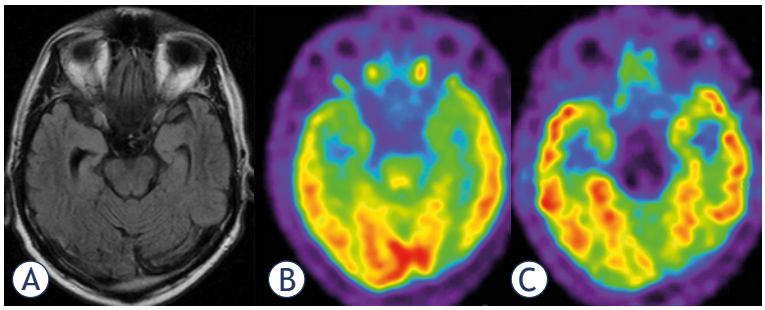

FIGURE 3. Focal epilepsy in 56 year old male patient. **(A)** MRI: astrogliosis of the right hippocampus (later proven by histology); **(B)** ^{18}F-FDG PET: minal temporomesial hypometabolism of both sides; **(C)** ^{18}F-Flumazenil PET: focal defect of tracer uptake at the right hippocampus.

Conclusions

Functional information delivered by PET and the morphologic information delivered by CT or MR are essential in presurgical evaluation of epilepsy. Nowadays ^{18}F-FDG PET/CT is a routinely performed imaging modality in localization of the ictal onset zone in patients with refractory epilepsy who are unresponsive to medication therapy. Unfortunately, ^{18}F-FDG is not an ideal PET tracer regarding the management of patients with epilepsy: areas of glucose hypometabolism do not correlate precisely with the proven degree of change within hippocampal sclerosis, as observed by histopathology or MRI. Benzodiazepine-receptor imaging is a promising alternative in nuclear medicine imaging of epileptogenic focus. The use of ^{11}C-FMZ in clinical practice has been limited by its short half-life and necessitating an on-site cyclotron for production. Therefore, ^{18}F-FMZ might be established as one of the tracers of choice for patients with refrac-

tory epilepsy because of better sensitivity and anatomical resolution.

Multiple new PET tracers for presurgical evaluation of patients with epilepsy are still under preclinical investigations; tracers for neurotransmitter and neuromodulator systems, including the GABA, serotonin, dopamine, glutamate, acetylcholine, adenosine and opioid systems.

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research article

Uncertainties in target volume delineation in radiotherapy – are they relevant and what can we do about them?

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Background. Modern radiotherapy techniques enable delivery of high doses to the target volume without escalating dose to organs at risk, offering the possibility of better local control while preserving good quality of life. Uncertainties in target volume delineation have been demonstrated for most tumour sites, and various studies indicate that inconsistencies in target volume delineation may be larger than errors in all other steps of the treatment planning and delivery process. The aim of this paper is to summarize the degree of delineation uncertainties for different tumour sites reported in the literature and review the effect of strategies to minimize them.

Conclusions. Our review confirmed that interobserver variability in target volume contouring represents the largest uncertainty in the process for most tumour sites, potentially resulting in a systematic error in dose delivery, which could influence local control in individual patients. For most tumour sites the optimal combination of imaging modalities for target delineation still needs to be determined. Strict use of delineation guidelines and protocols is advisable both in every day clinical practice and in clinical studies to diminish interobserver variability. Continuing medical education of radiation oncologists cannot be overemphasized, intensive formal training on interpretation of sectional imaging should be included in the program for radiation oncology residents.

Key words: target volume; interobserver variability; delineation uncertainties; imaging; training

Introduction

Modern radiotherapy techniques such as intensity modulated radiotherapy (IMRT), volumetric modulated arch therapy (VMAT) and image guided adaptive brachytherapy (IGABT) enable delivery of high doses to the target volume without escalating dose to organs at risk (OAR), offering the possibility of better local control while preserving good quality of life.[1,2] Highly conformal radiation techniques and sharp dose falloff make the accuracy and precision of every step in treatment planning and delivery extremely important. Uncertainties in the process of radiotherapy include patient set-up error, inter- and intra-fraction organ movement, patient movement and uncertainties in target volume delineation. Image guided radiation therapy (IGRT) addresses the uncertainties arising from patient set-up, patient and organ movement and improves target localisation during treatment. However, reduction of margins introduced with the use of IGRT is limited by the ability to adequately define the target. Accurate target volume delineation is a precondition for the use of IMRT, VMAT, IGABT and other high precision radiotherapy techniques, since all subsequent steps in treatment planning and delivery are based on target volume contours. Inadequate definition of the target introduces a systematic geographic miss that could potentially lead to reduction of the dose delivered to the tumour, lower local control and/or increased morbidity for an individual patient.[3-6]

In addition, such uncertainties can undermine meaningful comparison of treatments within and between institutions and interpretation of clinical studies.

Uncertainties in target volume delineation have been demonstrated for most tumour sites, and various studies indicate that inconsistencies in target volume delineation may be larger than errors in all other steps of the treatment planning and delivery process.[7-22]

The aim of this paper is to summarize the degree of delineation uncertainties for different tumour sites reported in the literature and review the effect of strategies to minimize them.

Magnitude of uncertainties

Direct comparison of published data is difficult, since a variety of methods is used to quantify interobserver variability. Most papers report parameters describing the distribution of delineated volumes including mean, range, standard deviation (SD), the ratio of the largest and the smallest delineated volume (V_{max}/V_{min}), coefficient of variation (COV) *etc.* Also commonly used are different concordance measures such as conformity, concordance or similarity index (CI, SI – ratio between common and encompassing volume), Dice-Jaccard coefficient (DJC), percent overlap, ratio of encompassing and common volume (1/CI), geographical miss index and mean discordance index or statistical measures of agreement *i.e.* kappa (κ) - statistics.[23] Less commonly, methods for local interobserver variation assessment are used *i.e.* local standard deviation (SD), inter-delineation distance or radial line measurement variation, all expressed in mm.[24-27]

A wide range of interobserver variability is observed for various tumour sites, the largest variation being reported for target volume delineation in oesophageal, head and neck and lung cancer, Hodgkin's lymphoma and sarcoma, where the V_{max}/V_{min} ratios are 6, 18.3, > 7, 15 and > 8, respectively.[3,18,28-30]

Gastrointestinal tumours

In rectal cancer, reported conformity indices are from 0.29 to 0.98 for clinical target volume (CTV) and from 0.26 to 0.81 for primary tumour gross target volume (GTV), depending on the use of consensus guidelines and chosen imaging modality.[19,31-33] The ratio of V_{max} to V_{min} is 1.93 – 2.65 for GTV and 1.75 – 4.71 for CTV depending on imaging modality used for treatment planning.[19] Interobserver variability in CTV and planning target volume (PTV) delineation for gastric cancer was assessed as a part of the CRITICS trial. Despite delineation atlas provided for participants V_{max}/V_{min} ratio was 3.4 for CTV and 2.6 for PTV and the authors speculated the reason was unfamiliarity with target volumes in the upper abdomen.[6] For oesophageal cancer, median Jaccard conformity index for GTV was 0.69 in a study by Gwynne *et al.*[34], with the highest observer agreement in the middle section of the GTV, which is a marked improvement compared to results reported by Tai *et al.*[18] at the start of 3D planning era, when V_{max}/V_{min} ratio was up to 6.

Cervix cancer

Considerable interobserver variability was described by Weiss *et al.*[10] in CTV for cervix carcinoma, with the ratio of common to encompassing volume from 0.11 to 0.57 and V_{max}/V_{min} ratio 1.3 – 4.9. The main reason for large variability was wide variation in caudal and cranial CTV borders, resulting from varying inclusion of specific nodal regions (para-aortic, iliac and inguinal) by the observers. In a study of cervix cancer IGABT, CI was 0.6 – 0.8 for high risk CTV (HR CTV) and 0.6 – 0.7 for GTV and intermediate risk CTV (IR CTV), demonstrating a relatively good interobserver agreement considering that CI is sensitive to volume size and volumes in brachytherapy tend to be much smaller than in EBRT. Mean inter-delineation distance was 4.2 mm, 3.8 mm and 5.2 mm for GTV, HR CTV and IR CTV, respectively.[25,35]

Head and neck tumours

Interobserver variability in CTV delineation in oropharyngeal cancer (tonsillar tumour) is one of the largest described in the literature. With the primary GTV already provided, V_{max}/V_{min} ratio for CTV reported by Hong *et al.*[30] was 18.3. Recommended PTV expansion from the contoured CTV also varied considerably in different institutions (mean 4.11 mm, range 0 – 15 mm). Smaller but still significant variability was reported by Thiagarajan *et al.*[9] for oropharyngeal primary tumour GTV with CI 0.54 – 0.62, depending on imaging modality. Agreement on nodal GTV was higher with CI > 0.75 for all imaging modalities. For nasopharyngeal carcinoma local SD was 3.3 – 4.4 mm for CTV (visible tumour + potential microscopic extension) and 4.9 – 5.9 mm for elective CTV (CTV + 1 cm margin and the entire nasopharynx), depending on imaging modality.[24]

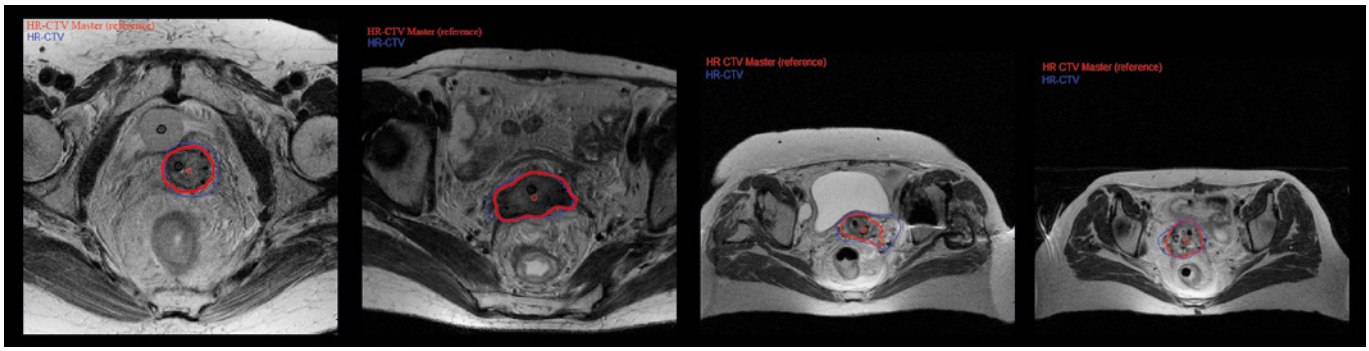

FIGURE 1. MR images showing interobserver variability between an unexperienced RO and the reference contour in IGABT of 4 cervix cancer patients (from a workshop for RO residents at the Institute of Oncology Ljubljana).

Lung tumours

In lung cancer the range of reported interobserver variability is quite large with V_{max}/V_{min} ratio from 1.8 to 2.3 for primary GTV alone and from 5.2 to > 7 for primary and nodal GTV. Reported conformity indices range from 0.04 to 0.70 for the same target volumes, depending on imaging modality, with some authors describing cases where there was no common volume for all observers.[3,14,15,27,36] Like in cervix cancer the reason for large variability is inclusion of different nodal regions in the target volume. In a study by Van De Steene *et al.*[3] the observers included only 63% of involved nodal regions in the target volume (generating 37% false negative nodes), on the other hand 22% of included nodal regions were considered false positive after a review. The authors suggested lack of knowledge being one of the main reasons for interobserver variability, beside problems of methodology (interpretation of GTV definition, drawing precision *etc.*) and difficulty in discriminating the tumour from surrounding pathological (*i.e.* atelectasis, peritumoral reaction) and normal structures (*i.e.* mediastinal vessels).

Other tumour sites

Interobserver variability for target delineation in brain tumours is similar to the one described for prostate with V_{max}/V_{min} ratio from 1.3 to 2.8 and CI from 0.14 to 0.47 depending on imaging modality.[16,37,38] Despite being one of the smallest reported variations, it is still larger than the patient set-up error and/or organ motion.

In prostate interobserver variability for CTV delineation seems to be smaller than in other tumour sites with V_{max}/V_{min} ratio from 1.2 to 1.6, which is probably due to a better circumscribed CTV.[21,26] The largest variation is described at the apex and the base of the prostate.[39,40] Valicenti *et al.*[21] found that interobserver variability is 4 times larger for seminal vesicles delineation compared to prostate delineation.

For breast cancer the largest interobserver variability is reported for lumpectomy cavity with CI from 0.19 to 0.56, followed by CTV with CI from 0.38 to 0.87 and PTV with CI from 0.45 to 0.92.[11-13,41,42] In partial breast brachytherapy CI for lumpectomy cavity ranges from 0.48 to 0.52 and for PTV from 0.55 to 0.59, with V_{max}/V_{min} ratio for all volumes 2.2. – 2.8.[43] Lower CI for lumpectomy cavity compared to other target volumes could be attributed to the fact that lumpectomy cavity is the smallest target volume in postoperative breast carcinoma and CI is sensitive to volume size.

How described interobserver variability affected delivered dose to the target and/or OAR is only reported in a few papers. Steenbakkers *et al.*[44] observed a reduction of mean dose to the rectal wall by 5.1 Gy and to the penile bulb by 11.6 Gy when reducing interobserver variability by using MRI for delineation in EBRT for prostate cancer. Allowing the same dose to OAR as in CT based delineation the dose prescribed to the target volume (prostate) could be escalated from 78 to 85 Gy. With improved target volume delineation due to the use of CT/MRI fusion in nasopharyngeal carcinoma, the mean PTV D_{95} improved from 60 to 69.3 Gy, while D_5 to the brainstem and spinal cord was reduced by 19%, dose to the parotid glands and cochlea was reduced below their dose constraint.[45] In lung cancer the probability of delivering at least 95% of prescribed dose to at least 95% of the target volume was reduced from 96% to 88% when using a plan designed to cover another observer's GTV. Mean interobserver range of irradiated normal tissue volume was 12%, with a maximum variability

of 66%.[3] In cervix IGABT, a mean relative SD of 8-10% in D_{90} for GTV and HR CTV was observed in a single fraction analysis. For bladder and rectum mean relative SD for D_{2cc} was 5 – 8%, whereas for sigmoid it was 11%. When taking into account the whole treatment course, interobserver variability generated an uncertainty of +/-5 Gy ($\alpha\beta$ = 10) for HRCTV and +/-2-3 Gy ($\alpha\beta$ = 3) for OAR.[46]

Strategies to improve target volume delineation

Several strategies to reduce uncertainties in target volume delineation have been proposed by different authors [7,8,25] and there have been a few attempts to implement those strategies to improve quality assurance in clinical trials in radiation oncology.[26,47-49] Three major areas that could contribute to improving the accuracy of target delineation have been identified: optimisation of imaging, implementation of standardized protocols and delineation guidelines and specialized training.

Optimisation of imaging

High quality imaging with reproducible protocols is a pre-requisite for accurate target volume delineation. In the last decades, radiotherapy planning was mostly CT based, recently, new imaging techniques *i.e.* MRI, PET-CT, functional MRI are increasingly being used to improve visibility of the target. Potential advantages of functional imaging modalities are reduction of interobserver variability, indentification of tumour extensions missed by CT and/or MRI and possibly identification of GTV subvolumes requiring higher radiation dose. Even in the absence of modern imaging modalities for treatment planning, simple measures such as the use of intravenous and/or intracavitary contrast, fiducial markers and reproducible imaging protocols can markedly increase the quality of imaging. When contouring, the use of zoom levels, simultaneous viewing in multiple planes (use of sagittal and coronal plane) and use of adequate level and window settings on the planning CT reduce interobserver variability.[50]

In a series of 42 patients with rectal cancer, the use of PET-CT significantly reduced the size of GTV compared to CT alone, better interobserver agreement was observed (mean CI 0.79 *vs.* 0.82 *vs.* 0.93 for CT, PET-CT and PET-CT with auto-contours, respectively). Additionally, in almost one third of patients GTV based on PET-CT extended outside CT based GTV. The addition of MRI to CT did not result in significant improvement of CI.[31] Patel *et al.*[33] also compared CT and PET-CT for delineation of primary and nodal GTV (GTVp and GTVn) in rectal cancer. Similarity index for GTVp was modestly better, but statistically significant on PET CT *e.g.* 0.81 *vs.* 0.77, and notably better for GTVn *e.g.* 0.70 *vs.* 0.22. Several studies show a good correlation between PET-CT and pathology based tumour length in oesophageal cancer[51,52], but to our knowledge, there are no studies comparing interobserver variability on different imaging modalities. The benefit of PEC-CT based delineation was also demonstrated for GTV in lung cancer patients, where registration of PET-CT images with the planning CT improved median interobserver percentage of concordance form 61% to 70% compared to CT alone.[36] In RTOG 0515 trial the lung cancer GTV volumes contoured on PET CT were significantly smaller when compared to CT derived volumes and nodal GTV was altered in over 50% of patients on PET-CT.[53] When compared to pathological findings both CT and PET-CT based contours overestimated tumour size for 46.6% and 32.5%, respectively. Both GTV volumes and maximal tumour diameters were larger on CT.[54]

There are several publications evaluating the effect of addition of PET-CT and/or MRI on interobserver variability in delineation of the GTV or CTV for head and neck tumours.[9,24,55-57] In a study by Daisne *et al.*[55] GTV was contoured on CT, MRI and

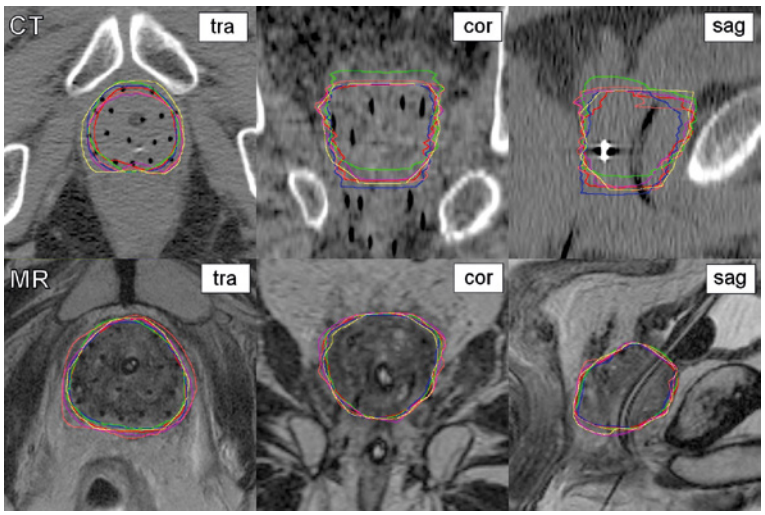

FIGURE 2. Interobserver variability in delineation of the prostate. MR and CT images in different planes of the same patient are shown. Ability to discriminate prostate apex, base and lateral borders is superior on MRI.

cor = coronal; sag = sagittal; tra = transverse[40]

PET-CT in 29 patients with head and neck tumours. Mean GTV volume was not significantly different on CT and MRI, mean GTVs on PET-CT were significantly smaller. For nine patients where surgical specimen after total laryngectomy was available, no imaging modality adequately depicted the extension of the tumour. The average GTVs for anatomic imaging were over 100% larger and for functional imaging almost 50% larger than the surgical specimen. For laryngeal and hypopharyngeal tumours mean GTV volume was 21.4 cm^3 for both CT and MRI, 16.4 cm^3 for PET-CT and 12.6 cm^3 in surgical specimen. PET-CT was the most accurate modality in patients where comparison with the surgical specimen was available. In a similar comparison Thiagajaran *et al.*[9] compared contouring GTVs in oropharyngeal carcinoma on CT + PET *vs.* CT + MR *vs.* CT + PET + MR to a reference contour and found no significant difference in the size of the GTV when contouring using any combination of two imaging modalities. Interobserver agreement between GTV_{CTPET} and GTV_{CTMR} was low, with CI = 0.62. When compared to the reference contour $CI_{CTPETMR}$ was low (0.62), but still significantly higher than CI for either CT + PET or CT + MR (0.54 and 0.55, respectively), which implicates that none of the imaging modalities should be used alone. For nodal GTV CI was > 0.75 for all tested imaging modalities compared to the reference contour, the added benefit to contrast enhanced CT alone was small. Anderson *et al.*[58] also compared CT, PET-CT and MRI for definition of GTV in head and neck tumours. Interobserver variability was present for all imaging modalities, with CT being least consistent. PET-CT derived target volumes were the smallest in size, interobserver agreement was the highest with CI = 0.46, compared to CI = 0.36 and 0.35 for MRI and CT, respectively. In nasopharyngeal carcinoma the use of CT and co-registered MRI decreased local SD from 4.4, to 3.3 mm and from 5.9 to 4.9 mm for CTV and elective CTV, respectively, and resulted in a higher agreement between observers.[24] Two published studies observed no significant difference between observers across imaging modalities when comparing CT to PET-CT and CT to MRI for GTV delineation in head and neck tumours.[56,57]

Giezen *et al.*[41,42] compared CT and MRI for delineation of CTV and lumpectomy cavity (LC) after breast-conserving surgery and found that both imaging modalities provided similar visibility of LC, CI was lower for MRI than for CT, but the difference was not significant. These results have to be interpreted with caution, as the participating radiologists had no experience in LC contouring and the radiation oncologists were not familiar with breast MRI, which gives the results limited value. In postoperative brain gliomas radiotherapy the use of registered CT and postoperative MR images reduced interobserver variability compared to contouring on CT with the aid of preoperative MRI (CI 0.47 *vs.* 0.14, respectively). However, in delineation of inoperable supratentorial brain tumours the addition of MRI did not reduce interobserver variability with V_{max} remaining up to 2.7 times larger than V_{min}.[38] For prostate cancer all studies demonstrate up to almost 75% larger volumes on CT compared to MRI, but while some found better interobserver agreement on MRI others found less interobserver variability on CT, demonstrating that current delineation guidelines might not be applicable to MRI planning.[40,59,60]

Implementation of delineation protocols and guidelines

Delineation guidelines have been published on a national or international level for several tumour sites both in EBRT and BT.[61-67] Different reports show that the use of site specific anatomical atlases, consensus delineation guidelines and standardized contouring protocols diminish variability between observers in various tumour sites.[32,68-71] In rectal carcinoma, the implementation of site specific consensus atlas significantly reduced interobserver variability in a pilot study[68], which was later confirmed in a larger study, in which Nijkamp *et al.*[32] demonstrated that the use of a digital delineation atlas twice or more during target volume contouring significantly improves CI. The addition of delineation guidelines significantly reduced interobserver variation in caudal CTV border (from 1.8 to 1.2 cm) and the size of average CTV volume by 25% (620 *vs.* 460 cc). In lung cancer, re-contouring of the GTV with the use of a protocol, aimed at minimizing variation, reduced the degree of interobserver variation from 20% to 13%. In the second contouring session the differences between observers were not statistically significant.[72] Comparison of contouring seroma cavity in partial breast radiotherapy with and without guidelines showed that radiation oncologists (ROs) contouring without guidelines contoured significantly larger CTVs and PTVs in more than 50% of patients.[69] When all participating ROs were provided with guidelines, the differences in sizes of the target volumes were no longer significant. In breast brachytherapy conformity indices increased significantly with the use of guidelines

TABLE 1. Interobserver variation for various tumour sites

Tumoursite	Target volume	No of pts	No of obs	Imaging modality	Results	Author (publication date)
Rectum	GTV, CTV	2	10	CT, PETCT	CI(GTV) = 0.26-0.33 CI(CTV) = 0.29-0.35	Krengli et al 2010
	GTV	52	5	CT, PETCT, MRI	CI = 0.79-0.93	Bujisen et al 2012
	CTV	8	10	CT	CI = 0.63-0.66	Nijkamp et al 2012
	GTV	6	4	CT, PETCT	SI(GTV-P) = 0.77-0.81 SI(GTV-N) = 0.22-0.70	Patel et al 2007
Stomach	CTV, PTV	1	10	CT	Vmax/Vmin(CTV) = 3.4 Vmax/Vmin(PTV) = 2.6	Jansen et al 2010
Oesophagus	GTV	1	50	CT	JCI = 0.69	Gwynne et al 2012
	GTV, CTV, PTV	1	48	CT	Vmax/Vmin(PTV) = 5.25-6.03	Tai et al 1998
Cervix EBRT	CTV	3	7	CT	CI = 0.11-0.57 Vmax/Vmin = 3.6-4.9	Weiss et al 2003
IGABT	GTV, HRCTV, IRCTV	6	10	MRI	CI(GTV) = 0.6-0.8 CI(HR&IRCTV) = 0.6-0.7	Petrič et al 2012, 2013
Head and neck	GTV,CTV,PTV	1	20	CT	Vmax/Vmin(CTV) = 18.3	Hong et al 2012
	GTV	41	3	CT, PETCT, MRI	CI(GTV-P) = 0.54-0.62 CI(GTV-N)>0.75	Thiagajaran et al 2012
	CTV, CTVE	10	10	CT, MRI	localSD(CTV) = 3.3-4.4mm localSD(CTVe) = 4.9-5.9mm	Rasch et al 2012
Lung	GTV	12	8	CT, CBCT	CI = 0.27-0.39 CIgen = 0.58-0.70	Altorjai et al 2012
	GTV	8	5	CT	Vmax/Vmin>7	Van De Steene et al 2002
	GTV	10	17	CT	Vmax/Vmin = 5.2 CI = 0.04-0.48	Giraud et al 2002
	GTV	22	11	CT, PETCT	meanCI = 0.17(CT),0.29(PETCT) localSD = 1cm(CT),0.4cm(PETCT)	Steenbakers et al 2006
	GTV	19	2	CT, PETCT	medianCI(CT) = 0.61, medianCI(PETCT) = 0.70	Fox et al 2005
Brain	CTV	7	5	CT + MRI	CI = 0.14-0.47	Cattaneo et al 2005
	GTV	5	9	CT, MRI	Vmax/Vmin(CT) = 1.7-2.8 Vmax/Vmin(MR) = 1.5-2.7	Weltens et al 2001
Prostate	Prostate, seminal vesicles (SV)	10	7	CT	Vmax/Vmin(P) = 1.18-1.63 Vmax/Vmin(SV) = 2.02-6.43	Valicenti et al 1999
	Prostate	3	2	CT	Vmax/Vmin = 1.39-1.65	Seddon et al 2000
	Prostate	5	5	CT, MRI	MeanCI(MR)CI = 0.83 MeanCI(CT) = 0.69	Segedin et al 2011
Breast	Lumpectomy cavity (LC), CTV	15	3	CT, MRI	CI(LC) = 0.32(MR),0.52(CT) CI(CTV) = 0.77(CT),0.79(MR)	Giezen et al 2011,2012
	Lumpectomy cavitiy	30	5	CT	MeanCI = 0.36	Boersma et al 2012
	Lumpectomy cavity, CTV, PTV	8	13	CT	CI(LC) = 0.19-0.77 CI(CTV) = 0.38-0.80 CI(PTV) = 0.45-0.81	VanMourik et al 2010
	Lumpectomy cavity, PTV	9 5	5 4	CT	CI(LC) = 0.48-0.52 CI(PTV) = 0.55-0.59 Vmax/Vmin = 2.2-2.8	Major et al 2015
	Lumpectomy cavity, CTV	18	5	CT	MeanCI(LC) = 0.56 MeanCI(CTV) = 0.87	Struikmans et al 2005

CI = conformity/concordance index; CTV = clinical target volume; GTV = gross target volume; local SD = local standard deviation; max = maximum; min = minimum; obs = observers; pts = patients; PTV = planning target volume; SI = similarity index; V = volume;

both for lumpectomy cavity contours and PTV. The increase was 14% and 11% for the cavity and 28% and 17% for PTV on preimplant and post-implant CT images, respectively.[43] Even for site-specialized ROs, a reduction in interobserver variability was noticed in CTV delineation for postprotatectomy radiotherapy when adhering to the RADICALS trial delineation protocol.[71] Mean V_{max}/V_{min} for all cases was reduced from 3.7 at first delineation to 2.0 at the second delineation.

Training

A survey of radiotherapy planning and delivery undertaken in the UK in 2007 showed a lack of formal education in target volume and OAR delineation in different staff groups.[73] Only 4% of NHS radiotherapy departments offered structured training on image interpretation, while 6% offered informal sessions with radiologists. 90% of participating ROs stated they wanted formal training in interpretation of cross sectional imaging and almost 85% were interested in online training modules. More than half of junior ROs considered their training in cross sectional imaging to be inadequate

Some publications evaluated the effect of clinical experience on interobserver variability, the results, however, were ambiguous.[15,37,74] While Hurkmans *et al.*[74] reported that more experienced ROs delineate smaller volumes than unexperienced in breast carcinomas, Giraud *et al.*[15] found experienced ROs to delineate larger volumes than their younger colleagues in lung carcinoma. In brain tumours, Leunens *et al.* found no significant difference between experienced and unexperienced ROs.[37] Only a few publications have addressed the subject of training, some in the course of pre-accrual quality assurance delineation exercises (dummy run).[26,34,47-49,75,76] In dummy run for a randomised multicentre PET-plan clinical trial in lung cancer, they found considerable differences despite providing detailed contouring guidelines. After a teaching session at a study group meeting, they observed an improvement in overall interobserver agreement, demonstrated by reduction of target volumes and an increase in kappa (κ) indices for GTV and two CTVs (0.63 *vs.* 0.71, 0.60 *vs.* 0.65 and 0.59 *vs.* 0.63, respectively).[48] Similarly, Khoo *et al.* reported reduced encompassing to intersecting volume ratio (VR) at re-contouring the prostate after education sessions focusing on MRI prostate anatomy with CT correlation. Mean VR was reduced by 15% for CT (from 2.74 to 2.33) and 40% for MRI (from 2.38 to 1.41).[49] Dewas *et al.*[75], however, found no significant difference for delineation of the target volumes in lung cancer before and after training. The residents κ- indices were lower compared to senior ROs both before and after the training, V_{20} for lung was higher in the residents group. The authors speculated there was no improvement because initial delineations by the residents were good. However, they offered no hands-on training for the residents and most reports showing improvement included hands-on training in their educational sessions. During training, special attention needs to be payed to predilection areas for larger interobserver variability, identified in available literature.[25,26,30,39,40]

Conclusions

The main goal of improving accuracy in radiotherapy treatment planning and delivery is better local control with less morbidity, resulting in better quality of life. Our review shows that interobserver variability in target volume contouring represents the largest uncertainty in the process for most tumour sites, potentially resulting in geographic miss in dose delivery, which could hamper local control for individual patients. Studies on use of multimodality imaging and image co-registration show promising results, however, for most tumour sites the optimal combination of imaging modalities still needs to be determined. Strict introduction and use of imaging and delineation protocols and guidelines reduces interobserver variability, therefore it is advisable in every day practice and mandatory in the frame of clinical studies. Especially in multicentric studies, efforts to unify target volume delineation in different institutions in a dummy run should be maximized as interobserver variability influences reliability of dose reporting, comparison of treatment outcomes and interpretation of study results, hence diminishing the value of a study. To assure adherence to study protocols and delineation guidelines, a central reviewing board for contour correction is useful. Continuing medical education of ROs cannot be overemphasized, intensive formal training on interpretation of sectional imaging should be included in the program for radiation oncology residents. In the fields, where the other conditions are fulfilled (recommendations on imaging for treatment planning, delineation guidelines), a study systematically assessing the effect of training on interobserver variability is warranted.

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research article

Non-contrast computed tomography in the diagnosis of cerebral venous sinus thrombosis

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Background. The aim of the study was to investigate the sensitivity and specificity of non-contrast computed tomography (NCCT) in the diagnosis of cerebral venous sinus thrombosis (CVST).

Methods. Screening our neurological department database, we identified 53 patients who were admitted to neurological emergency department with clinical signs of CVST. Two independent observers assessed the NCCT scans for the presence of CVST. CT venography and/or MR venography were used as a reference standard. Interobserver agreement between the two readers was assessed using Kappa statistic. Attenuation inside the cerebral venous sinuses was measured and compared between the patient and the control group.

Results. CVST was confirmed in 13 patients. Sensitivity and specificity of NCCT for overall presence of CVST were 100% and 83%, respectively, with Kappa value of 0.72 (a good agreement between observers). The attenuation values between CVST patients and control group were significantly different (73.4 ± 14.12 HU *vs.* 58.1 ± 7.58 HU; p = 0.000). The ROC analysis showed an area under the curve (AUC) of 0.916 (95% CI, 0.827 – 1.00) and an optimal cutoff value of 64 HU, leading to a sensitivity of 85% and specificity of 87%.

Conclusions. NCCT as a first-line investigation has a high value for diagnosis of CVST in the emergency setting. The additional measurement of the sinus attenuation may improve the diagnostic value of the examination.

Key words: cerebral venous sinus thrombosis; computed tomography; stroke

Introduction

Due to the diversity of underlying factors and the absence of a uniform treatment approach, diagnosis and management of patients with cerebral venous sinus thrombosis (CVST) remain a challenging task.[1] CVST represents 0.5% to 1% of all strokes and affects approximately 5 patients per million every year, but has a higher frequency among younger patients.[1-3] Typical acquired risk factors include recent surgery, trauma, pregnancy, postpartum state, antiphospholipid syndrome, cancer and use of oral contraceptives. Cases of inherited thrombophilia include Antithrombin III, Protein C and Protein S deficiency, factor V Leiden positivity, prothrombin gene mutation and hyperhomocisteinemia.[1-4] Infection of parameningeal spaces (ears, paranasal sinuses, oral cavity, face and neck) is common cause of CVST in pediatric population[2,5,6], but rare in adults.[6]

The symptoms of CVST are not specific. The most common complaint is headache which occurs in up to 90% of patients.[7] Additionally, abnormal vision, any of the symptoms of stroke and seizures have been described.[7] In the past D-dimer levels appeared to be of value as an initial screening test. A study in 2004 evaluated the sensitivity of D-dimer to be 97.1% and specificity 99.1%.[8] However, later studies showed that up to 10% of patients with CVST have a normal D-dimer.[9]

As it is fast, affordable and widely available, non-contrast computed tomography (NCCT) is the most frequently performed imaging study for evaluation of patients with new headache, focal neuro-

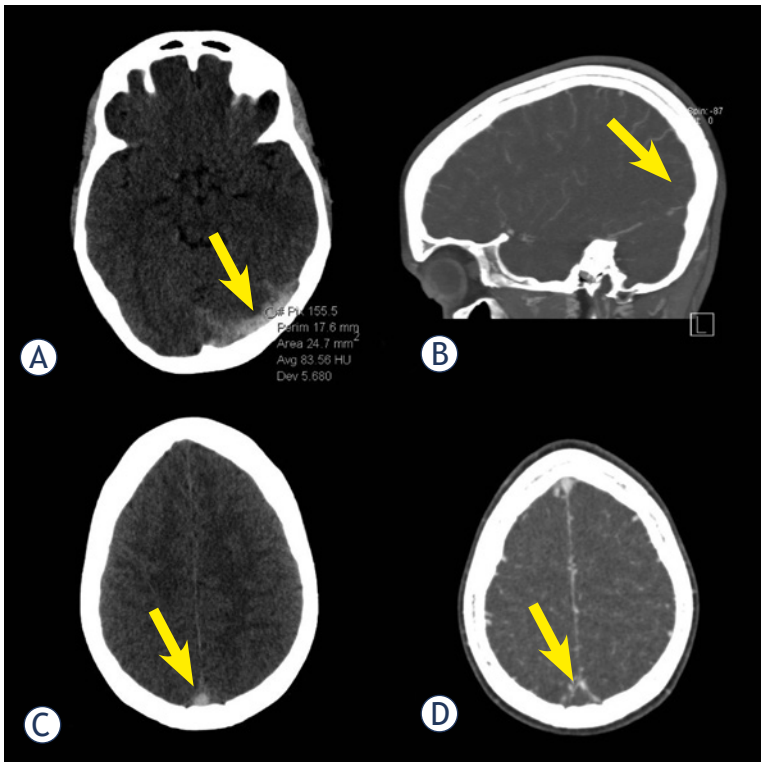

FIGURE 1. 19-year-old female with thrombosis (arrow) of the left transverse sinus (LTS) **(A)** and superior sagittal sinus **(C)**, confirmed by CT venography in sagittal **(B)** and axial **(D)** reconstruction. Average attenuation inside the LTS was 83.6 HU.

logical abnormalities, seizure, or change in mental status. A typical imaging finding in patients with CVST is direct visualization of a hyperattenuating thrombus in the occluded sinus (dense sinus sign).[2,3] Occasionally, NCCT may only show indirect signs of thrombosis, including diffuse brain edema and parenchymal hemorrhage.[10] Sensitivity of NCCT in the diagnosis of CVST was previously considered rather poor.[2,10-13] However, using modern multidetector row CT scanners, recent studies report higher sensitivity and specificity values.[14] In addition, Buyck *et al.* suggest measurement of the venous sinus attenuation to increase the diagnostic yield of the examination.[15] Therefore, the goal of the present study was to evaluate the diagnostic accuracy of NCCT in the diagnosis of CVST in the emergency setting.

Patients and methods

The study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board Committee.

Subjects

Screening our neurological department database, we retrospectively identified 53 patients (37 women, 16 men; mean age, 42 years; age range, 17—82 years) who were admitted to neurological emergency department from July 2008 to May 2013 with clinical signs of CVST. The following inclusion criteria were defined: NCCT had to be performed on admission and at least one of the reference imaging modalities, *i.e.* CT venography and/or MR venography had to be performed in 24 hours from admission.

CT image acquisition

All CT images were acquired on Somatom Sensation 40 Open system (Siemens, Erlangen, Germany). The following scanning parameters were used: 120 kV, 220 mAs, section thickness of 3 mm below the tentorium and 120 kV, 260 mAs and section thickness of 4.8 mm above the tentorium.

Image interpretation

NCCTs were assessed for the presence of direct and indirect signs of CVST by two experienced neuroradiologists, using a standard picture archiving and communication system (PACS) workstation. Observers were blinded to the clinical data and patient identification. Readings were randomized, and standardized evaluation forms were used to ensure the systematic evaluation of the following structures: superior sagittal sinus (SSS), straight sinus (SS), inferior sagittal sinus (ISS), right and left transverse sinuses (RTS, LTS), and right and left sigmoid sinuses, (RSS, LSS). Additionally, right and left internal cerebral vein (RICV, LICV), vein of Galen (VG), right and left basal vein of Rosenthal (BVR), right and left thalamostriate vein (TSV) and cortical veins were assessed. Attenuation inside the thrombosed venous sinus was measured. If no venous structure was classified as thrombosed, mean attenuation of up to three venous sinuses that could be reliably differentiated from surrounding brain parenchyma was documented. Presence of parenchymal hemorrhage or edema was also noted. Finally, observers had to decide regarding the overall presence or absence of CVST and rate their diagnostic confidence on a scale from 1 (absolutely certain) to 5 (uncertain).

After having evaluated all NCCTs, readers reviewed all available imaging data, including follow-ups of any respective patient to obtain a ref-

erence standard. In this manner, they determined the overall presence of CVST, the involvement of individual venous structures and the presence of parenchymal hemorrhage or edema.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Macintosh, Version 20.0. (IBM Corp., Armonk, NY, USA) software package. Based on collected data, we calculated the specificity and sensitivity of NCCT for diagnosis of CVST. Agreement between the two observers was assessed using Kappa statistic.[16]

Patients in whom the diagnosis of CVST was excluded were used as a control group for the second part of the study. Average venous sinus attenuation value of individual subject from the CVST patient group and the control group was used for statistical evaluation. Comparison between the two groups was made by unpaired t-test. Receiver operating characteristic curves (ROC) were used to define optimal cutoff value for which sensitivity and specificity was calculated. A p value < 0.05 was considered significant.

Results

CVST was confirmed in 13 patients (9 women, 4 men; mean age, 36.5 years; age range, 19 – 82 years). Patients presented with headache (12 patients, 93%), papiloedema (7 of 9 evaluated patients, 78%), objective neurological deficit (8 patients, 62%), vomiting (8 patients, 62%), somnolence or stupor (6 patients, 46%), confusion (5 patients, 38%), disturbance of vision (3 patients, 23%) and epileptic seizures (2 patients, 15%). Risk factors of patients with CVST included oral contraception (6 of 9 female patients), pregnancy, puerperium or recent abortion (4 of 9 female patients). Laboratory values were retrospectively available for 12 of 13 patients. Thrombophilia was found in 6 of 12 evaluated patients (50%). Average D-dimer value was 2644 mmol/L, but it was normal in 2 of 12 patients (17%). CRP levels were elevated in 9 of 12 patients (75%).

Transverse sinus was the most commonly thrombosed structure, followed by sigmoid sinus (Table 1). Typical NCCT findings of CVST are depicted in Figure 1. All 13 patients with CVST were accurately diagnosed by both observers. Therefore, the calculated sensitivity was 100% in both readings, with specificity of 80 and 87.5%, respectively. Kappa value regarding the presence or absence of thrombosis was 0.72, which is considered to represent a good agreement between observers. Sensitivity and specificity of NCCT for overall presence of thrombosis were 100% and 83%. Average diagnostic confidence level regarding the presence or absence of CVST was 2.1 ± 1.2 (very certain). A case with false positive finding is depicted in Figure 2.

TABLE 1. Location of thrombus and parenchymal changes in 13 patients with cerebral venous sinus thrombosis (CVST)

Patient No.	Location of thrombus	Parenchymal changes
1	RTS, RSS	None
2	LTS	Hemorrhage, edema
3	SSS, RTS, RSS	None
4	RTS	None
5	SSS, RTS, RSS	None
6	SSS, LTS, LSS, right ICV, left ICV, VG	None
7	RTS, RSS	None
8	RTS, RSS	None
9	LTS, LSS	None
10	SSS, RTS, RSS, cortical veins	None
11	SSS, LTS, LSS	Hemorrhage, edema
12	RTS	Hemorrhage, edema
13	SSS, cortical veins	Hemorrhage, edema

ICV = internal cerebral vein; LSS = left sigmoid sinus; LTS = left transverse sinus; RSS = right sigmoid sinus; RTS = right transverse sinus; SSS = superior sagittal sinus; VG = vein of Galen

Attenuation values were available for all 13 CVST patients and 23 controls and significant difference was found between the two groups (Figure 3). The ROC analysis of the attenuation showed an area under the curve (AUC) of 0.916 (95% CI 0.827, 1.00) and an optimal cutoff value of 64 HU, leading to a sensitivity of 85% and specificity of 87%.

Discussion

CVST is a distinct cerebrovascular disorder that, unlike arterial stroke, most often affects young adults and children and is associated with significant morbidity and mortality, especially when the diagnosis is not made in time.[17] In order to initiate appropriate therapy as soon as possible, early diagnosis is essential.

The clinical presentation of CVST is highly variable. In our series, headache was the most frequently reported symptom. Similar to recent study by Linn *et al.*[14], there was no single symptom or sign, present in all patients with CVST.

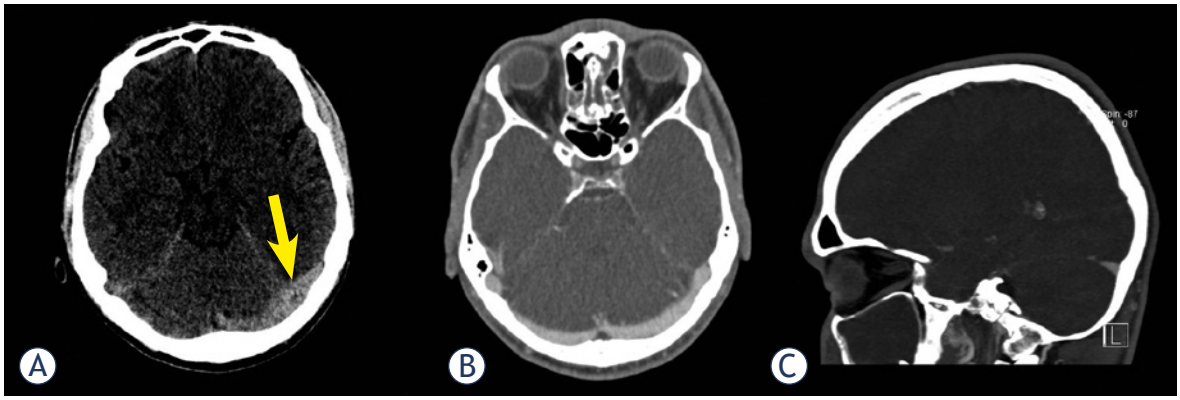

FIGURE 2. Left transverse sinus (LTS) of a 26-year-old male appeared hyperattenuated on non-contrast computed tomography (arrow) **(A)** and was interpreted as thrombosed by both readers. However, CT venography in axial **(B)** and sagittal **(C)** reconstruction showed patency of the LTS. Average attenuation inside the LTS was 60 HU.

Normal D-dimer levels have previously been considered to have a high negative predictive value in patients with suspected CVST.[7] Recently, normal D-dimer levels were reported in patients with isolated thrombosis of deep cerebral venous system and were explained by the relatively small thrombus volume.[14] However, we found normal D-dimer levels in 2 patients with CVST, suggesting that D-dimer is of limited value in excluding the diagnosis.

The American Heart Association (AHA)/ American Stroke Association (ASA) 2011 Scientific Statement on diagnosis and management of cerebral venous thrombosis recommends imaging of the cerebral venous system in patients with suspected cerebral venous thrombosis.[1] Recently, MRI in combination with MR venography has largely replaced digital subtraction angiography as a gold standard for imaging of CVST[17] and is currently considered the most sensitive examination technique.[18,19] In situations when MRI is not readily available, CT venography has been shown to be equivalent to MRI in establishing the diagnosis.[20] Nevertheless, in most institutions NCCT remains the first-line imaging method in the emergency evaluation of patients with unspecific neurological symptoms[14], because of its cost-effectiveness and availability.[15]

Using a blinded reader approach, we examined the value of NCCT in diagnosis of CVST, based on presence or absence of classic hyperattenuating signs. These are observed on NCCT scans when an acute thrombus has formed in a blood vessel. The increase in attenuation is caused by clot retraction, eliminating water and thereby raising the concentrations of red blood cells and hemoglobin. This mechanism results in increased attenuation of the thrombus to 60 – 90 HU.[15] In CVST, hyperattenuating sign (dense sinus sign) can serve as a unique finding indicating an acute stage, at a time when treatment is most likely to be effective and to have a significant effect on clinical outcome.[21] In the past, sensitivity and specificity of NCCT was considered rather low[11-13], and according to the literature, direct signs of CVST were present in only one third of patients.[11] A study from 1987 reported a sensitivity of approximately 25%.[22] However, using modern multidetector row CT scanner, recently reported sensitivity of the direct signs for CVST was 64.6%, which was higher than reported in older studies[13,22], but still insufficient in excluding the diagnosis.[14] Authors of this work emphasized the value of NCCT in diagnosis of deep venous thrombosis, reporting the 100% sensitivity and 99.4% specificity in this subgroup of patients. In our series however, there was only one patient who, in addition to venous sinus thrombosis, also had deep venous system thrombosis (Table 1).

Our study revealed higher sensitivity (100%) of NCCT for overall presence of thrombosis than previously reported (25% – 64%).[13,14,22] Specificity, on the other hand, was lower than expected (83.8%). The observers, although blinded to clinical and imaging data, knew the purpose of the study, which may partially explain these results. Also, if the venous structure was recognized as thrombosed, it was considered a positive finding even in cases, where diagnostic confidence was low (4 or 5 on our scale). Interestingly enough, if we interpreted these cases as negative, the sensitivity and specificity would be 88.5% and 95%, which is closer to previously published data.

Apparent increase in attenuation of venous sinuses can be misleading and is not always visually

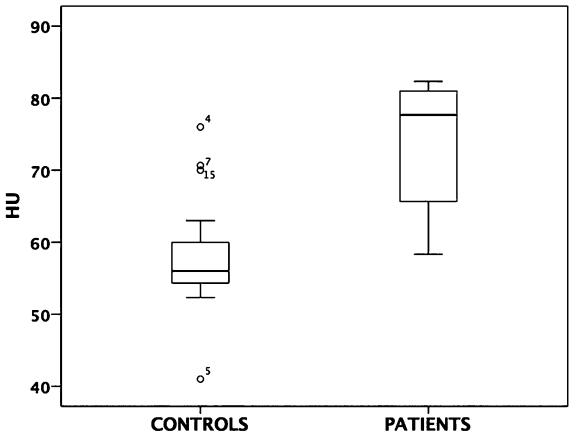

FIGURE 3. The attenuation values between cerebral venous sinus thrombosis (CVST) patients and control group were significantly different (73.4 ± 14.12 HU *vs.* 58.1 ± 7.58 HU; p = 0.000). HU, Hounsfield units.

recognized, therefore recently additional measurement of the sinus attenuation was proposed to increase the sensitivity of the examination in the diagnosis of CVST.[15] In our series, mean attenuation of 73.4 HU was found in thrombosed venous sinuses. This degree of increased attenuation was previously found in clotted sinuses of patients with CVST[15,23], as well as in acute thrombosis elsewhere in the body.[23] Significant difference in the average sinus attenuation was found between the CVST patient group and the control group. However, an overlap between the two groups and the presence of outliers in the control group (Figure 3) may limit the reliability of this method in some patients. Our results are similar to the findings of Buyck *et al.*, who suggested a threshold of 62 HU to differentiate the patients with CVST from those without.[15] Based on our data, the optimal threshold was 64 HU. The drawback of this method is the possibility of false negative finding in patients with anemia who have low attenuation of blood due to low hemoglobin.[15] Similarly, the most common cause of false positive readings is the high attenuation of blood in patients with high hemoglobin or hematocrit level.[24] These limitations may partially be avoided by calculating the Hounsfield unit-to-hematocrit ratio. However, the improvement in accuracy with this method has been shown to be minor.[15] Additionally, such calculation may often be impractical in emergency setting and was therefore not included in our study.

We acknowledge the following limitations of our work. Relatively small size of the studied population was a drawback. However, CVST is a relatively uncommon disease and the size of our group is comparable to previously published studies. Furthermore, the impact of relatively low number of patients was minimized using the blind and multiple observer approach. DSA was not performed and so the consensus reading of CT venography or MR venography was used as a reference standard. Nonetheless, the diagnostic value of these methods for the diagnosis of CVST has been shown to be very high.[25,26]

In conclusion, prompt therapy of CVST has a profound impact on clinical outcome, therefore early diagnosis is important. Our study has shown high sensitivity of NCCT for diagnosis of CVST and thus confirmed the role of NCCT as a investigation of choice in the emergency setting. The additional measurement of the sinus attenuation may improve the diagnostic value of the examination and help decide on the need for confirmatory study.

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research article

Recurrence rate in regional lymph nodes in 737 patients with follicular or Hürthle cell neoplasms

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Background. Preoperative ultrasound (US) evaluation of central and lateral neck compartments is recommended for all patients undergoing a thyroidectomy for malignant or suspicious for malignancy cytologic or molecular findings. Our aim was to find out how frequent was recurrence in regional lymph nodes in patients with follicular or Hürthle cell neoplasm and usefulness of preoperative neck US investigation in patients with neoplasm.

Patients and methods. Altogether 737 patients were surgically treated because of follicular or Hürthle cell neoplasms from 1995 to 2014 at our cancer comprehensive center, among them 207 patients (163 females, 44 males; mean age 52 years) had thyroid carcinoma.

Results. Carcinoma was diagnosed in follicular and Hürthle cell neoplasm in 143/428 and 64/309 of cases, respectively. A recurrence in regional lymph nodes occurred in 12/207 patients (6%) during a median follow-up of 55 months. Among patients with carcinoma a recurrence in regional lymph nodes was diagnosed in follicular and Hürthle cell neoplasms in 2% and 14%, respectively (p = 0.002). Recurrence in regional lymph nodes was diagnosed in 3/428 of all patients with follicular neoplasm and 9/309 of all patients with Hürthle cell neoplasm.

Conclusions. Recurrence in lymph nodes was diagnosed in 0.7% of patients with a preoperative diagnosis of follicular neoplasm and 3% of patients with a Hürthle cell neoplasm. A recurrence in regional lymph nodes is rare in patients with carcinoma and preoperative diagnosis of follicular neoplasm. Preoperative neck ultrasound examination in patients with a follicular neoplasm is probably not useful, but in patients with Hurtle cell neoplasm it may be useful.

Key words: thyroid neoplasms; ultrasonography; recurrence; diagnosis; pathology

Introduction

High-resolution ultrasound (US) examination with a 10–13 MHz linear probe detected thyroid nodules in 68% of randomly selected individuals and 18% of nodes were larger than 10 mm.[1] Diagnostic fine needle aspiration biopsy (FNAB) and cytology of a thyroid nodule is recommended for nodules ≥ 1cm with a high suspicion US pattern.[2,3] The Bethesda system for reporting thyroid cytopathology is used to report thyroid nodule FNAB cytology.[4,5] The Bethesda IV category comprises follicular neoplasm or suspicious for a follicular neoplasm and also encompasses the diagnosis of Hürthle cell neoplasm/suspicious for Hürthle cell neoplasm. Confirmation of malignancy in these types of primary thyroid tumors is possible only with histological examination of the tumor.[4,5] Demonstration of transcapsular and/or vascular invasion confirms malignancy.[4,5] Risk of malignancy in Bethesda IV tumors was 26% among 2751 patients.[6] At our institute the risk of malignancy was 33% and 25% in follicular and Hürthle cell neoplasms, respectively.[7,8]

According to the National Cancer Comprehensive Network guidelines for patients with thyroid cancer a preoperative US examination of central and

lateral neck compartments is recommended for all patients undergoing thyroidectomy for malignant or suspicious for malignancy cytological findings.[5] Our aim was to find out the frequency of recurrence in regional lymph nodes in patients with carcinoma who had preoperative diagnosis of follicular or Hürthle cell neoplasm and to estimate usefulness of preoperative US examination of the neck region in patients with follicular or Hürthle cell neoplasm.

Patients and methods

Altogether 737 patients were surgically treated because of follicular (N = 428) or Hürthle cell (N = 309) neoplasms as shown by cytology from 1995 to 2014 at our cancer comprehensive center. Altogether 207 patients (163 females, 44 males; mean age 52 years, range 12–84 years) had thyroid carcinoma in a dominant nodule as shown by a definitive histopathology. According to the TNM classification system using the UICC criteria from 2009 pT1, pT2, pT3 and pT4 tumor was diagnosed in 69 patients, 68, 64 and 6 patients, respectively.[9] Metastases in regional lymph nodes and distant metastases were diagnosed in 2 and 10 patients, respectively. A majority of patients were treated by total or near-total thyroidectomy (87%) and radioiodine (RAI) ablation of the thyroid remnant (91%) followed by a suppressive therapy with L-thyroxine. None of our patients had central or lateral neck dissection during thyroidectomy.

All the patients with carcinoma were followed-up at our institute at least once a year (median follow-up period 55 months, range 6–180 months). The follow-up consisted of a medical history, physical examination and determination of serum thyroglobulin (Tg) concentration and Tg antibodies. The criteria for disease-free survival were: Tg levels of less than 1 ng/mL, negative whole-body RAI scans, and exclusion of cervical lymph node metastases detected by US as defined by 2009 American Thyroid Association guidelines.[2] Neck US was always performed within first 6 months after surgery because of thyroid cancer. Neck US was performed at least once per year in case of positive Tg antibodies. Imaging (X-ray, US, CT, MRI, bone scintigraphy, PET-CT and/or RAI scintigraphy) was performed whenever Tg concentration was elevated or clinical symptoms of possible recurrence were present in order to determine the site and extent of the suspected recurrence.

A retrospective chart review of all patients with neoplasms was carried out and data about preoperative US neck examination and recurrence in regional lymph nodes was collected for this study. Predictive factors for the presence of carcinoma in follicular (N = 388) and Hürthle cell (N = 279) neoplasms in our patients have already been published.[7,8] Twelve of them had thyroid lesions incidentally detected by 18F-FDG PET-CT.[10]

A follicular or Hürthle cell neoplasm was diagnosed by FNAB and cytology. FNABs were performed by an endocrinologist, radiologist, and/or cytopathologist using a 21–23-gauge needle attached to a 10-mL syringe. All cytological slides were examined by cytopathologists and histological slides by pathologists experienced in thyroid pathomorphology. Routine cytological and final pathology reports from our Institute were used in this study.

Preoperative US examination of central and lateral neck compartments were performed in 27 patients with carcinoma who had a preoperative diagnosis of follicular or Hürthle cell neoplasm. Benign cervical LN were sonographically seen as oval shaped, well defined structures with different amount of hilar fat, hilar type of vascularization and transversal diameter in upper regions limited to 9 mm. US criteria for malignant lymph node were: marked hypoechogenicity, rounded shape, absent hilum and irregular, blurred, angular or invasive margins, and presence of microcalcifications. Doppler criteria for malignant lymph node were: peripheral flow, multiple vascular pedicles, chaotic vascular pattern and high impedance values. Probability of malignancy is higher with increasing number of malignant features.

The study was reviewed and approved by the Institutional Review Board and Medical Ethics Committee and was performed in accordance with the medical ethics standards laid down in an appropriate version of the 1964 Declaration of Helsinki. All our patients were asked during the first admission to our institute or a follow-up visit to give consent to use their charts and biopsy material for scientific purposes. Since the Institutional Review Board of the Institute of Oncology Ljubljana approved this specific study, our patients were not asked to give written consent on this specific study.

Statistical analysis

The chi-square test was used to compare the observed and expected frequencies of recurrence in regional and lymph nodes in patients with follicular and Hürthle cell neoplasms. A p-value < 0.05

was considered as statistically significant. SPSS 16.0 for Windows was used for statistical analysis.

Results

Carcinoma was diagnosed in follicular and Hürthle cell neoplasms in 143/428 (33%) and 64/309 (21%) of cases, respectively. A follicular variant of papillary thyroid carcinoma, Hürthle cell carcinoma, follicular carcinoma, classical type of papillary carcinoma and other types of papillary carcinoma were diagnosed in 90, 50, 39, 21 and 7 cases, respectively (Table 1). Mean size of carcinoma was 3.46 cm (range 0.4–11 cm). Microcarcinoma in a dominant nodule was diagnosed in only 8.2% of patients.

Twelve patients had focal thyroid lesions incidentally detected by 18F-FDG PET-CT. Five of them had carcinoma (2 follicular carcinoma, 2 papillary carcinoma and 1 medullary carcinoma), while seven patients had benign tumor (5 adenoma, 2 multinodular goiter). Median tumor size in malignant and benign tumor was 30 mm and 15 mm, respectively. Median maximal standardized uptake value in malignant and benign tumor was 11 and 6.4, respectively.

Recurrence in regional lymph nodes

Recurrence in lymph nodes was diagnosed in 3/428 (0.7%) of patients with a preoperative diagnosis of follicular neoplasm and 9/309 (3%) of patients with a Hürthle cell neoplasm.

Among patients with carcinoma, a recurrence in lymph nodes was detected in 2% and 14% of those who had preoperative diagnosis of follicular and Hürthle cell neoplasms, respectively (p = 0.002). Recurrence rate in regional lymph nodes according to subtype of carcinoma, tumor size and pT tumor stage is shown in Table 2.

Neither primary tumor diameter or pT tumor stage was correlated with a recurrence in regional lymph nodes. Primary tumor diameter was 4 cm or more in 4/12 patients with a recurrence in regional lymph nodes. A recurrence in regional lymph nodes was diagnosed in only 2/12 patients earlier than three years after a thyroidectomy and radioiodine ablation of the thyroid remnant. These two patients had a Hürthle cell neoplasm and concentration of Tg remained elevated after initial treatment. One of them had a very aggressive Hürthle cell carcinoma which had a regional recurrence in lymph nodes 6 months after a thyroidectomy and radioiodine ablation and bone metastases 11 months thereafter.

TABLE 1. Distribution of follicular and Hürthle cell neoplasms and carcinoma subtype

Carcinoma type	Type of neoplasm		Total
	Follicular neoplasm	Hürthle cell neoplasm	
Follicular	31	8	39
Hürthle cell	12	38	50
Papillary - classical variant	16	5	21
Papillary - follicular variant	81	9	90
Papillary - other variant*	3	4	7
Total	143	64	207

* Oncocytic variant in 4 cases, trabecular variant in 2 cases, poorly differentiated thyroid carcinoma in 1 case

TABLE 2. Recurrence rate in regional lymph nodes and subtype of carcinoma, pT, pN, M and tumor size in 207 patients with malignant follicular or Hürthle cell neoplasm

Carcinoma type	Total	Recurrence in regional lymph nodes		p-value
		No	Yes	
Follicular	39	37	2	0.008
Hürthle cell	50	42	8	
Papillary – classical variant	21	20	1	
Papillary – follicular variant	90	89	1	
Papillary – other variant	7	7	0	
pT1	69	69	0	0.067
pT2	68	62	6	
pT3	64	59	5	
pT4	6	5	1	
pN0	205	194	11	0.113
pN1	2	1	1	
M0	197	188	9	0.014
M1	10	7	3	
Tumor diameter 0.4-4 cm	145	137	8	0.75
Tumor diameter 4.01-11 cm	62	58	4	
Total	207	195	12	–

TABLE 3. Preoperative neck US and recurrence in regional lymph nodes in patients with follicular and Hürthle cell neoplasms (Fisher's exact test p = 0.296)

Type of neoplasm	Recurrence in regional lymph nodes		Total
	No	Yes	
Follicular	19	0	19
Hüthle cell	7	1	8
Total	26	1	27

From our cohort of patients, these two cases are the only ones in whom a preoperative US examination would have shown the presence of metastases in regional lymph nodes and therefore the treatment would have been changed.

After initial treatment altogether 8/12 patients had suppressed thyroglobulin (Tg) < 1 ng/mL, TSH-stimulated Tg < 10 ng/ml, or stable or decreasing levels of Tg antibodies and an absence of structural

disease. Two of the remaining four patients had distant metastases soon after a regional relapse.

Preoperative neck US examination

Preoperative US examination of the neck region was performed in 27 patients with thyroid carcinoma (Table 3): in 12 , 6, 5, 1 and 3 cases with a follicular variant of papillary carcinoma, Hürthle cell carcinoma, follicular carcinoma, classical type of papillary carcinoma and other types of papillary carcinoma, respectively. In patients who underwent preoperative US examination of the neck region no pathological lymph nodes were detected. However, a recurrence in regional lymph nodes was diagnosed in one of 26 patients six years after thyroid surgery. This patient had a follicular variant of papillary carcinoma.

In only 2 of 12 patients with a recurrence in regional lymph nodes it was diagnosed earlier than three years after thyroidectomy. After modified radical neck dissection in one of them, there is no evidence of disease. However, the other one had a very aggressive Hürthle cell carcinoma in whom a preoperative US would probably have shown the presence of metastases in regional lymph nodes which could have changed the treatment and possibly his outcome. However, it is very doubtful if, in the remaining 10 patients with a recurrence, metastases in regional lymph could have been detected with a preoperative US examination. Among patients with a recurrence in regional lymph nodes after a thyroidectomy and radioiodine ablation of the thyroid remnant, altogether 8/12 patients had suppressed thyroglobulin (Tg) < 1 ng/mL, TSH-stimulated Tg < 10 ng/ml, or stable or decreasing Tg antibody levels or the presence of structural disease. Therefore we believe that there are only two patients from our cohort of patients in whom a preoperative US examination would have shown the presence of metastatic lymph nodes which would probably have changed the treatment and his outcome.

Discussion

Preoperative US examination of central and lateral neck compartments is recommended for all patients undergoing a thyroidectomy because of malignant or suspicious for malignancy cytology. Namely, cervical lymph node metastases were found in as many as 50% of patients with carcinoma.[11-13] Because preoperative US examination of the neck region detects metastases in cervical lymph nodes in 23–33% of patients the surgical approach is changed.[14,15] In the literature, there is very limited data on the frequency of involvement of cervical lymph nodes in patients with follicular or Hürthle cell neoplasms. Paunovic *et al.* reported the presence of regional lymph node metastases in 2% of patients with a Hürthle cell neoplasm.[16]

Our study was observational and not randomized, thus it is not possible to draw conclusions about the impact of preoperative US examination on the surgical approach or patients' outcomes. However, it has shown in a large dataset of patients that recurrence in regional lymph nodes was detected in only 0.7% of patients with preoperative diagnosis of a follicular neoplasm and 3% of patients with a Hürthle cell neoplasm. Based on these findings our opinion is that preoperative US examination of the neck region is not useful in patients with a follicular neoplasm. On the other hand, in patients with a Hürthle cell neoplasm preoperative US examination of the neck region may be useful.

A recurrence in regional lymph nodes occurred in 6% of our patients with a carcinoma during a median follow-up of 55 months. The outcomes of our patients are in accordance with reports from the literature. During a median follow-up period of 7 years after a total thyroidectomy and radioiodine ablation of the thyroid remnant a recurrence rate was reported to occur in low risk patients in 3%, in intermediate risk patients in 21%, and in high risk patients in 68%.[17] In papillary thyroid carcinoma (PTC), a recurrence was diagnosed in papillary microcarcinoma in 1–3% of patients.[18,19] The ten-year regional recurrence rate of T1, T2, and T3 patients with PTC was 1.9, 4.6, and 8.1%, respectively.[20] In our patients with PTC a recurrence in regional lymph nodes was diagnosed in only 2 of 118 (1.7%) patients. But it should be stressed that the median follow-up in our study was only 55 months. It is well known that in thyroid carcinoma recurrences occur years after initial treatment.[20-22]

Our study confirmed the well-known fact that the recurrence rate in regional lymph nodes is common in Hürthle cell carcinoma.[23-26] It was diagnosed in as much as 16% of patients with Hürthle cell carcinoma. The recurrence rate in our patients is comparable to reports in the literature. Khafif *et al*., Stojadinovic *et al*. and Mills *et al*. reported that locoregional recurrence occurred in 10.5%, 16% and 34% of cases, respectively.[23-25]

Conclusions

Preoperative neck ultrasound examination in patients with a follicular neoplasm is probably not useful, but in patients with Hurtle cell neoplasm it may be useful. Recurrence in lymph nodes was diagnosed in 0.7% of patients with a preoperative diagnosis of follicular neoplasm and 3% of patients with a Hürthle cell neoplasm. A recurrence in regional lymph nodes is rare in patients with carcinoma and preoperative diagnosis of follicular neoplasm.

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research article

Electrochemotherapy with bleomycin is effective in BRAF mutated melanoma cells and interacts with BRAF inhibitors

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Background. The aim of the study was to explore the effectiveness of electrochemotherapy (ECT) during the treatment of melanoma patients with BRAF inhibitors. Its effectiveness was tested on BRAF mutated and non-mutated melanoma cells *in vitro* and in combination with BRAF inhibitors.

Materials and methods. ECT with bleomycin was performed on two human melanoma cell lines, with (SK-MEL-28) or without (CHL-1) BRAF V600E mutation. Cell survival was determined using clonogenic assay to determine the effectiveness of ECT in melanoma cells of different mutation status. Furthermore, the effectiveness of ECT in concomitant treatment with BRAF inhibitor vemurafenib was also determined in BRAF mutated cells SK-MEL-28 with clonogenic assay.

Results. The survival of BRAF V600E mutated melanoma cells was even lower than non-mutated cells, indicating that ECT is effective regardless of the mutational status of melanoma cells. Furthermore, the synergistic interaction between vemurafenib and ECT with bleomycin was demonstrated in the BRAF V600E mutated melanoma cells.

Conclusions. The effectiveness of ECT in BRAF mutated melanoma cells as well as potentiation of its effectiveness during the treatment with vemurafenib *in vitro* implies on clinical applicability of ECT in melanoma patients with BRAF mutation and/or during the treatment with BRAF inhibitors.

Key words: electrochemotherapy; BRAF inhibitors; vemurafenib; melanoma

Introduction

Recently, there have been great advances in the treatment of metastatic melanoma, including targeted therapy with BRAF and MEK inhibitors, and immunomodulation either with anti-CTLA-4 or anti-PD-1 or anti-PD-L1 antibodies.[1-5]

BRAF inhibitors are based on the fact that 50% of melanoma tumors harbor BRAF V600 mutations, which cause increased activation of MAP kinase signaling pathway that results in melanoma cell proliferation.[6] Inhibitors of BRAF V600 mutated melanoma, such as vemurafenib and dabrafenib, increase progression-free survival and overall survival[1,7,8], but unfortunately the resistance mechanisms usually appear to re-establish the signaling pathway and the disease progresses in the matter of months after the start of treatment.[9]

For the progressive disease after treatment with BRAF inhibitors, other locoregional treatments are usually needed to control tumor relapses. Electrochemotherapy (ECT) provides this approach. It is a combined local treatment in which locally-applied high voltage electric pulses are used to facilitate the uptake of non-permeant chemotherapeutic drugs.[10] It is used mainly for the treatment of cutaneous and subcutaneous metastases of different tumor histology, with a complete and objective response rate of 59.4% and 84.1%, respectively.[11] Specifically for melanoma, the com-

plete and objective response rates of 56.8% and 80.6% were obtained.[11]

The unexplored question is whether ECT is effective in BRAF mutated melanoma cells, and whether ECT is effective as concomitant treatment to therapy with BRAF inhibitors. In the first reported case regarding treatment of a patient undergoing dabrafenib treatment, ECT proved to be effective on progressing tumor nodules.[12] Based on this report, studies on effectiveness of ECT with bleomycin in BRAF V600 mutated, compared to non-mutated melanoma cells are warranted. Besides this aim, we also investigated whether the concomitant vemurafenib treatment of cells can affect the effectiveness of ECT in melanoma cells *in vitro*.

Materials and methods

Cell lines and culturing

Human melanoma cell line SK-MEL-28 (American Type Culture Collection (ATCC), Manassas, VA, USA) with BRAF V600E mutation was cultivated in an Advanced MEM medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 5% fetal bovine serum (FBS, Gibco), 10 mM L-glutamine (GlutaMAX, Gibco), 100 U/ml penicillin (Grünenthal, Aachen, Germany) and 50 μg/ml gentamicin (Krka, Novo mesto, Slovenia) in a 5% CO_2 humidified incubator at 37°C.

Human melanoma cell line CHL-1 (ATCC) without BRAF V600 mutations was cultured in an Advanced RPMI 1640 medium (Gibco), supplemented with 5% FBS, 10 mM L-glutamine, 100 U/ml penicillin and 50 μg/ml gentamicin in a 5% CO_2 humidified incubator at 37°C.

Electrochemotherapy (ECT)

Melanoma cells were grown as a monolayer until they reached at least 80% confluence. The medium was removed and the cells were washed with phosphate-buffered saline (PBS, Merck Millipore, Darmstadt, Germany). After that, cells were detached from the surface with 0.25% trypsin/EDTA in Hank's buffer (Gibco). After detachment, trypsin was inactivated with an equal amount of cell culture medium with FBS and cells were collected and centrifuged. For electroporation, 88 μl of cell suspension (25×10^6 cells/ml) was prepared in electroporation buffer (125 mM sucrose, 10 mM K_2HPO_4, 2.5 mM KH_2PO_4, 2 mM $MgCl_2 \times 6H_20$) at 4°C. Cell suspension was mixed with 22 μl of different stock concentrations of bleomycin (Bleomycin medac, Medac, Wedel, Germany) to reach a final concentration of 1.4×10^{-12} M, 1.4×10^{-11} M, 1.4×10^{-10} M, 1.4×10^{-9} M, 1.4×10^{-8} M, 1.4×10^{-7} M, 1.4×10^{-6} M. Out of 110 μl of mixture, 50 μl served as a control for bleomycin treatment and other 50 μl was pipetted between two stainless steel parallel plate electrodes (2 mm apart) and 8 square-wave electric pulses (amplitude over distance ratio of 1300 V/cm, duration of 100 μs and frequency of 1 Hz) were applied. Electric pulses were generated with the electric pulse generator GT-01 (Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia). The cells were incubated 5 min after electroporation at room temperature, and then cell culture medium was added. Afterwards, clonogenic assay was performed. Experimental groups were denoted: BLM (different concentrations of bleomycin); BLM + EP (ECT with bleomycin of different concentrations). Each group was normalized to the control group of same treatment regimen with 0 M bleomycin (Ctrl, EP).

Clonogenic assay

After the treatment, cells were plated in 6 cm petri dishes with 4 ml of culture medium for clonogenic assay. The number of plated cells was in a range of 300-4000 cells, based on predicted cytotoxicity of the treatment. Colonies were formed after 12 and 8 days for SK-MEL-28 and CHL-1, respectively. After the colonies were formed, they were fixed and stained with crystal violet solution (Sigma-Aldrich, St. Louis, MO, USA) and counted. The colonies containing less than 50 cells were disregarded. Plating efficiency was calculated for each experimental group as the ratio between counted colonies and the number of plated cells. Plating efficiency of each treatment group was normalized to the untreated cells group, representing cell survival. IC_{90} value was determined (drug concentration required to reduce cell survival for 90%).

Vemurafenib sensitivity

Cell lines were tested for their sensitivity to vemurafenib treatment in order to confirm the selectivity of vemurafenib on BRAF V600E mutated cells and to determine the optimal concentration for the combination treatment. Cells were detached from cell culture dishes and prepared for clonogenic assay. Vemurafenib (MedChem Express, Monmouth Junction, NJ, USA) was obtained in 10 mM DMSO solution and was added to 6 cm culture dishes with 4 ml of culture medium to reach a final concentra-

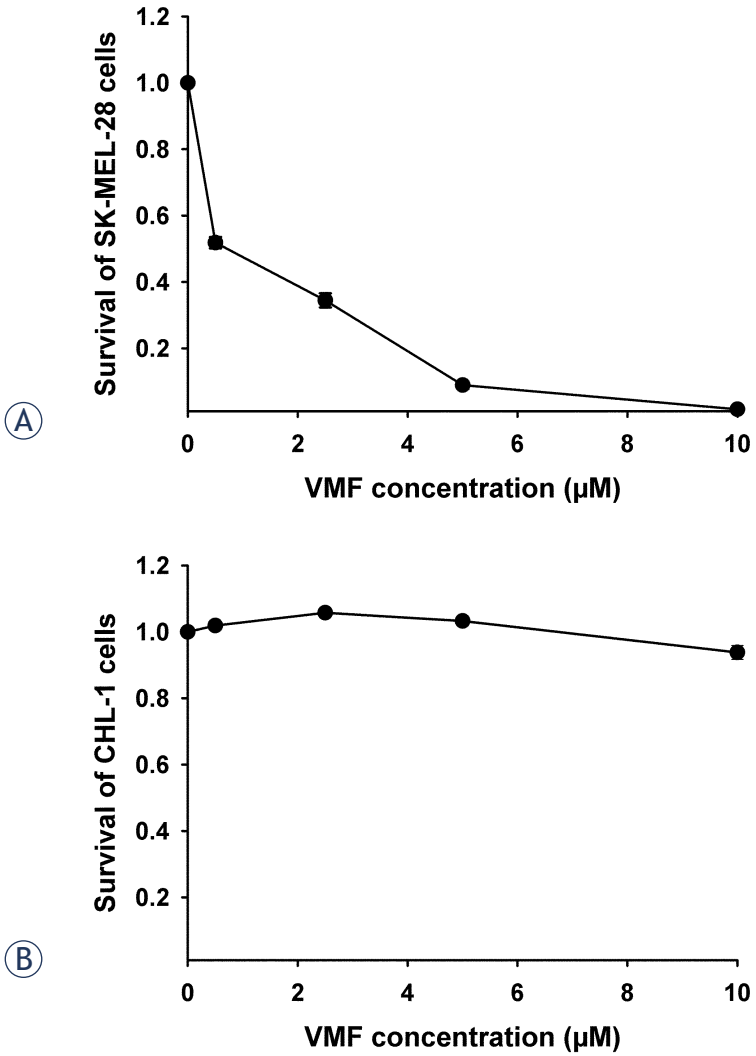

FIGURE 1. Survival of melanoma cells after vemurafenib (VMF) treatment. Survival of **(A)** BRAF V600E mutated melanoma cells SK-MEL-28 and **(B)** non-mutated melanoma cells CHL-1.

tion of 0.5 μM, 2.5 μM, 5 μM and 10 μM. The final amount of DMSO in the cell culture medium represented 20000x — 1000x dilution and was not toxic to the cells. Afterwards, cells were cultured in a 5% CO_2 humidified incubator at 37°C until the formation of colonies, which were counted and analyzed as described. Each group was normalized to the control group with 0 μM vemurafenib.

Combination of ECT and vemurafenib

ECT was performed as described. After that, cells were plated in 6 cm petri dishes for clonogenic assay and vemurafenib was added in each dish to reach a final concentration of 0.5 μM. Afterwards, cells were cultured in a 5% CO_2 humidified incubator at 37°C until the formation of colonies, which were counted and analyzed as described. Experimental groups were denoted: BLM (different concentrations of bleomycin); BLM + EP (ECT with bleomycin of different concentrations); BLM + VMF (bleomycin of different concentrations and addition of 0.5 μM vemurafenib), BLM + EP + VMF (ECT with bleomycin of different concentrations and addition of 0.5 μM vemurafenib). Each group was normalized to the control group of same treatment regimen with 0 M bleomycin (Ctrl, EP, Ctrl + VMF, EP + VMF).

Statistical analysis

The mode of interaction between the treatments with independent mode of action was calculated at the level of IC_{90} by the method developed by Spector.[13] For the analysis and graphical representation, SigmaPlot Software (version 12.0, Systat Software, London, UK) was used.

Results

Sensitivity of BRAF V600E mutated melanoma cells to vemurafenib

Vemurafenib treatment was tested for selective cytotoxicity to BRAF V600E mutated cells. Selectivity of BRAF inhibitor vemurafenib was confirmed on SK-MEL-28 BRAF V600E mutated melanoma cells. Cell survival was reduced with increasing vemurafenib concentration with IC_{90} 5 μM (Figure 1A). In contrast, vemurafenib treatment did not reduce the survival of BRAF V600E non-mutated cell line CHL-1. The cell survival was higher than 94%, also with the highest concentration of vemurafenib used (Figure 1B). These results also provided bases for the combined vemurafenib and ECT treatment of cells, the concentration of 0.5 μM was selected, which reduced cell survival to 50%.

BRAF V600E mutated melanoma cells are more sensitive to ECT than non-mutated melanoma cells

The effectiveness of ECT was tested on BRAF V600E mutated melanoma cells (SK-MEL-28) and non-mutated melanoma cells (CHL-1). ECT effectively reduced survival of both cell lines with IC_{90} 3.8×10^{-10} M and 7.7×10^{-10} M for SK-MEL-28 and CHL-1, respectively. ECT was even more effective on BRAF V600E mutated SK-MEL-28 cells that required 2 times lower concentration of BLM

at IC_{90},compared to non-mutated cells CHL-1 (Figure 2), confirming that ECT is an effective method for treatment of also BRAF V600E mutated melanoma cells.

Concomitant vemurafenib treatment increased ECT effectiveness

Concomitant treatment with ECT and vemurafenib was simulated *in vitro* on cells in the way, that ECT treated cells were seeded into dishes containing 0.5 μM vemurafenib. The vemurafenib treatment decreased survival of SK-MEL-28 cells for 50%. If the effect of the vemurafenib was eliminated (normalized to control groups with added vemurafenib for groups BLM + VMF and BLM + EP + VMF), as shown in Figure 3, then an increased effectiveness of ECT was observed on BRAF mutated SK-MEL-28 cells. A 4.5 times lower concentration of BLM was needed at the IC_{90} for cells treated with vemurafenib (BLM + EP + VMF; IC_{90} value 8.5 x 10^{-11} M) compared to cells without vemurafenib treatment (BLM + EP; IC_{90} value 3.8 x 10^{-10} M). The potentiation was more than additive (Figure 3), in fact, according to the method developed by Spector *et al.* was synergistic.[13]

Discussion

This study demonstrates the effectiveness of ECT with bleomycin on BRAF V600E mutated melanoma cells. The effectiveness was higher to that on BRAF non-mutated cells. Furthermore, an interaction of ECT and vemurafenib treatment was observed in BRAF mutated melanoma cells, indicating on more than additive or synergistic effectiveness.

BRAF inhibitors provide a clear benefit to patients with disseminated disease. The effect is mediated by inhibition of cell proliferation through the inhibition of the MAPK pathway.[14] Patients often present with multiple metastases, that may not all respond to the treatment either due to their bigger size or due to the development of the resistance to BRAF inhibitors. In such cases additional therapy is needed, that has proven effectiveness also in BRAF mutated melanoma cells. Our study demonstrates that ECT with BLM is as effective, or even more effective on BRAF mutated, compared to non-mutated melanoma cells, although the exact biological mechanism still needs to be explored. The data support the recent observation on melanoma patient undergoing dabrafenib treatment

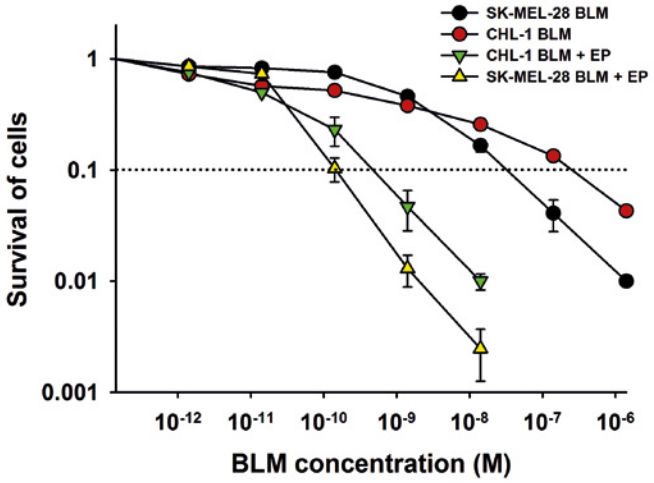

FIGURE 2. Survival of BRAF V600E mutated SK-MEL-28 melanoma cells and non-mutated CHL-1 cells after ECT with bleomycin. BLM (bleomycin); BLM + EP (ECT with bleomycin). Dotted line represents the IC_{90} value.

where some nodules were treated with ECT. ECT proved effective even on tumor nodules, which were in progression during the dabrafenib treatment[12], which indicates that it could be effective also on dabrafenib resistant tumor clones.

The other aspect that was clarified is that ECT can be successfully implemented also during the treatment with BRAF inhibitors. The *in vitro* results demonstrated that vemurafenib and ECT treatment has synergistic effectiveness. This is of clinical importance, since the *in vitro* data indicate that there is no need to wait for the discontinuation of

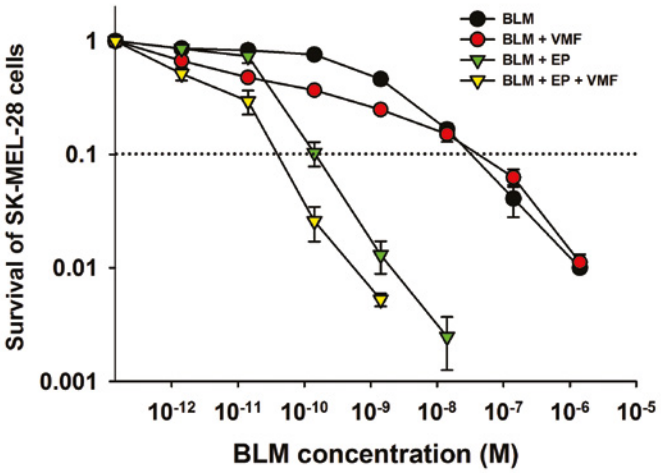

FIGURE 3. Survival of BRAF V600E mutated SK-MEL-28 melanoma cells after concomitant treatment with ECT and vemurafenib. BLM (bleomycin); BLM + VMF (bleomycin and 0.5 μM vemurafenib); BLM + EP (ECT with bleomycin); BLM + EP + VMF (ECT with bleomycin and 0.5 μM vemurafenib). Dotted line represents the IC_{90} value.

treatment with BRAF inhibitors and can be given concomitantly. Based on the observation of the Valpione *et al*, the tolerability of the combined treatment is of great importance, as was observed in the described case.[12] The possibility of the enhanced effectiveness further supports the approach to use ECT concomitantly during the treatment with BRAF inhibitors; however, further clinical studies with larger number of patients are needed to fully support the fact that the combined treatment does not cause additional undesired side effects. If the interaction of the treatments will be observed also *in vivo*, this may lead also to reduction of BLM dosage in ECT.

The interaction of BRAF inhibitors with radiotherapy has also been demonstrated, both on tumor and normal tissue. *In vitro* study demonstrated radiosensitization of BRAF mutated melanoma cells with BRAF inhibitor PLX-4032 by clonogenic and invasion assay and was associated with enhancement of G1 cell cycle arrest.[15] Furthermore, the combination of BRAF inhibitor and irradiation was proven to be effective also in high-grade gliomas, harboring BRAF V600E mutation. Radiosensitization was observed by PLX-4720 BRAF inhibitor *in vitro*, whereas in BRAF non-mutated glioma cells the radiosensitizing effect was not observed.[16] The clinical studies, however, demonstrated the radiosensitization of normal tissue as well. There is still not consensus whether the normal tissue damage is acceptable[17], or that they are so severe that this requires profound investigations in the future.[18]

Eventually ECT will most probably find its place in combination with systemic treatments, with targeted therapies as well as with recently emerging immune checkpoint inhibitors. ECT was recently evaluated also combined with the immunotherapeutic approaches, immune checkpoint inhibitors. The first clinical study of Mozillo *et al.* reported on safety of the combined ipilimumab and ECT with good therapeutic responsiveness.[19] The second report also demonstrated effectiveness and safety of a sequential treatment with ECT plus ipilimumab, which induced a durable complete response of multiple cutaneous metastases with vitiligo-like lesions indicating on involvement of immune response.[20] Obviously ECT is progressing into concomitant treatment with melanoma targeted therapies and immunotherapies, therefore its safety and effectiveness needs to be established. Specifically, due to induction of the immunogenic cell death induced in tumors[21], ECT of tumors may serve as *in situ* vaccination that could be boosted by concomitant immunotherapeutic approach.[22] The consequences may be both, the potentiated local response and also increased side effects.

In conclusion, the effectiveness of ECT in BRAF mutated cells implies on clinical applicability of ECT in BRAF mutated melanoma tumors. Furthermore, its effectiveness also during the treatment with BRAF inhibitors was demonstrated, with synergistic effectiveness. These results are encouraging, but need to be extended to more cell lines, and *in vivo* studies on experimental tumors, evaluating both the tumor and normal tissue response. The study should also be extended to patient-derived melanoma cell lines and also on clones which develop resistance to the therapy with BRAF inhibitors to verify if ECT maintains its effectiveness on the resistant clones. Such studies will then predict the tumor response, and possible side effects.

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research article

Discovery of 'click' 1,2,3-triazolium salts as potential anticancer drugs

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Background. In order to increase the effectiveness of cancer treatment, new compounds with potential anticancer activities are synthesized and screened. Here we present the screening of a new class of compounds, 1-(2-picolyl)-, 4-(2-picolyl)-, 1-(2-pyridyl)-, and 4-(2-pyridyl)-3-methyl-1,2,3-triazolium salts and 'parent' 1,2,3-triazole precursors.

Methods. Cytotoxic activity of new compounds was determined by spectrophotometric MTT assay on several tumour and one normal cell line. Effect of the selected compound to bind double stranded DNA (ds DNA) was examined by testing its influence on thermal stability of calf thymus DNA while its influence on cell cycle was determined by flow cytometric analysis. Generation of reactive oxygen species (ROS) was determined by addition of specific substrate 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA).

Results. Parent triazoles were largely inactive, while some of the triazolium salts were highly cytotoxic for HeLa cells. Triazolium salts exhibited high cell-type dependent cytotoxicity against different tumour cells. Selected compound (4-(4-methoxyphenyl)-3-methyl-1-(2-picolyl)-1*H*-1,2,3-triazolium hexafluorophosphate(V) (2b) was significantly more cytotoxic against tumour cells than to normal cells, with very high therapeutic index 7.69 for large cell lung carcinoma H460 cells. Additionally, this compound was similarly cytotoxic against parent laryngeal carcinoma HEp-2 cells and their drug resistant 7T subline, suggesting the potential of this compound in treatment of drug resistant cancers. Compound 2b arrested cells in the G1 phase of the cell cycle. It did not bind ds DNA, but induced ROS in treated cells, which further triggered cell death.

Conclusions. Our results suggest that the 'click' triazolium salts are worthy of further investigation as anti-cancer agents.

Key words: triazoles; triazolium salts; anticancer activity; cell cycle; ROS

Introduction

Cancer is one of the major causes of death in developed countries.[1] Despite the fact that several decades of investigations and massive funding have been devoted to the cancer research, the decrease in cancer mortality is relatively modest.[2] Based on numerous different genes implicated in sustained proliferative signalling, evading growth suppression, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis, cancers exhibit

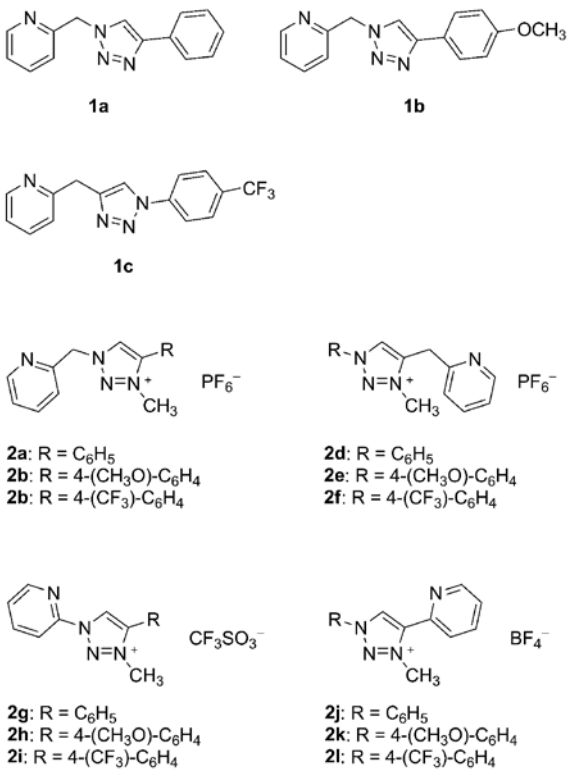

FIGURE 1. The structures of triazoles 1 and triazolium salts 2.

considerable genetic complexity and genome instability, which generates the genetic diversity.[2,3] Consequently, amongst all diseases, cancer is one of the most difficult to treat and cure, because it is not a single disease but rather consists of numerous different types and subtypes.

Targeted therapy with novel drugs directed at specific molecular pathways opens promising new avenues to improve the efficacy of therapy. However, in spite of the initial enthusiasm, the clinical application of such target oriented anticancer drugs did not fulfil the expectations. Although the targeted therapy has been successful, it is still limited to some very specific types of tumours.[3] Hence, in order to increase the effectiveness of cancer treatment, new compounds with potential anticancer activities have to be synthesized and screened.

Modern synthetic chemistry is powered by facile synthetic protocols that allow for a rapid generation of compound scaffolds. Catalyzed azide-alkyne cycloaddition reaction producing 1,2,3-triazoles ('click' triazoles) is one of such example.[4,5] Moreover, this chemistry paves the way to a variety of derivatives including 1,3,4-trisubstituted 1,2,3-triazolium salts ('click' triazolium salts).[6] 1,2,3-Triazole has drug-like properties[7] and its derivatives are recognized for their broad range of biological activities, including antiviral, antibacterial, antifungal, anti-inflammatory and analgesic, anticonvulsant, antiparasitic, antidiabetic, antiobesitic, antihistaminic, antineuropathic, antihypertensive, and anticancer activities[8], presenting a promising group of potential anticancer drugs. Despite the fact that there are numerous examples on cytotoxic activities of compounds with a triazole subunit[9-15], to our knowledge, only one such report exists for the 1,2,3-triazolium salts. Namely, Shrestha and Chang reported an interesting anticancer activity of the compounds having triazolium ring fused to 1,4-naphthoquinone.[16] No cytotoxic activity of the 1,3,4-trisubstituted 1,2,3-triazolium salts has been reported to date.

We recently developed a strategy for the 'click' triazolium salts synthesis, and prepared a library of twelve isomeric and homologous pyridine tethered 1,2,3-triazoles (1), which were then subjected to the selective N-methylation into the 3-methyl-1,4-disubstituted 1,2,3-triazolium salts 2a – l.[17,18] The library constituents differed in the 1,4-substitution pattern consisting of phenyl, 4-methoxyphenyl and 4-(trifluoromethyl)phenyl functionalized 1-(2-picolyl) (2a – c), 4-(2-picolyl) (2d – f), 1-(2-pyridyl) (2g – i) and 4-(2-pyridyl) (2j – l) isomers (Figure 1).

The unique properties of this class of compounds, including the charge and hydrogen bonding ability, prompted us to evaluate their anticancer activity and to gain more insight into the mode of action that underlies their antiproliferative activity.

Materials and methods

Triazoles and triazolium salts

Triazoles 1 and triazolium salts 2 were prepared as described previously.[17,18] Compounds 1b, 1f, 2b, 2c, 2f – i and 2l were dissolved in ethanol while compounds 1a, 2a, 2d, 2e, 2j and 2k were dissolved in DMSO. The solutions were stored at -20°C, and diluted to the appropriate concentrations with growth medium just before use.

Cell culture

Human cervical carcinoma HeLa and laryngeal carcinoma HEp-2 cells were obtained from cell culture bank (GIBCO BRL, Invitrogen, Grand Island, NY, USA). HEp-2 cell line was recently recognized and categorized by ATCC as human laryngeal carcinoma cell line cross-contaminated with HeLa cells. The development of HEp-2 subline resist-

ant to carboplatin (7T) has been published previously.[19] These cells are cross-resistant to anticancer drug cisplatin, transplatin, mitomycin C and the natural compound curcumin as well.[19-21] Large cell lung carcinoma H460 cells and colorectal carcinoma HCT-116 were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Normal human skin fibroblasts were isolated from the upper arm of a 7-years-old female donor at the Neurochemical Laboratory, Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb. They were used for the cytotoxicity assay at 32 and 36 population doublings. All cell lines were grown as a monolayer culture in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) in a humidified atmosphere of 5% CO_2 at 37°C and were sub-cultured every 3 – 4 days.

Cytotoxicity assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cytotoxic activity of triazoles and triazolium salts was determined by MTT assay[22], modified as described. Cells were seeded into 96-well tissue culture plates (3×10^3 cells/0.18 mL medium/well). The next day different concentrations of triazoles or triazolium salts were added (0.02 mL) to each well and each concentration was tested in quadruplicate. Following 72 h incubation at 37°C, the medium was aspirated, and 20 μg of the MTT dye (Sigma-Aldrich) /0.04 mL medium/well was added. Three hours later, formazan crystals were dissolved in DMSO (0.17 mL/well), the plates were mechanically agitated for 5 min and the optical density at 545 nm was determined on a microtiter plate reader (Awareness Technology Inc, Palm City, FL, USA). To examine the effect of reactive oxygen species (ROS) scavengers on survival of 2b treated cells, the same procedure was used as described above, except that two hours prior to addition of 2b, 5 mM of N-acetyl-cysteine (NAC, Sigma-Aldrich), or 1mM tempol (Santa Cruz Biotechnology, Dallas, TX, USA) was added in wells. Experiment was repeated at least three times.

Colony-forming assay

For determination of colony formation 1000 cells were seeded in 6 cm dish. The next day the cells were pretreated either with 5 mM NAC or with 1 mM (HEp-2 cells) or 0.125 mM (H460 cells) tempol. Two hours later different concentrations of 2b were added in dishes with or without ROS scavengers. The effect of antioxidants on colony formation alone was determined as well. After ten days of continuous treatment, the colonies were washed with PBS, fixed with methanol, stained with Giemsa-crystal violet and counted. Untreated samples were used for determination of plating efficiency. Each concentration was tested in triplicate. Experiment was repeated at least three times.

Cell cycle analysis

Human large cell lung carcinoma H460 cells were seeded into 6-well tissue culture plates (10^5 cells/2 mL medium/well) and treated with 29.7 and 110 μM of 2b on the following day for 24, 48 and 72 hours. Thereafter, both adherent and floating cells were collected, washed with PBS and fixed overnight in 70% ethanol at -20°C. Fixed cells were treated with RNase A (0.1 mg/mL, Sigma-Aldrich) for 1 h at room temperature and afterward stained with propidium iodide (50 μg/mL, Sigma-Aldrich) for 30 min in the dark. The DNA content was analysed by flow cytometry (FACS Calibur, Becton Dickinson, Mountain View, CA, USA). Data were analysed with ModFitLTTM program (Verity Software House Inc., Topsham, ME, USA). Experiment was repeated more than three times.

Determination of DNA binding

The calf thymus (ct)-DNA was purchased from Sigma-Aldrich, dissolved in Na-cacodylate buffer, I = 0.05 mol/dm^3, pH = 7, additionally sonicated and solution filtered through a 0.45 mm filter. Polynucleotide concentration was determined spectroscopically as the concentration of phosphates by $\varepsilon_{260\ nm}$ = 6600 dm^3/mol^1 cm^{-1}. Thermal denaturation curves for ct-DNA and its complexes with studied compounds were determined in Na-cacodylate buffer, I = 0.05 mol/dm^3, pH = 7 by following the absorption change at 260 nm as a function of temperature, as previously described.[23,24] The absorbance of the compound was subtracted from each curve and the absorbance scale was normalized. Measured T_m values are the midpoints of the transition curves determined from the maximum of the first derivative and checked graphically by the tangent method. The DT_m values were calculated subtracting T_m of the free nucleic acid from T_m of the complex. Every ΔT_m value reported here was an average of at least two measurements. The error in DT_m is ± 0.5°C.

TABLE 1. IC_{50} values of triazolium salts and some parent triazoles against cervical carcinoma HeLa cells

Cmpd.	IC_{50} (μM)[a]
1a	> 100
1b	> 100
1f	> 100
2a	91.6 ± 3.9
2b	57.0 ± 12.9
2c	> 100
2d	> 100
2e	55.4 + 9.4
2f	–[b]
2g	–[c]
2h	88.9 + 7.5
2i	54.4 + 14.7
2j	54.9 + 3.5
2k	> 100
2l	–[c]

[a] IC_{50} is the concentration of the triazoles and triazolium salts inducing 50% cell growth inhibition after 72 h incubation. The results are shown as mean values of at least three experiments (± SD).

[b] Triazolium salt precipitated promptly after the addition to the growth medium and thus the cytotoxicity could not be measured accurately.

[c] The range of concentrations 10 – 1000 μM reduced survival from about 60 to 40%, and therefore the exact IC_{50} was difficult to determine.

Induction of reactive oxygen species (ROS)

Generation of ROS was determined by addition of 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) (Invitrogen). Briefly, logarithmically growing H460 cells were incubated with 10 mM CM-H_2DCFDA for 1 hour according to manufacturer's instructions. Afterward, cells were incubated with or without different concentrations of 2b during indicated time periods. After trypsinization and centrifugation, the cells were fixed in cold 80% methanol. Shortly before measurement, they were centrifuged and resuspended in PBS. The fluorescence of the product, developed by removal of the acetate groups from CM-H_2DCFDA by intracellular esterases and oxidation, was measured by flow cytometry on BC Navios instrument (Beckman Coulter, Inc., Miami, FL, USA).

To further examine whether toxicity of 2b is coupled with formation of ROS, two ROS scavengers were used: NAC, a drug that has been known for years to directly reduce the level of ROS[25] or the new ROS scavenger tempol.[26] Their effect was determined by MTT assay or colony-forming assay as described in *Cytotoxicity assay*.

Statistical analysis

All data were analysed by unpaired Student's t-test, and expressed as the mean ± standard error of the mean. Data were considered significant when P values were lower than 0.05, and in the figures these are designated as * = $P < 0.05$ or ** = $P < 0.01$. Experiments were repeated at least three times.

Results and discussion

Cancer is the second leading cause of death in developed countries.[1] Primary or acquired drug resistance and heavy side-effects strongly limit the effectiveness of classical chemotherapy. The success of advanced, target-oriented cancer therapy is at present limited only to the special types of cancers.[3] This provides a great impetus for investigation of new compounds with potential anticancer activities. 1,2,3-Triazoles are very important class of heterocycles, which have been well-recognized for their broad range of biological activities, including anticancer activity.[8-16]

This and the fact that no cytotoxic activity of the unfused 1,3,4-trisubstituted 1,2,3-triazolium salts has yet been reported encouraged us to examine the cytotoxic activity of compounds 2a – l along with some selected parent 1,4-disubstituted triazoles 1a, 1b and 1f.

Antiproliferative effects of triazoles and triazolium salts

The effect of tested triazoles and triazolium salts was first evaluated in HeLa cells, the cell model system that we previously found suitable for screening of new compounds.[27,28] The results are collected in Table 1.

It appears that in the picolyl series of the triazolium cations (2a – f) the aryl substituent modulated the cytotoxicity against the tumour cells, with the electron donating 4-methoxyphenyl group being greater as compared to the electron neutral phenyl and the electron withdrawing 4-(trifluoromethyl)-phenyl groups. In this series of the compounds the pyridine ring was separated from the triazole core by a methylene bridge and thus less likely influences the electron density at the latter. The situation dras-

tically changes in the pyridyl series (2g – l) were the pyridine ring was attached directly to the triazole, now playing an important role in the cytotoxicity. In 2g – l the triazole core was functionalized with pyridine in two different ways, either through the triazole N1 nitrogen atom as in 2g – i, or through the triazole C4 carbon atom (2j – l). Interestingly, in the former (2g – i), the electron deficient 4-(trifluoromethyl)phenyl group (2i) increased the cytotoxicity whereas in the latter series of compounds the member with the electron neutral phenyl group (2j) was identified as the most active.

Apparently, no clear structure-activity correlation, based purely on the electronic considerations of the triazolium cations, could be drawn at this point. Noteworthy, different chemical reactivity patterns of the four isomeric triazolium cations have been previously observed in some chemical transformations, with the structure-reactivity relation still remaining to be fully understood.[29] It is reasonable to expect that in a far more complex system such as the living cell, the structure-activity relation is likely to be a result of combined stereoelectronic effects and could be addressed after considerably larger library of derivatives have been assayed.

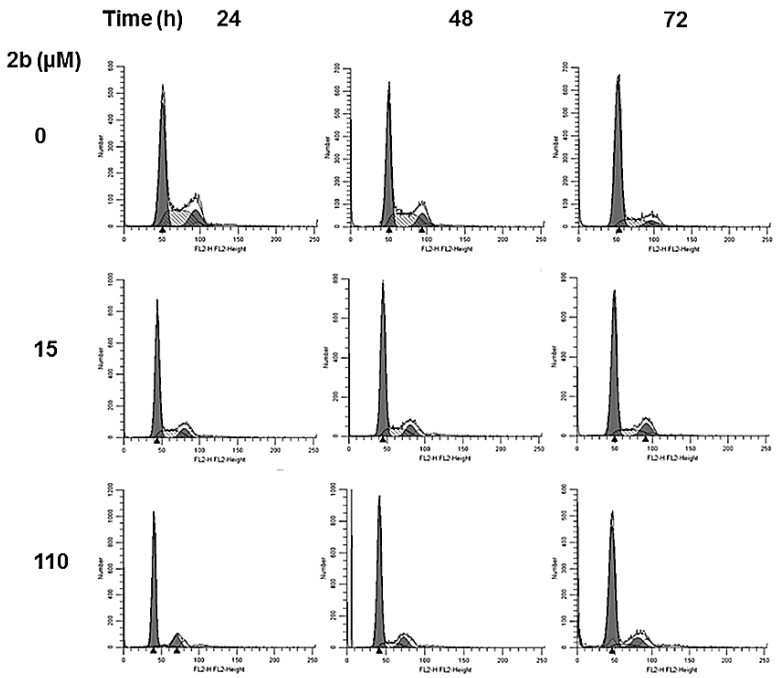

FIGURE 2. Effects of 2b on the cell cycle of H460 cells. Logarithmically growing H460 cells were treated with 29.7 and 110 μM of 2b for indicated period of time. Afterwards they were harvested for cell cycle analysis measured by FACS as described in Materials and methods. Representative data of three experiments are shown.

TABLE 2. Cytotoxic activity of 2b against different cell lines

Cell line	IC_{50} (μM)[b]	T.I.[c]
HeLa	57.0 ± 12.9	4.01
HEp-2	87.0 ± 28.5	2.63
7T	111.7 ± 7.6	2.05
H460	29.7 + 4.5	7.69
HCT-116	51.9 ± 7.2	4.40
Fibroblasts	228.5 ± 5.6	–

HeLa = cervical carcinoma cells; HEp-2 = laryngeal carcinoma cells; 7T = carboplatin and cisplatin-resistant HEp-2 subline; H460 = large cell lung carcinoma cells; HCT-116 = colorectal carcinoma cells; Fibroblasts = normal primary fibroblasts. The results are shown as mean values of three experiments (±SD).

[b] IC_{50} is the concentration of 2b that induces 50% cell growth inhibition after 72 h incubation.

[c] T.I. refers to therapeutic index, calculated from the ratio of cytotoxicity (IC_{50}) on normal fibroblasts and cytotoxicity (IC_{50}) on tumour cells.

In order to compare the results with those for the triazolium salts 2, three parent triazoles 1a, b, f were also selected for the biological screening. None of them exhibited cytotoxic activity against HeLa cells with IC_{50} below 100 μM.

To shed more light on the mechanisms responsible for the cytotoxic effect of above examined compounds, we decided to proceed further with compound 4-(4-methoxyphenyl)-3-methyl-1-(2-picolyl)-1*H*-1,2,3-triazolium hexafluorophosphate(V) (2b) as a representative compound. First, we explored its antiproliferative activity on several tumour cell lines from different origin, as well as on the normal human fibroblasts. The results are shown in Table 2. Compound 2b strongly inhibited the growth of all examined tumour cell lines and this effect was cell-type specific. Human large cell lung carcinoma H460 cells were the most sensitive toward 2b (and were selected for further studies), while HEp-2 cells were most resistant. Difference in sensitivity between most sensitive H460 and most resistant 7T cells (for IC_{50} value) was almost 4 times.

Drug resistance is the major cause of failure in successful treatment of cancer patients.[30,31] It is based on the variety of complex mechanisms.[30-35] The compounds that might be efficient against drug-resistant cells could be of great help in improvement of cancer treatment. Therefore we also included carboplatin, cisplatin and mitomycin C resistant 7T subline of HEp-2 cells in our study. As shown in Table 2, both, parental HEp-2 and drug-resistant 7T cells are similarly sensitive to 2b compound, suggesting a potential future application of

TABLE 3. Effect of compound 2b on the cell cycle of H460 cells

Conc. (μM)	24 h				48 h				72 h			
	G1	S	G2/M	subG1	G1	S	G2/M	subG1	G1	S	G2/M	subG1
0	54	31	15	2	59	26	15	2	73	13	14	2
29.7	61	24	15	2	61	26	13	5	65	17	18	6
110	78	9	13	3	78	9	13	15	79	5	16	35

H460 cells were treated for the indicated time period with 2b, stained with propidium iodide and analysed by flow cytometry. Cell cycle distribution was assessed as described in the Materials and methods section.

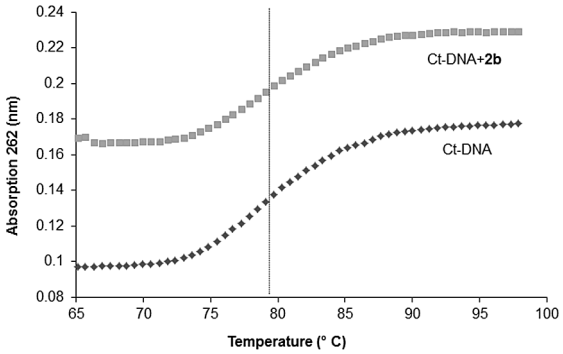

FIGURE 3. DNA as possible target of compound 2b. Thermal denaturation curves of ct-DNA (c(ct-DNA) = 2 x 10^{-5} M, $r_{[compound]/[ct-DNA]}$ =0.3) at pH 7.0 (sodium cacodylate buffer, I = 0.05 M) upon addition of compound 2b. Error in DTm values: ±0.5°C.

2b compound in clinical treatment of cisplatin and carboplatin resistant tumours. For comparison, 7T cells were 3.3 fold more resistant to cisplatin as shown previously.[19]

One of the most requested characteristics of potential anticancer drug is its higher efficacy against tumour than the normal cells. In this study all examined tumour cell lines were more sensitive to compound 2b than normal cell line, with the therapeutic index higher than 2 (Table 2). Specifically, for H460 cells it was particularly high, 7.69, fulfilling the request of this essential characteristic for a potential anticancer compound.

Effect of compound 2b on the cell cycle of H460 cells

To gain more insight into the mode of action that underlies the antiproliferative activity of 2b, we investigated its effect on the cell cycle in H460 cells. The flow cytometric analysis is presented in Figure 2, and Table 3. They show that compound 2b arrested the cells in the G1 phase of the cell cycle in dose-dependent manner, even after 24 hours of treatment. At later time points a dose- and time-dependent increase was detected in a fraction of cells with reduced DNA content (subG1), which represents the apoptotic cells subG1 fraction. These results suggest that 2b induces apoptosis in treated cells.

DNA as possible target of compound 2b

According to their structure, triazolium salts resemble the structures of DNA minor groove binders like those studied by Chenoweth and Dervan[36], that show precise recognition of the DNA sequence by thermodynamically controlled "H-bond based reading" of predesigned heterocycle-polyamide molecules. Some non-condensed heterocyclic molecules also proved to be DNA intercalators.[8,37] Therefore we studied the interactions of 2b with double strand (ds) DNA. Thus, in thermal denaturation experiment compound 2b was mixed with ct-DNA in a ratio $r_{[compound]/[ct-DNA]}$ = 0.3, at which any DNA binding mode should give a measurable change in DNA melting point transition. However, in the thermal denaturation experiment no measurable change in DNA melting point transition was observed, and no influence on the thermal stability of ct-DNA (Figure 3), indicating that at biologically relevant conditions (pH 7, c(compound) = 6 x 10^{-6} M) stable non-covalent complex with ds DNA was not formed, suggesting that DNA was not the target of 2b compound.

Formation of ROS by compound 2b

Although 2b does not bind to DNA, alternative mechanisms of 2b action and cytotoxicity were examined. Literature data indicate that diverse compounds can induce cell damage due to formation of ROS.[38,39] ROS may irreversibly oxidize DNA, nucleic acids, proteins, and lipids, thereby representing the primary source of damage in biological systems that may eventually lead to cell death.[40,41] Accordingly, we directly measured the induction of ROS formation following the treatment with 2b. For this, we stained the cells with 10 mM CM-

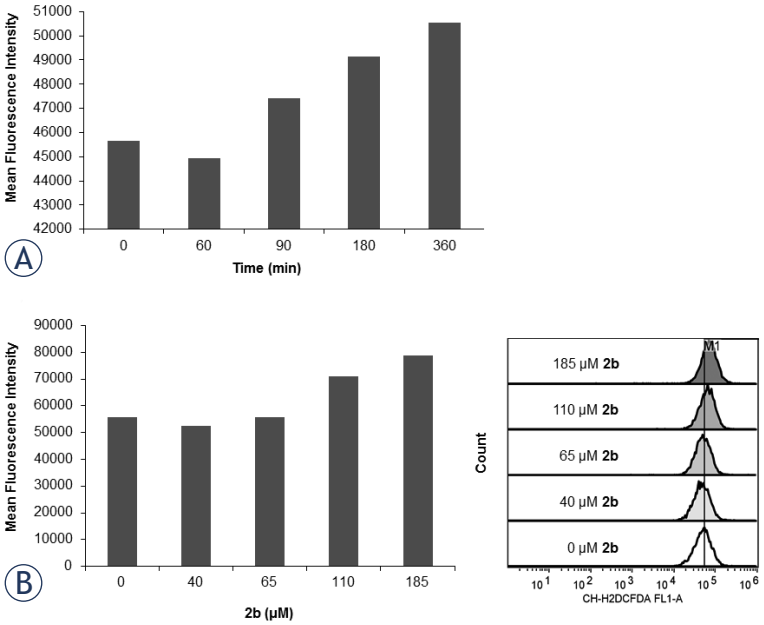

FIGURE 4. Formation of ROS by 2b in H460 cells. Logarithmically growing H460 cells were stained for 1 hour with 10 mM CM-H_2DCFDA and then either treated with 110 μM 2b during indicated time points **(A)** or treated with indicated concentrations of 2b for 180 min (3 hours) **(B)**. Afterward ROS formation was determined by flow cytometry as described in Materials and methods section. Dose-dependent formation of ROS was additionally presented by cell count and fluorescence intensity of CM-H_2DCFDA. M1 line is positioned to designate MFI value of the non-treated sample (white histogram) compared to signals obtained upon cell treatment with indicated concentrations of 2b.

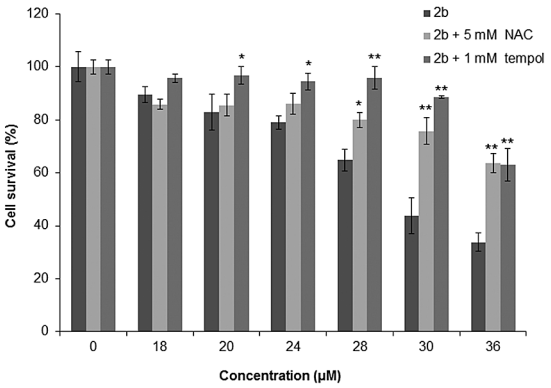

FIGURE 5. The effects of ROS scavenger on survival of H460 cells treated with 2b determined by MTT assay. H460 cells were seeded and next day pretreated for 2 hours with 5 mM of NAC or 1 mM tempol. Afterwards different concentrations of 2b were added. The cell survival was determined 72 hours later by MTT assay as described in Materials and methods section. Each point represents the mean ±SD of at least three independent experiments. All data are expressed as the average percentage of survival values relative to an untreated control ± SD or samples treated with antioxidants alone. The significance in differences is indicated (*, $P < 0.05$; **, $P < 0.01$).

H_2DCFDA for one hour and then treated them either with 110 μM 2b during different time points (Figure 4A) or with different concentrations of 2b during 3 hours (Figure 4B). For better illustration of ROS formation upon 2b treatment during 3 hours we presented additionally results as CM-H_2DCFDA fluorescence intensity compared to cell number (count). As shown in Figure 4, 2b induced ROS in time- and dose-dependent manner. Dose-dependent skewing of signals toward higher fluorescence intensity with increased concentration of compound is noticeable (Figure 4B). In order to confirm the formation of ROS and validity of detection we incubated the cells in each experiment with 0.01% H_2O_2 for 30 min (data not shown).

To approve this result and additionally examine the possible role of 2b-induced formation of ROS in cell toxicity, we pretreated for 2 hours H460 cells with two different ROS scavengers: NAC and tempol. As shown in Figure 5, the pretreatment of cells with either NAC or tempol increased survival of 2b treated cells compared to the cells treated only with 2b, indicating that 2b induced ROS and that the cytotoxicity of 2b can be reduced by addition of ROS scavenger. To confirm the data that we obtained by MTT assay (Figure 5), we have done additional experiments using colony-forming assay and chronical exposure to 2b with ROS scavenger. The preteratment of HEp-2 cells (a less responsive cell line to 2b compound) with either NAC or tempol increased the survival of 2b treated cells compared to the survival of cells treated only with 2b (Figure 6A). Similar results were obtained with the most sensitive cell line to 2b compound, *i. e.* H460 cells treated with NAC. Again, the pretreatment with this ROS scavenger increases the survival of cells as compared to the survival of cells treated only with 2b. However H460 cells were highly sensitive to tempol. The highest non-toxic concentration of tempol for chronic treatment of H460 cells was 0.125 mM, which is 8 times lower concentration than could be applied for HEp-2 cells (see Figure 6B). It is possible that this concentration of tempol was too low to scavenge ROS induced by 2b. Perhaps, higher concentration could protect the cells from toxic effect of 2b, but higher concentration used during 10 days incubation was too toxic for H460 cells. H460 and HEp-2 cells differ in their p53 status: H460 has wild type p53, while HEp-2 cells have mutated p53. While p53 has important role in cell response to oxidative stress and apoptosis[42], we can assume that wild type p53 can be involved in increased H460 sensitivity to chronic treatment with tempol.

Thus, experiments in which the cells were pretreated with ROS scavengers suggest that 2b indeed induced ROS in treated cells and that ROS induction was involved in cytotoxicity, but that induction of ROS is not the only mechanism of action of the selected compound 2b.

Conclusions

In conclusion, pyridine tethered 'click' triazolium salts have been tested for their anticancer activity for the first time. As revealed on human cervical carcinoma HeLa cells, selected compound (4-(4-methoxyphenyl)-3-methyl-1-(2-picolyl)-1*H*-1,2,3-triazolium hexafluorophosphate(V) (2b), exhibits high cytotoxicity. Its antiproliferative activity was cell type dependent, being mostly cytotoxic against large cell lung carcinoma H460 cells. It is of utmost importance that 2b was significantly more cytotoxic against tumour cells than normal cells, having very high therapeutic index, such as 7.69 for H460 cells. Additionally, this compound was similarly cytotoxic against parental laryngeal carcinoma HEp-2 cells and their drug resistant 7T subline which is, having in mind the importance of inhibitory effect of drug resistance on the success of cancer treatment, a very valuable result. Compound 2b arrested tumour cells in the G1 phase of the cell cycle and induced programmed cell death. This compound does not form a complex with ds DNA, but rather induced ROS in treated cells which further triggers cell death. In short, our results suggest that the 'click' triazolium salts are simple to make compounds that are worth of further investigation as anticancer agents. Work is in progress to design and examine an extended library of their analogues.

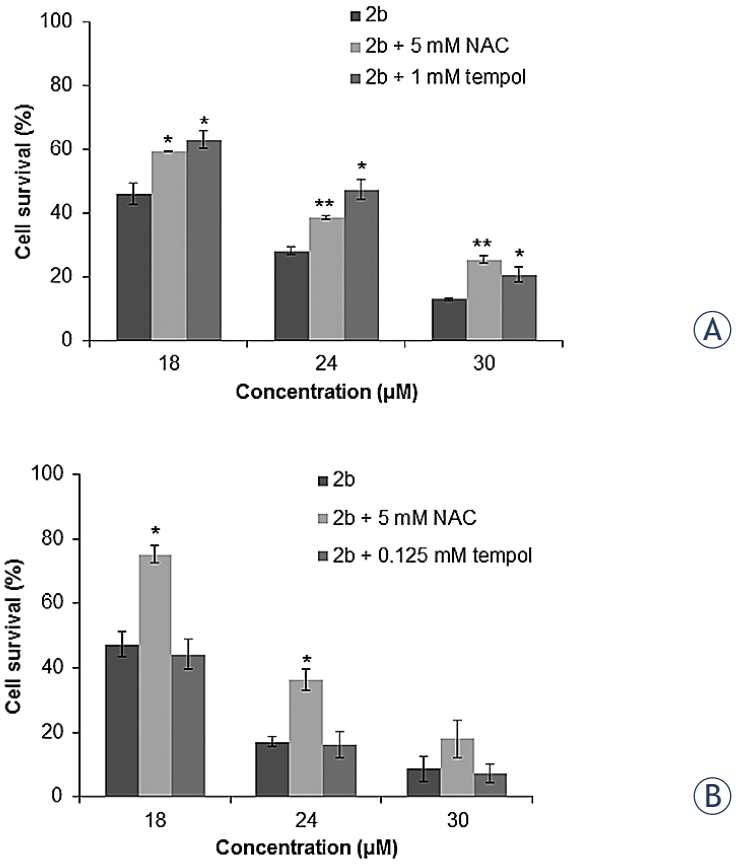

FIGURE 6. The effect of ROS scavenger on survival of HEp-2 **(A)** and H460 **(B)** cells treated with 2b determined by colony-forming assay. HEp-2 and H460 cells were seeded and next day pretreated for 2 hours with 5 mM of NAC or 1 mM (HEp-2 cells) or 0.125 mM (H460 cells) tempol. Afterwards different concentrations of 2b were added. Ten days later the colonies were counted. Non-treated cells and cells treated with antioxidants alone were used as controls. Each point represents the mean ±SD of at least three independent experiments. All data are expressed as the average percentage of survival values relative to an untreated control ± SD or samples treated with antioxidants alone. The significance in differences is indicated (*, $P < 0.05$; **, $P < 0.01$).

Acknowledgments

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research article

Functional polymorphisms in antioxidant genes in Hurthle cell thyroid neoplasm - an association of *GPX1* polymorphism and recurrent Hurthle cell thyroid carcinoma

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Background. Hurthle cells of the thyroid gland are very rich in mitochondria and oxidative enzymes. As a high level oxidative metabolism may lead to higher level of oxidative stress and can be associated with an increased risk for cancer, we investigated whether common functional polymorphisms in antioxidant genes (*SOD2*, *CAT*, *GPX*, *GSTP1*, *GSTM1* and *GSTT1*) are associated with the development or clinical course of Hurthle cell thyroid carcinoma (HCTC).

Methods. A retrospective study was performed in 139 patients treated by thyroid surgery for a Hurthle cell neoplasm. HCTC, Hurthle cell thyroid adenoma (HCTA) or Hurthle cell thyroid nodule (HCTN) were diagnosed by pathomorphology. DNA was extracted from cores of histologically confirmed normal tissue obtained from formalin-fixed paraffin-embedded specimens and genotyped for investigated polymorphisms. Logistic regression was used to compare genotype distributions between patient groups.

Results. HCTC, HCTA and HCTN were diagnosed in 53, 47 and 21 patients, respectively. Metastatic disease and recurrence of HCTC were diagnosed in 20 and 16 HCTC patients, respectively. Genotypes and allele frequencies of investigated polymorphisms did not deviate from Hardy-Weinberg equilibrium in patients with HCTC, HCTA and HCTN. Under the dominant genetic model we observed no differences in the genotype frequency distribution of the investigated polymorphisms when the HCTA and HCTN group was compared to the HCTC group for diagnosis of HCTC or for the presence of metastatic disease. However, *GPX1* polymorphism was associated with the occurrence of recurrent disease ($p = 0.040$).

Conclusions. *GPX1* polymorphism may influence the risk for recurrent disease in HCTC.

Key words: Hurthle cell thyroid carcinoma; Hurthle cell neoplasm; thyroid; oxidative stress; antioxidant genes

Introduction

Hurthle cell thyroid carcinoma (HCTC) is a rare type of differentiated thyroid cancer (DTC). Traditionally, HCTC was regarded as a subtype of follicular thyroid cancer, while new evidence indicates that HCTCs may have a distinct molecular profile compared to other DTCs.[1]

Clinically, and compared to other DTCs, HCTCs are considered more aggressive, with worse prognosis, requiring more stringent follow-up. HCTCs are also more likely to metastasize to neck soft tissue and distant sites, are more iodine resistant and have higher tumour-related mortality.[1-4] A definitive way to differentiate a HCTC from a benign Hurthle cell thyroid adenoma (HCTA) is based on vascular and/or transcapsular invasion.[5-9] For HCTA, a lobectomy is a sufficient surgical procedure. However, if a HCTC is diagnosed on histologic sections after a lobectomy, then a complete

thyroidectomy is performed as a second surgical procedure. Therefore, when a follicular neoplasm is detected with a cytological analysis of material obtained by fine-needle aspiration biopsy, the use of predictive clinical[4,10] or genetic markers[11] has been proposed, before deciding on the extent of the thyroid surgical procedure.

A Hurthle (oncocytic) cell has abundant granular eosinophilic cytoplasm, which has such an appearance because of the accumulation of a large number of mitochondria. A full-blown Hurthle cell has 4000 to 5000 mitochondria, while a human cell rich in mitochondria (oocyte) has about 1500 mitochondria only.[7,12] Enzyme histochemistry studies have shown that Hurthle cells contain high concentrations of oxidant enzymes.[13]

The respiratory redox chain in the mitochondria is considered the major source of reactive oxygen species (ROS) and other free radicals in the cell.[14] ROS and other free radicals can oxidize target cellular proteins, membrane lipids, nucleic acids and damage their cellular structure and function. Effective protective mechanisms, comprising antioxidative molecules and compartmentalization of potentially toxic molecules, have been developed to maintain a balance between generation and detoxification of reactive oxygen species (ROS) under physiological conditions. In case of excessive ROS oxidative stress occurs.[15,16] To prevent this, complex defence mechanisms including many enzymes, proteins and antioxidants are involved. Antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD), glutathione peroxidase (GPX) and catalase (CAT) directly eliminate ROS, while glutathione-S-transferases (GSTs) detoxify cytotoxic secondary metabolites. Numerous functional polymorphisms in the genes coding for antioxidant enzymes have been described that may also modify their ROS detoxification capacity.[17]

Oxidative stress and ROS have been associated with several cancers and also many complex diseases like cardiovascular disease, diabetes mellitus and neurodegenerative disorders.[15,18] Several studies also found a connection between oxidative stress and thyroid diseases including neoplasia and thyroid cancer.[16,19-25] However all these studies have been done on papillary thyroid carcinoma and/or follicular thyroid carcinoma. As Hurthle cells are very rich in mitochondria and oxidative enzymes, it is possible that antioxidant enzymes may have an important role in defence against oxidative stress. To our knowledge, there are no data in the literature about oxidative stress and HCTC or HCTA. Furthermore, there are no data about the association between HCTC/HCTA and polymorphisms of genes coding for antioxidant enzymes.

Patients and methods

Patients

A retrospective study included Slovenian patients treated by thyroid surgery for a Hurthle cell neoplasm at the Institute of Oncology Ljubljana. The medical records of all the patients were reviewed and a total of 167 patients with cytological features for a Hurthle cell neoplasm were selected for molecular analysis. As 28 patients had no sufficient formalin-fixed and paraffin-embedded (FFPE) material for DNA extraction, they were excluded from the study. Eventually, 139 patients were included.

All the patients had a Hurthle cell neoplasm diagnosed by fine-needle aspiration cytology and the majority of fine-needle aspiration biopsies were ultrasound guided.[4,10] The cytological criteria for Hurthle cell neoplasms were hypercellularity, with a predominance of Hurthle cells (at least 75%), few or no lymphocytes, and scant or no colloid.[26] Cytological slides were examined by cytopathologist, experienced in thyroid pathomorphology.

Final diagnosis of HCTC/HCTA/other was obtained by definitive histology of thyroid tissue obtained by surgical procedure. The histological features for HCTC were based on vascular invasion and/or transcapsular invasion.[26] Histology slides were examined by a pathologist, experienced in thyroid pathomorphology.

All patients with HCTC diagnosis were regularly monitored for possible recurrent or metastatic disease. The median follow-up time was 105 (1–337) months.[4]

The study was reviewed and approved by the Slovenian Ethics Committee for Research and Medicine (No: KME 32/12/11) and was carried out according to the Declaration of Helsinki. The study was also approved by the Institute of Oncology Ljubljana Protocol Review Board.

Methods

Hematoxylin and eosin (H&E) stained slides from FFPE samples were examined by a pathologist, experienced in thyroid pathomorphology, to confirm the diagnosis and to select areas representative of normal tissue. Two to three cores (1 mm in diameter) of histologically confirmed normal tissue were obtained from each specimen for DNA extraction using a QiaAmp Mini kit (Qiagen, Hilden,

Germany) according to the manufacturer's instructions.

Genotyping of SNPs in *SOD2* rs4880 (c.47C>T; p.Val16Ala), *CAT* rs1001179 (c.-262C>T; c.-262G>A), *GPX1* rs1050450 (c.599C>T; p.Pro200Leu), *GSTP1* rs1695 (c.341C>T; p.Ile105Val) and *GSTP1* rs1138272 (c.313A>G; p.Ala114Val) was carried out using a fluorescence-based competitive allele-specific (KASPar) assay (Kbiosciences, Herts, UK) according to the manufacturer's instructions. Amplifications were performed in a PCR system 9700 AB (Applied Biosystems, California, USA) as recommended by the manufacturer (Kbiosciences). Fluorescence was measured on a 7500 Real Time PCR System AB and allele discrimination data analyzed with 7500 System SDS Software (both Applied Biosystems).

GSTM1 and *GSTT1* gene deletions were detected using a multiplex PCR simultaneously amplifying *GSTM1*, *GSTT1* and *BGLO* genes as described previously.[27] With this approach, we could identify homozygous *GSTM1* or *GSTT1* gene deletion, but we were not able to distinguish between carriers of one or two copies of each gene. Genotyping was repeated in 20% of samples to check for genotyping accuracy.

Statistical analysis

Median and interquartile ranges were used to describe central tendency and variability of continuous variables, while frequencies were used to describe the distribution of categorical variables. A standard chi-square test was used to assess the deviation from Hardy-Weinberg equilibrium (HWE). Logistic regression was used to compare genotype distributions between patient groups and to calculate odds rations (ORs) and 95% confidence intervals (CIs). All statistical analyses were carried out using IBM SPPS Statistics version 19.0 (IBM Corporation, Armonk, NY, USA). A dominant genetic model was used in all statistical analyses and the level of statistical significance was set at 0.05. Haplotype analysis was performed using Thesias software[28] as previously described.[29]

Results

In total 139 patients with cytological features for Hurthle cell neoplasm were included in the study. The female to male sex ratio was 3.8:1. Median (range) age was 54 (42–66) years. Median diameter of the tumour was 28 (20–45) mm. The final diagnosis was established by definitive histology of the thyroid tissue obtained by a surgical procedure. Patients were diagnosed as follows: 53 (38.1%) had HCTC, 47 (33.8%) HCTA, 21 (15.1%) Hurthle cell thyroid nodule (HCTN), 11 (7.9%) multi nodular goiter, 4 (2.9%) follicular thyroid adenoma, while 2 (1.4%) patients had lymphocyitic thyroiditis. In 46 (33%) patients, concomitant disease was recorded: 31 (22.3%) had Hashimoto thyroiditis, 12 (8.6%) diabetes mellitus, 7 (5.0%) Graves' disease, and 4 (2.9%) patients had other malignant disease not present in the thyroid tissue.

Only patients with a final diagnosis of HCTC, HCTA or HCTN were selected for molecular analysis. The group of patients with HCTA or HCTN was compared to the group of patients with HCTC. Altogether 20 of 53 (37.7%) patients with HCTC had metastatic disease. Recurrent disease was observed in 16 (30.0%) patients with HCTC. The clinical and demographic characteristics of those patients are summarized in Table 1.

The patients from the HCTC group had a different gender (F/M) ratio ($p = 0.043$), were older ($p = 0.004$) and had a larger tumour diameter ($p < 0.001$) in comparison to the patients from the HCTA or HCTN group (Table 2). In the HCTC group, independent risk factors for both metastatic disease and recurrent disease were the patient's age and tumour diameter as shown by logistic regression analysis (Table 2).

Genotype frequencies of the investigated polymorphisms in patients with HCTC, HCTA and

TABLE 1. Clinical and demographic characteristics of patients with Hurthle cell neoplasms

	HCTA + HCTN	HCTC
Number [N] (%)	68 (56.2)	53 (43.8)
Median age [years] (range)	49.5 (38.5–57.8)	62 (45.5–70.5)
Gender F/M [N] (%)	58/10 (85.3/14.7)	37/16 (69.8/30.2)
Median tumor diameter [mm] (range)	26.0 (16.0–34.8)	40.0 (25.5–65.0)
Metastasis (%)	/	20 (37.7)
Recurrence (%)	/	16 (30.2)
Concomitant disease N (%)	16 (23.5)	20 (37.7)
Hashimoto thyroiditis	11 (16.2)	12 (22.6)
Diabetes mellitus	1 (1.5)	7 (13.2)
Graves' disease	2 (2.9)	3 (5.7)
Non-thyroid Malignancy	2 (2.9)	2 (3.8)

F= female; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; M = male

TABLE 2. Association of clinical and demographic characteristics with Hurthle cell thyroid neoplasms, metastatic disease and recurrent disease

	HCTA+HCTN versus HCTC		Metastatic disease		Recurrent disease	
	OR (95% CI)	p[a]	OR (95% CI)	p[a]	OR (95% CI)	p[a]
Gender	2.51 (1.03–6.12)	0.043	2.08 (0.63–6.90)	0.230	2.42 (0.70–8.37)	0.163
Age	1.04 (1.01–1.06)	0.004	1.07 (1.02–1.12)	0.005	1.05 (1.01–1.10)	0.026
Tumor diameter	1.05 (1.02–1.07)	< 0.001	1.09 (1.04–1.14)	< 0.001	1.04 (1.01–1.07)	0.005
Concomitant disease	1.97 (0.90–4.34)	0.092	0.83 (0.26–2.63)	0.749	0.83 (0.26–2.63)	0.523

CI = confidence interval; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio; [a] = p less than 0.05 was considered statistically significant

HCTN are shown in Table 3. The observed genotype frequencies did not deviate from Hardy-Weinberg equilibrium in the whole cohort of patients (p > 0.050, Table 3).

The association of *SOD2*, *CAT*, *GPX1* and *GST* polymorphisms with diagnosis of Hurthle cell neoplasm and with the presence of metastatic or recurrent disease are presented in Table 4. These associations were also adjusted for clinical parameters. Since gender, age and tumour diameter were correlated in a multivariable model, only tumour diameter was used for adjustment.

Under the dominant genetic model, no significant differences in the genotype frequency distribution of the investigated polymorphisms were observed when the HCTA and HCTN group was compared to the HCTC group (all p > 0.050). These polymorphisms were also not associated with metastatic disease (all p > 0.050). However, *GPX1* polymorphism was associated with the presence of recurrent disease (p = 0.040). The association of *GPX1* polymorphism and recurrent disease was even greater when adjusted for tumour diameter (p = 0.036).

TABLE 3. Genotype frequencies in patients with Hurthle cell neoplasms

Gene	Polymorphism	Genotype	All patients (%)	P_{HWE}	HCTA+HCTN (%)	HCTC (%)
SOD2	rs4880; c.47C>T; p.Val16Ala	CC	26 (21.7)	0.903	12 (17.9)	14 (26.4)
		CT	59 (49.2)		34 (50.7)	25 (47.2)
		TT	35 (29.2)		21 (31.3)	14 (26.4)
CAT	rs1001179; c.-262C>T; c.-262G>A	CC	70 (58.3)	0.907	35 (52.2)	35 (66.0)
		CT	43 (35.8)		30 (44.8)	13 (24.5)
		TT	7 (5.8)		2 (3)	5 (9.4)
GPX1	rs1050450; c.599C>T; p.Pro200Leu	CC	63 (52.1)	0.424	35 (51.5)	28 (52.8)
		CT	51 (42.1)		32 (47.1)	19 (35.8)
		TT	7 (5.8)		1 (1.5)	6 (11.3)
GSTP1	rs1695; c.341C>T; p.Ile105Val	CC	54 (44.6)	0.653	28 (41.2)	26 (49.1)
		CT	52 (43.0)		32 (47.1)	20 (37.7)
		TT	15 (12.4)		8 (11.8)	7 (13.2)
GSTP1	rs1138272; c.313A>G; p.Ala114Val	AA	103 (85.1)	0.159	58 (85.3)	45 (84.9)
		AG	16 (13.2)		8 (11.8)	8 (15.1)
		GG	2 (1.7)		2 (2.9)	0 (0)
GSTM1	Gene deletion	Wild type	55 (50.9)	/[a]	33 (50.8)	22 (51.2)
		Gene deletion	53 (49.1)		32 (49.2)	21 (48.8)
GSTT1	Gene deletion	Wild type	93 (86.1)	/[a]	54 (83.1)	39 (90.7)
		Gene deletion	15 (13.9)		11 (16.9)	4 (9.3)

HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; HWE = Hardy-Weinberg equilibrium
[a] HWE could not be evaluated for GSTM1 and GSTT1 as we were not able to distinguish between carriers of one or two copies of each gene.

TABLE 4. Association of *SOD2*, *CAT*, GPX1 and *GST* polymorphisms with diagnosis of Hurthle cell neoplasm, presence of metastatic disease and occurrence of recurrent disease

Gene	Genotype	Diagnosis (HCTA+HCTN vs. HCTC)				Metastatic disease				Recurrent disease			
		OR (95% CI)	p[a]	OR-adj[b] (95% CI)	p-adj[b]	OR (95% CI)	p[a]	OR-adj[b] (95% CI)	p-adj[b]	OR (95% CI)	p[a]	OR-adj[b] (95% CI)	p-adj[b]
SOD2 rs4880	CC CT+TT	0.61 (0.25–1.46)	0.264	0.65 (0.25–1.67)	0.373	1.12 (0.32–4.00)	0.856	0.72 (0.12–4.09)	0.706	1.11 (0.29–4.26)	0.878	0.82 (0.18–3.62)	0.788
CAT rs1001179	CC CT+TT	0.56 (0.27–1.18)	0.129	0.81 (0.36–1.81)	0.600	0.34 (0.09–1.24)	0.102	0.57 (0.11–2.91)	0.499	1.25 (0.37–4.25)	0.721	2.95 (0.66–13.1)	0.155
GPX1 rs1050450	CC CT+TT	0.95 (0.46–1.94)	0.882	1.02 (0.46–2.24)	0.962	0.63 (0.20–1.93)	0.417	0.72 (0.15–3.52)	0.682	0.25 (0.07–0.94)	0.040	0.19 (0.04–0.89)	0.036
GSTP1 rs1695	CC CT+TT	0.73 (0.35–1.50)	0.388	0.82 (0.37–1.82)	0.628	1.30 (0.43–3.96)	0.646	2.40 (0.47–12.13)	0.291	0.46 (0.14–1.52)	0.202	0.49 (0.13–1.89)	0.300
GSTP1 rs1138272	AA AG+GG	1.03 (0.38–2.83)	0.952	1.15 (0.39–3.45)	0.800	0.99 (0.21–4.67)	0.988	1.24 (0.17–9.19)	0.836	0.29 (0.03–2.54)	0.261	0.24 (0.02–2.64)	0.244
GSTM1	Wild type Gene deletion	0.98 (0.46–2.13)	0.968	0.91 (0.39–2.12)	0.819	1.59 (0.47–5.39)	0.456	1.40 (0.23–8.57)	0.716	1.32 (0.38–4.64)	0.666	1.24 (0.28–5.41)	0.774
GSTT1	Wild type Gene deletion	0.50 (0.15–1.70)	0.269	0.44 (0.11–1.82)	0.257	1.44 (0.18–11.29)	0.730	0.83 (0.02–39.34)	0.923	2.00 (0.25–15.85)	0.512	1.42 (0.1–20.98)	0.798

CI = confidence interval; HCTA = Hurthle cell thyroid adenoma; HCTC = Hurthle cell thyroid carcinoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio; [a] = p less than 0.05 was considered statistically significant; [b] = adjusted for tumor diameter

TABLE 5. Association of *GSTP1* haplotypes and diagnosis of Hurthle cell neoplasm, presence of metastatic disease and occurrence of recurrent disease

Haplotype	Estimated frequency	Diagnosis (HCTA+HCTN vs. HCTC)		Metastatic disease		Recurrent disease	
		OR (95% CI)	p[a]	OR (95% CI)	p[a]	OR (95% CI)	p[a]
AC	0.68	Reference		Reference		Reference	
GC	0.25	0.88 (0.49–1.60)	0.686	1.04 (0.38–2.86)	0.935	0.45 (0.13–1.64)	0.230
GT	0.07	0.83 (0.33–2.13)	0.704	0.99 (0.21–4.72)	0.988	028 (0.03–2.89)	0.288

CI = confidence interval.; HCTC = Hurthle cell thyroid carcinoma; HCTA = Hurthle cell thyroid adenoma; HCTN = Hurthle cell thyroid nodule; OR = odds ratio
[a] - p less than 0.05 was considered statistically significant

Haplotype analysis was performed to assess the combined effect of SNPs within the *GSTP1* gene. As shown in Table 5, no associations were observed between *GSTP1* haplotypes and diagnosis of HCTA/HCTN versus HCTC, the presence of metastatic disease or the occurrence of recurrent disease.

Discussion

In the present study, we investigated whether common functional polymorphisms in antioxidant genes could be used as molecular markers for the development of HCTC or its clinical course in patients with Hurthle cell neoplasms.

In patients with cytological features for Hurthle cell neoplasm, different final diagnoses are made by definitive histology of thyroid tissue obtained by a surgical procedure. In our study group, 87% of patients with cytological features for Hurthle cell thyroid neoplasm had HCTC, HCTA or HCTN and were eligible for our study. Patient groups with benign HCTA and HCTN were combined and compared to a group with HCTC. The malignancy rate in our HCTC group was 44% and within the incidence rate of malignancy reported in the literature, where it ranged from 13%[30] up to 70%.[31] A significant difference in age was observed between the HCTA+HCTN group and the HCTC group, with patients in the HCTC group being nearly 12 years older and having a significantly larger median size of initial tumour (26 versus 40 mm). These findings are consistent with previous reports.[10,32,33] We also found a small gender difference, with a significantly larger F/M ratio in the HCTA+HCTN group as compared to the HCTC group. The two groups did not differ regarding the presence of concomitant disease. Metastases were diagnosed in 38% of patients with HCTC. Furthermore, 30% of patients developed a recurrent disease. These two groups of patients had a significantly larger initial

tumour diameter (69 versus 30 mm and 62 versus 30 mm, respectively) or were significantly older (67 versus 53 years and 65 versus 54 years, respectively) at initial diagnosis than the HCTC patients that did not have metastatic or recurrent disease. However, it has to be noted that our HCTC group with metastatic or recurrent disease was relatively small compared to non-metastatic or non-recurrent HCTC group.

To establish whether common functional polymorphisms in genes coding for antioxidant genes could be used as molecular markers for the development of HCTC or its clinical course, we investigated associations between *SOD2*, *CAT*, *GSTP1*, *GSTM1*, *GSTT1* and *GPX1* genotypes and the clinical characteristics of patients with definite diagnosis of HCTC, HCTA or HCTN.

CAT -262C>T genotype frequencies observed in our patient group were in accordance with those previously published for a healthy population.[34-36] In our study *CAT* -262C>T polymorphism was not associated with HCTC, or metastatic or recurrent disease. To our knowledge *CAT* -262C>T has not been studied in HCTC, but higher CAT activity has been associated with papillary thyroid carcinoma and follicular carcinoma.[24,37] It has been demonstrated that *CAT* -262C>T polymorphism influences the binding of transcriptional factors and is associated with a decrease in enzyme expression[35,38,39], but also with higher CAT activity.[34,40]

Also *SOD2* Val16Ala genotype frequencies in our patients with Hurthle cell neoplasms were similar to frequencies previously reported in Caucasian patients.[41-44] In our study *SOD2* Val16Ala polymorphism was not associated with the occurrence of HCTC, or with metastatic or recurrent disease. To our knowledge this polymorphism has not been studied in HCTC yet. *SOD2* Val16Ala polymorphism leads to less efficient transport of SOD2 into mitochondrial matrix in vitro[45], but association studies of SOD2 in thyroid cancers gave inconclusive results. Two groups showed an increased SOD2/SOD level or activity in follicular and papillary thyroid cancer, while one group found no change of SOD activity in papillary thyroid cancer.[37,46,47] On the other hand a reduced level of SOD2 was found in poorly differentiated thyroid cancers.[48]

Frequencies of *GSTP1*, *GSTM1* and *GSTT1* polymorphisms in our patients were similar to previously reported studies.[49] However, in the HCTC group we noticed a lower percentage of *GSTT1* gene deletion, compared to the HCTN/HCTA group. *GSTP1* genotypes and haplotypes as well as *GSTT1* and *GSTM1* deletions were not associated with the occurrence of HCTC and neither with metastatic nor recurrent disease. Our findings are in agreement with a previous study that also found no association between *GSTM1* and *GSTT1* polymorphisms and HCTC.[50] Both *GSTP1* Ile105Val and *GSTP1* Ala114Val decrease enzymatic activity[51,52], while *GSTM1* and *GSTT1* deletion polymorphisms result in the complete loss of enzymatic activity in homozygous carriers.[53] Some previous studies have shown possible associations of *GSTP1*, *GSTM1* or *GSTT1* polymorphisms, or a combination of *GSTT1* and *GSTM1* null allele with papillary and/or follicular thyroid cancer[54-58], while others found no association between these polymorphisms and primary or secondary thyroid cancers.[59-62]

Frequencies of *GPX1* Pro198Leu genotypes in our study group were also similar to the ones previously reported.[63] We did not find any association of *GPX1* Pro198Leu polymorphism with the occurrence of HCTC or with metastatic disease, even though several groups have found decreased activity or decreased expression of GPX1 in thyroid carcinomas[24,47,64,65], while one group reported increased levels of GPX1 in papillary thyroid carcinoma.[37] Several groups also reported that Leu variant could lead to lower GPX1 activity in patients with lung cancer, breast cancer, prostate cancer, bladder cancer and some other cancers.[66-68] We observed an interesting association between *GPX1* Pro198Leu polymorphism and lower probability for recurrent disease. Our findings are consistent with a previous report on the association of *GPX1* 198Leu variant with lower risk of recurrence in cancer patients.[69] A possible explanation may be that some HCTC therapies (radioiodine ablation and radiotherapy) are large ROS generators with antineoplastic effects and may also influence the patient's prognosis after these treatments. As GPX1 198Leu variant is associated with reduced removal of ROS and their secondary products produced by some HCTC therapies, patients with variant allele may have a better prognosis and longer recurrence-free survival time.

To sum up, in our study we did not find any association between common functional polymorphism antioxidant genes (*SOD2*, *CAT*, *GPX1*, *GSTP1*, *GSTM1*, and *GSTT1*) and the development of HCTC. A possible explanation could be that these polymorphisms may influence an initial and shared phase of HCTC and HCTA/HCTN development. Common functional polymorphisms in *SOD2*, *CAT*, *GSTP1*, *GSTM1* or *GSTT1* were also

not associated with metastatic or recurrent disease development, while *GPX1* Pro198Leu polymorphism may modulate the risk of HCTC recurrence. However, the group of patients with recurrent disease was relatively small, so it is possible that the results may result from sampling error. Ideally, our findings relating both to statistically significant associations and not significant associations, should be confirmed in an independent sample cohort. Because of the rarity of these tumours, it was impossible to perform a validation study in a single institution. Further research in a larger group is needed before we can conclude that *GPX1* Pro198Leu polymorphism could be used as an additional molecular marker in clinical practice to support decisions about follow-up procedures in patients with HCTC.

Conclusions

In conclusion, *GPX1* Pro198Leu polymorphism may influence the risk for recurrent disease in HCTC, however, these results must be validated in an independent sample cohort.

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research article

Association between polymorphisms in segregation genes BUB1B and TTK and gastric cancer risk

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Background. Malignant transformation of normal gastric cells is a complex and multistep process, resulting in development of heterogeneous tumours. Susceptible genetic background, accumulation of genetic changes, and environmental factors play an important role in gastric carcinogenesis. Single nucleotide polymorphisms (SNPs) in mitotic segregation genes could be responsible for inducing the slow process of accumulation of genetic changes, leading to genome instability.

Patients and methods. We performed a case-control study of polymorphisms in mitotic kinases *TTK* rs151658 and *BUB1B* rs1031963 and rs1801376 to assess their effects on gastric cancer risk. We examined the *TTK* abundance in gastric cancer tissues using immunoblot analysis.

Results. C/G genotype of rs151658 was more frequent in patients with diffuse type of gastric cancer and G/G genotype was more common in intestinal types of gastric cancers (p = 0.049). Polymorphic genotype A/A of rs1801376 was associated with higher risk for developing diffuse type of gastric cancer in female population (p = 0.007), whereas A/A frequencies were increased in male patients with subserosa tumour cell infiltration (p = 0.009). T/T genotype of rs1031963 was associated with well differentiated tumours (p = 0.035). TT+CT genotypes of rs1031963 and GG+AG genotypes of rs1801376 were significantly associated with gastric cancer risk (dominant model; OR = 2,929, 95% CI: 1.281–6.700; p = 0.017 and dominant model; OR = 0,364, 95% CI: 0.192–0.691; p = 0.003 respectively).

Conclusions. Our results suggest that polymorphisms in mitotic kinases *TTK* and *BUB1B* may contribute to gastric tumorigenesis and risk of tumour development. Further investigations on large populations and populations of different ethnicity are needed to determine their clinical utility.

Key words: cancer susceptibility; chromosomal instability; chromosome segregation; mitotic checkpoint; serine/threonine kinase; genetic association

Introduction

Gastric cancer is one of the major contributors to cancer-related deaths worldwide with estimated 989 600 new cases and 738 000 deaths in 2008.[1,2] It is believed that complex interplay of genetic and environmental factors triggers the accumulation of numerous genetic and epigenetic alterations in cells, resulting in deregulation of normal cell functions and disruption of stomach linen homeostasis.[3-6] Individual genetic factors probably contribute to aberrant processes in the genesis of malignant phenotype. Among them, single nucleotide polymorphisms (SNPs) and other genetic variants play an important role as the main genetic elements in the aetiology of several complex diseases, including gastric cancer.[7-11] In gastric carcinogenesis this is further supported by the fact that only

a small proportion of individuals exposed to the known environmental risk factors develop adenocarcinoma.[5,10] Therefore, there is continuing interest for determining simple genetic tests for identifying individuals at high risk for the development of gastric tumours and for identifying patients with high risk for recurrence in order to ensure improved and early diagnosis as well as better survival of patients.

A majority of gastric cancer patients show chromosomal instability (CIN) resulting in aneuploidy.[4,12,13] It has been suggested that tumour cells acquire aberrant chromosome numbers and other chromosomal defects as a result of deregulation of mechanisms responsible for maintaining the chromosomal number stability, such as spindle assembly checkpoint and chromosome segregation.[14,15] However, mutations in mitotic genes are rare, due to the fact that severe defects of these genes would trigger cell death by cell-surveillance early in the development.[14-17] Studies revealed that subtle changes in mitotic segregation genes, controlling chromatids separation or regulating the progress of mitosis, could be prime candidates for inducing the slow process of accumulation of genetic changes, leading to CIN.[15,18-20] The novel hypothesis is further supported by the fact that this process is slow, and explains the late onset of sporadic epithelial cancers[21,22], as well as heterogeneous mutation load observed in different sections of tumours from individual patients.

The multidomain protein kinase BUB1B (BUB1-related kinase, known as MAD3 in yeast) plays a central role in the process of spindle assembly checkpoint (SAC), which prevents defects in the segregation of sister chromatids by delaying their separation until all chromatids have achieved correct attachments to the mitotic spindle.[23,24] BUB1B is part of the mitotic checkpoint complex (MCC), which together with BUB3, MAD2 and CDC20 inhibits the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation.[25] The protein BUB1B has also been localized to the kinetochores and is important for stabilizing the kinetochore-microtubule interactions and chromosome alignment.[26] A dual specificity protein kinase TTK (alias MPS1) is crucial for the spindle assembly checkpoint, for chromosome biorientation on the mitotic spindle and for ensuring accurate chromosome segregation.[27,28] Inhibitor and chemical genetics studies showed that TTK activity facilitates the conformational activation of MAD2 from open to closed form (C-MAD2) capable of CDC20 binding and inhibition, thus delaying the onset of anaphase.[29] TTK is probably implicated in the recruitment of the MAD1–C-MAD2 complex to kinetochores and during mitosis its activity is continuously required to recruit O-Mad2 to the Mad1–C-Mad2 core.[30] Furthermore, TTK is required for CENP-E recruitment, whose activity is essential for metaphase chromosome alignment.[30]

In the present study we examined polymorphisms rs151658 (C>G) in *TTK* gene, rs1031963 (C>T) and rs1801376 (A>G) in *BUB1B* gene in the population of Slovenian patients with an advanced gastric cancer and their impact on gastric cancer risk. We also examined the associations of these genetic variants with clinico-histopathological features of patients.

Patients and methods

Research subjects

The study population (n = 284) consisted of 108 Slovenian patients with gastric cancer and 176 control subjects who at the time of peripheral blood extraction did not have cancer. Tumour and corresponding non-tumour tissues at least 7 cm away from the edge of the adenocarcinoma were collected from patients who were admitted to the Clinical Department for Abdominal Surgery at the University Medical Centre Ljubljana and Department for Pathology at the Institute of Oncology Ljubljana during the years 2000–2008. Samples were macrodissected by pathologist, frozen in liquid nitrogen and stored at -70°C. Comprehensive medical data were obtained from registries and pathologist's evaluation. The following clinico-histopathological parameters were recorded: tumour differentiation (grade), location, blood and lymphatic vessel invasion (vascular invasion, perineural invasion), occurrence of tumour cells in the lymphatic vessels (lymphatic invasion), depth of invasion (pT), lymph node involvement (pN), and presence of distant metastases (pM). The gastric cancer cases were classified into diffuse type (n = 46) and intestinal (n = 58) according to Lauren classification. The mean age ± standard deviation (SD) of patients was 66.12 ± 12.02 (range, 33–87 years), and the percentage of men was 63.0%. Cases lost to follow-up (n = 6) and those, who died within 30 days after surgery (n = 2), were excluded from survival analyses. The control population was randomly selected during the years 1999–2007 and shared the ethnic and geographic background of the gastric cancer patients. The research was ap-

proved by the National Medical Ethics Committee of the Republic of Slovenia and confidentiality of personal medical data as well as other data relating to individual identification has been assured in accordance with the Helsinki Declaration.

Genotyping

Genomic DNA from gastric tumour and non-tumour tissues was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and QuickGene™ DNA Tissue Kit S (Fujifilm Corporation, Tokyo, Japan) on QuickGene-810 DNA isolation system (Fujifilm) according to manufacturer's protocol. Genomic DNA from control population was extracted from peripheral blood samples using Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. The DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc.). Genotyping for polymorphism rs151658 (C>G) in *TTK* gene, and polymorphisms rs1031963 (C>T) and rs1801376 (A>G) in *BUB1B* gene was performed using TaqMan-based allele-specific polymerase chain reaction assays on the ABI Prism 7000 Sequencing Detection System apparatus (Applied Biosystems, Foster City, CA, USA) according to the procedure recommended by Applied Biosystems. The 10 μL reaction volume contained 100 ng of DNA. Assay IDs were: C_3181603_10, C_1237153_10, and C_3052718_1. In order to confirm the veracity of the results, the polymorphisms were re-genotyped by direct sequencing on a randomly selected smaller batch of samples.

Immunoblot analysis

A total of 21 paired gastric adenocarcinoma (GA) and adjacent control tissue samples were ground with a mortar and pestle in liquid nitrogen and lysed with 7 mol/L urea, 2 mol/L thiourea, 40 g/L CHAPS, with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). For every 10 mg tissue, 50 μl lysis buffer was added. After sonication on ice (3 x 10 s), the samples were incubated for 1 h on ice with occasional vortexing, and then centrifuged at 20,000×g for 1 h at 4°C. The supernatants were collected and the protein concentrations were determined using the commercial Bradford reagent (Thermo Fisher Scientific, Waltham, MA, USA) with BSA used as the standard. Immunoblot analysis was performed on 42 samples. A total of 30 μg protein per sample was loaded onto 10% gels, separated using SDS-PAGE, and transferred onto PDVF membranes (Millipore, Billerica, MA, USA), which were then blocked in 50 g/L skimmed milk 1 h. The primary antibody was used in the following dilution: anti-Mps1 (anti-TTK) antibody, 1 μg/ml (ab11108, Abcam, Cambridge, UK). Horseradish peroxidase-conjugated secondary antibody was used in the following dilution: goat anti-mouse antibody, 1:5000 (115-035-062, Jackson ImmunoResearch, Newmarket, Suffolk, UK). The proteins were revealed by chemiluminescence using LAS-4000 CCD camera (Fujifilm, Tokyo, Japan). The blots were then quantified with Multi Gauge software (Fujifilm) and the intensities were normalized to Ponceau-S-stained membranes, to allow for loading and transfer variations.

Statistical and bioinformatic analyses

Statistical evaluation of the genotyping data was carried out using the χ^2 or Fischer's exact tests to compare the groups regarding genotype frequencies. Hardy-Weinberg (HW) equilibrium was calculated with an online program (http://www.genes.org.uk/software/hardy-weinberg.shtml).[31] Survival was assessed by the Kaplan-Meier method and differences between groups were evaluated using the log-rank test. Multivariate survival analyses were further performed using the Cox proportional-hazards regression model. In the Cox multivariate analyses, forced entry procedure was used to determine the predictor variables. Only the variables that resulted in p-values < 0.05 in the Kaplan-Meier test were entered into the Cox proportional hazard model for the determination of independent prognostic factors for gastric cancer. The postoperative period was measured from the date of surgery to the date of the last follow-up or death. Statistical software used for calculations was IBM® SPSS® Statistics Version 20. For all statistical tests, a probability level (p-value) of less than 0.05 was considered significant.

To assess the statistical significance of altered protein abundance in the immunoblotting (as the tumour vs. non-tumour paired samples), non-parametric Wilcoxon signed-rank test was used. The tests were double-sided and the values with $p < 0.05$ with a confidence level of 95% were considered to be statistically significant. To assess the correlation of the altered protein abundance from the immunoblotting with the histopathological parameters, repeated measures ANOVA was used. The values with $p < 0.05$ were considered to be statistically significant. Bonferroni post-tests were used to deter-

TABLE 1. Clinicopathological characteristics of patients with gastric cancer

Parameter	Number of patients (%)
Age (years ± standard deviation) (n = 108)	66.12 ± 12.02
Gender (n = 105)	
Male	68 (63.0)
Age (years ± standard deviation) (n = 66)	65.07 ± 12.03
Female	40 (37.0)
Age (years ± standard deviation) (n = 39)	67.90 ± 11.94
Lauren's classification (n = 104)	
Intestinal	58 (55.8)
Diffuse	46 (44.2)
Location (n = 101)	
Upper	40 (39.6)
Lower	34 (33.7)
Mixed	27 (26.7)
Grade/differentiation (n = 105)	
Well	9 (8.6)
Moderate	24 (22.9)
Poor	72 (68.6)
Vascular invasion (n = 80)	
Present	27 (33.8)
Not present	53 (66.3)
Perineural invasion (n = 95)	
Present	44 (46.3)
Not present	51 (53.7)
Lymphatic invasion (60)	
Present	53 (88.3)
Not present	7 (11.7)
pN (n = 105)	
0	24 (22.9)
1–2	15 (14.3)
3–6	20 (19.0)
> 7	46 (43.8)
pT (n = 105)	
Muscularis propria	6 (3.7)
Subserosa	50 (42.6)
Serosa	49 (36.1)

pN = number of positive regional lymph nodes; pT = tumour invasion

TABLE 2. Multivariate survival analysis of clinic-pathological variables in gastric cancer patients

Variable	B*	SE (B)	OR (95% CI)	P
pN	0.670	0.127	1.954 (1.525–2.504)	0.000
Lauren's classification	0.591	0.246	1.807 (1.116–2.925)	0.016

*Predicted change in the hazard for a unit increase in the predictor.
CI = confidence interval; OR = odds ratio; pN = number of positive regional lymph nodes; SE = standard error

mine where the differences were significant. All of the analyses were performed using Microsoft Office Excel 2007 (Microsoft Corporation, Washington, USA) and GraphPad Prism 5 (GraphPad Software, Inc., California, USA).

In order to functionally evaluate intronic polymorphisms we identified their effect in the context of polymorphic biological sequences on protein binding motifs. We used web-based software PROMO, which is part of the ALGGENE web-server.[32,33] The search for putative binding sites was performed using the following parameters: human species, all motifs, and all factors. The data for comparisons of genotype frequencies in European populations of examined SNPs in this study was extracted from the 1000 Genomes Project data platform using a specific version of the Ensembl browser (http://browser.1000genomes.org).[34]

Results

Patients' survival is associated with certain clinico-pathological features

The clinical information and demographic characteristics of selected patients with gastric cancer in this study are summarized in Table 1. At the end of a period of up to 11 years of follow-up, a total of 69 patients out of 100 have died.

The overall 5-year survival was 33.5%. No statistically significant association between tested genetic variations and survival was observed ($p > 0.05$). Univariate survival analysis showed that only Lauren's classification and lymph node involvement (pN) were significant prognostic factors. Diffuse type predicted shorter 5-year survival (log-rank test, $\chi^2 = 5.516$, $p = 0.019$) with overall mean estimate of survival for patients with intestinal type 64.67 months ± 7.74 (SE) (CI = 49.50–79.84) and 39.73 months ± 6.83 (SE) (CI = 26.75–53.11) for patients with diffuse type of gastric cancer. Regarding the parameter pN, patients with 7 or more positive lymph nodes had shorter survival time of 21.93 months ± 4.30 (SE) with CI = 13.5030–36 (log-rank test, $\chi^2 = 34.169$, $p = 0.000$). Multivariate analysis was performed for the same set of patients with complete clinical data sets. Cox regression model included both significant variables, pN and tumour classification. The enter method showed significant improvement ($p < 0.05$) if both parameters were entered into the model (Table 2).

SNPs in *TTK* and *BUB1B* are associated with type, grade, and location of gastric cancer

Associations between clinicopathological parameters and genotypes of SNPs are presented in Table 3. Statistical analysis revealed a weakly significant association for rs151658 genotypes C/G and risk of developing diffuse type of gastric cancer, and genotype G/G and risk of developing intestinal type of

cancer (p = 0.049). Similar results were obtained for both male and female populations of patients (p = 0.047 and p = 0.024, respectively). Genotype A/A of rs1801376 polymorphism was significantly associated with higher risk of developing the diffuse type of gastric cancer in total and female populations of patients (p = 0.007). Interestingly, A/G genotype was under-represented in populations with diffuse type of gastric cancer. Genotype A/A of this polymorphism was also associated with the invasion of tumour cells into subserosa layer of stomach in male population (p = 0.009). A/A genotype was also associated with tumour location, namely, A/A frequencies were increased in patients with tumours disseminated across the whole stomach (p = 0.035).

Genotype T/T of rs1031963 was associated with well differentiated tumours in total population (p = 0.035); however, when we stratified it into female and male populations, we observed a significant association of this genotype with moderately differentiated tumours in the female population (p = 0.004). Clinico-pathological features lymph node involvement (pN), depth of invasion (pT), vascular invasion, perineural invasion and lymphatic invasion did not show significant associations with investigated polymorphisms.

SNPs in BUB1B are associated with gastric cancer risk

The analyses of genotype frequencies in selected SNPs between cases and controls are shown in Tables 4 and 5. The frequencies of all genotypes in cases and control groups were in Hardy-Weinberg equilibrium.

The tested polymorphisms did not show significant differences between gastric cancer patients and control group. In contrast, when we stratified the population for gender, we found significant association between *BUB1B* rs1801376 genotypes and higher risk for developing gastric tumours (p = 0.029). Similarly, dominant model combining genotypes A/G and G/G showed comparable results (p = 0.010; p [Yates correction] = 0.017). Furthermore, tests for association showed analogous results and confirmed significantly higher frequency of G allele in female population of patients with gastric cancer (0.41 *vs.* 0.28 in control group). We also observed allele frequency difference in male patient population for *BUB1B* rs1031963. The dominant model, combining genotypes TT+CT versus CC, showed that patients with C/C homozygous allele had significantly higher risk for developing gastric cancer.

TABLE 3. Comparison of clinic-pathological features and genotypes *TTK* rs151658, *BUB1B* rs1031963, and *BUB1B* rs1801376 in patients with gastric cancer

Parameter		Subject	Variant/Genotype			P
			***TTK* rs151658**			
			GG	CG	CC	
Lauren's classification	Intestinal	Total	17	21	20	**0.049**
	Diffuse		5	25	16	χ^2=6.033
	Intestinal	Male	9	10	14	**0.047**
	Diffuse		5	20	8	χ^2=6.113
	Intestinal	Female	8	11	6	**0.024**
	Diffuse		0	5	8	F=7.499
			***BUB1B* rs1801376**			
			AA	AG	GG	
	Intestinal	Total	18	33	7	**0.007**
	Diffuse		28	13	5	χ^2=9.951
	Intestinal	Male	15	14	4	0.472
	Diffuse		20	9	4	F=1.836
	Intestinal	Female	3	19	3	**0.007**
	Diffuse		8	4	1	F=9.688
			***BUB1B* rs1031963**			
			CC	CT	TT	
Tumour differentiation	Well	Total	1	4	4	**0.035**
	Moderate		7	9	7	F=9.642
	Poor		23	39	7	
	Well	Male	0	2	3	0.139
	Moderate		6	7	2	F=6.439
	Poor		19	20	6	
	Well	Female	1	2	1	**0.004**
	Moderate		1	2	5	F=12.549
	Poor		19	20	6	
			***BUB1B* rs1801376**			
			AA	AG	GG	
pT	Muscularis propria	Total	1	4	1	0.232
	Subserosa		27	18	5	F=5.250
	Serosa		18	25	6	
	Muscularis propria	Male	0	3	1	**0.009**
	Subserosa		22	6	2	F=11.832
	Serosa		13	14	5	
	Muscularis propria	Female	1	1	0	0.816
	Subserosa		5	12	3	F=1.967
	Serosa		5	11	1	
			***BUB1B* rs1801376**			
			AA	AG	GG	
Tumour location	Upper	Total	14	23	3	**0.035**
	Lower		13	15	6	F=10.104
	Whole		18	6	3	

pT = tumour invasion

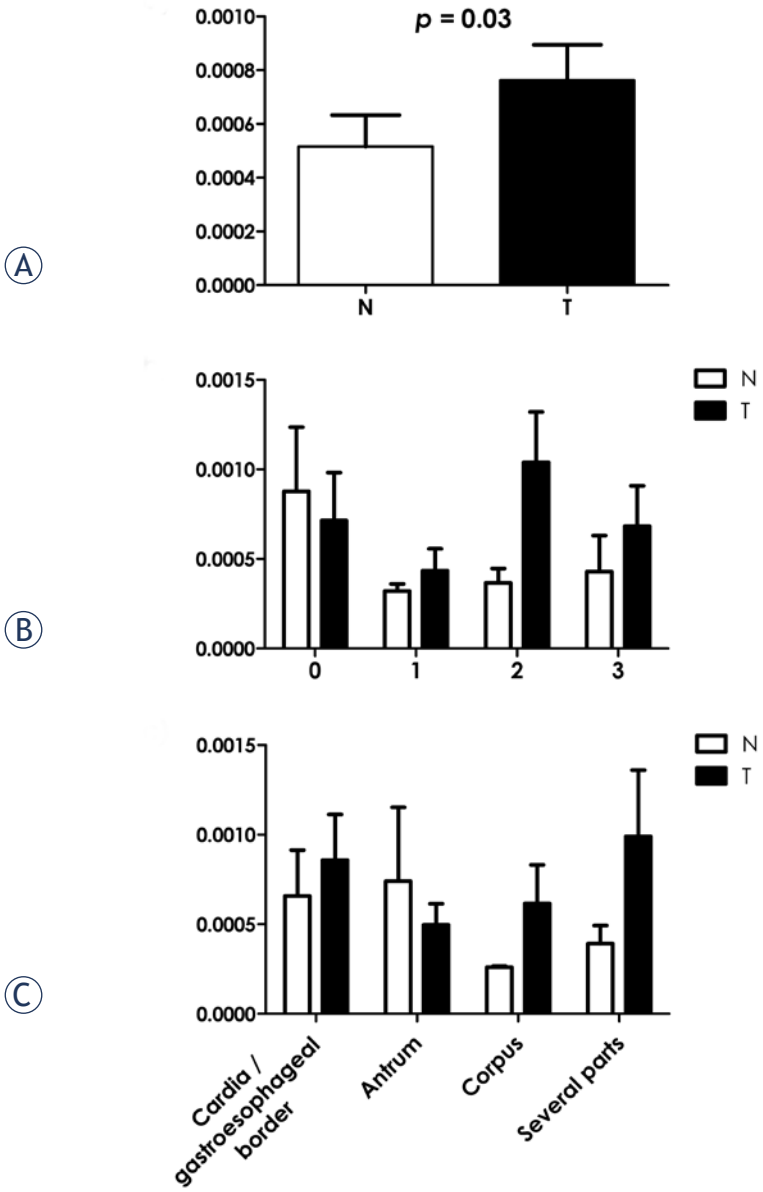

FIGURE 1. Immunoblotting of TTK. (A) Densitometry quantification analysis for the relative band densities from the protein abundance immunoblotting for the indicated protein in the non-tumour (N) and tumour (T) gastric tissues. The p value given (Wilcoxon signed-rank test) indicates the significance of the difference between the non-tumour (N) and tumour (T) gastric tissue samples. (B, C) Densitometry quantification analysis for the relative band densities from the protein abundance immunoblotting for TTK in the non-tumour (N) and tumour (T) gastric tissues samples according to lymph node involvement (pN) and location of the tumours.

Distribution and genotype frequencies of SNPs in *TKK* and *BUB1B* between European and Slovenian populations

Comparisons of the SNPs' genotype frequencies between our test groups and European populations are presented in Figure 3. Genotype frequencies of rs151658, rs1031963 and rs1801376 in our groups of populations showed significant differences from European population (Table 6). The frequency of rs151658 C/C genotype was higher than expected in the Slovenian population of patients compared to total European population ($p = 0.015$). Similarly, we observed more rs1031963 C/C genotypes in the male population of Slovenian patients with gastric cancer ($p = 0.042$) compared with total European population and European population stratified for males. The rs1801376 A/G genotype was higher and A/A genotype was under-represented in female population of patients with gastric cancer compared to the total European population ($p = 0.034$) and female European population ($p = 0.014$).

TTK abundance is altered in tumour tissues of gastric cancer patients

Immunobloting data on individual samples (Figure 1A) demonstrated statistical significance for the increased abundance of TTK ($p = 0.03$) in the tumour tissues. No statistically significant correlation of TTK abundance with clinical histopathological parameters or rs151658 genotypes was observed. However, some trends were observed (Figures 1B and C) for lymph node involvement (pN) and antral tumour location: TTK abundance was higher in normal tissues compared to tumour tissues when no regional nodes were invaded with tumour cells (pN = 0) and when the tumours were located at the bottom of the stomach (antrum).

Prediction of binding motifs showed that polymorphic sites in *TTK* and *BUB1B* bind different transcription factors

To determine if different intronic polymorphisms could affect binding of transcription factors, we performed *in silico* analysis of conserved human motifs using polymorphic sequences as templates (Figure 2). We identified distinct recognition sites for different proteins for both *TTK* rs151658 and *BUB1B* rs1031963. TFII-I, c-Myb, NFI/CTF, and HNF-4 alpha binding motifs were recognized if rs151658 polymorphic site contained C allele. In contrast, if G allele was present, GR, TFII-I, NCFI/CTF, and HNF-4 alpha were identified. Comparison of rs1031963 alleles showed that if allele T was present, several binding motifs were predicted, whereas in the case of allele C, there were no recognizable binding patterns.

Discussion

In this study, we investigated the effects of selected polymorphisms in mitotic kinases *TTK* and *BUB1B* and risk of developing gastric cancer in Slovenian population. We also determined the associations between tested polymorphisms and clinic-pathological features of patients. The results provide evidence that *TTK* rs151658, *BUB1B* rs1031963, and rs1801376 could potentially serve as prognostic biomarkers for determining tumour differentiation and invasion. Furthermore, rs1801376 G allele could be used as one of determinants for gastric cancer screening in female population and CC genotype in rs1031963 could be used for selection of male population at higher risk for developing gastric cancer.

TTK gene harbours more than 600 different one-nucleotide polymorphisms (data obtained from GeneCards, http://www.genecards.org/). We investigated intronic polymorphism, because this gene has many alternative transcripts, and intronic one-nucleotide variants could have an effect on splicing and/or ubiquitination.[35,36] SNP rs151658 lies between exons 12 and 13. In its vicinity there are binding sites for TFII-I, c-Myb, NFI/CTF, and HNF-4 alpha transcription factors, if C allele is present and binding sites for GR, TFII-I, NFI/CTF, and HNF-4 alpha, if G allele is present

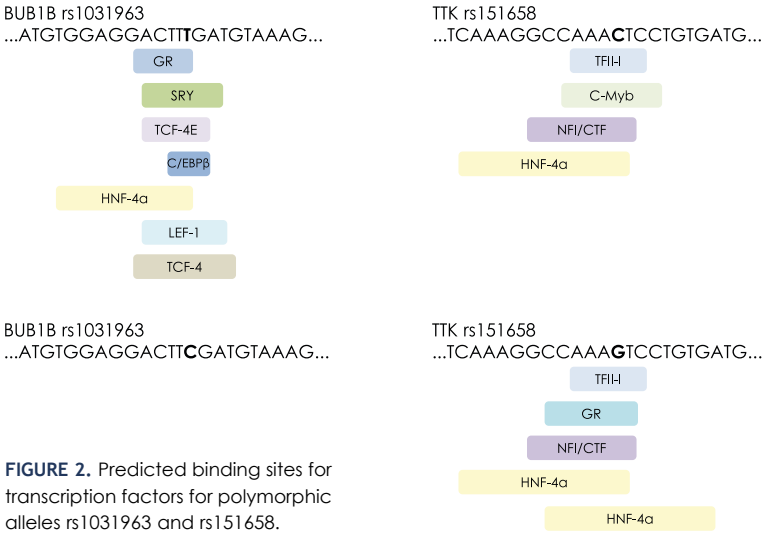

FIGURE 2. Predicted binding sites for transcription factors for polymorphic alleles rs1031963 and rs151658.

TABLE 4. Distribution of genotype frequencies of *TTK* rs151658, *BUB1B* rs1031963, and *BUB1B* rs1801376 between gastric cancer patients and control subjects

Variants	Genotype	Cases (n)	Controls (n)	P	HWE (cases)	HWE (controls)
TTK rs151658	GG	24	55	χ^2=3,628 0.163	χ^2=1.08 0.299	χ^2=2.87 0.090
	CG	48	76			
	C/C	36	44			
BUB1B rs1031963	CC	32	48	χ^2=1.059 0.589	χ^2=0.345 0.557	χ^2=0.035 0.852
	CT	54	89			
	TT	18	39			
BUB1B rs1801376	AA	47	89	χ^2=2.291 0.318	χ^2=0.021 0.885	χ^2=0.005 0.941
	AG	49	69			
	GG	12	13			
Male population	AA	36	49	χ^2=1.186 0.553	χ^2=1.530 0.216	χ^2=0.040 0.841
	AG	24	39			
	GG	8	7			
Female population	AA	11	40	F=6.955 **0.029**	χ^2=3.352 0.067	χ^2=0.013 0.909
	AG	25	30			
	GG	4	6			

F = Fisher statistics; HWE = Hardy-Weinberg Equilibrium; χ^2 = chi-square statistics;

TABLE 5. Odds ratios for *TTK* rs151658, *BUB1B* rs1031963, and *BUB1B* rs1801376 between the cases and controls and their effect on gastric cancer risk

Genotype model	Cases (n)/Control group (n)	OR (95% CI)*	P	P_Y
***BUB1B* rs1031963**				
Dominant TT+CT vs. CC	72/120 vs. 32/48	0.900 (0.527-1.536)	χ^2=0.149 0.699	χ^2=0.062 0.803
Recessive TT vs. CT+CC	18/36 vs. 86/137	0.797 (0.426-1.491)	χ^2=0.507 0.476	χ^2=0.309 0.578
Heterozygous CT vs. CC	54/89 vs. 32/48	0.910 (0.520-1.594)	χ^2=0.108 0.742	χ^2=0.035 0.853
Male population				
Dominant TT+CT vs. CC	41/101 vs. 26/26	0.364 (0.192-0.691)	χ^2=9.848 **0.002**	χ^2=8.834 **0.003**
Recessive TT vs. CT+CC	11/26 vs. 56/101	0.763 (0.351-1.659)	χ^2=0.467 0.494	χ^2=0.241 0.623
Heterozygous CT vs. CC	30/75 vs. 26/26	0.400 (0.201-0.797)	χ^2=6.959 **0.008**	χ^2=6.057 **0.014**
Female population				
Dominant TT+CT vs. CC	31/57 vs. 6/22	1.994 (0.731-5.437)	χ^2=1.862 0.172	χ^2=1.281 0.258
Recessive TT vs. CT+CC	7/13 vs. 30/66	1.185 (0.429-3.269)	χ^2=0.107 0.743	χ^2=0.004 0.949
Heterozygous CT vs. CC	24/44 vs. 6/22	2.000 (0.714-5.606)	χ^2=1.775 0.183	χ^2=1.188 0.276
***BUB1B* rs1801376**				
Dominant GG+AG vs. AA	61/82 vs. 47/89	1.409 (0.868-2.287)	χ^2=1.927 0.165	χ^2=1.601 0.206
Recessive GG vs. AA+AG	12/13 vs. 96/158	1.519 (0.666-3.465)	χ^2=0.999 0.318	χ^2=0.615 0.433
Heterozygous AG vs. AA	49/69 vs. 47/89	1.345 (0.808-2.237)	χ^2=1.304 0.253	χ^2=1.025 0.311
Male population				
Dominant GG+AG vs. AA	32/46 vs. 36/49	0.947 (0.508-1.766)	χ^2=0.029 0.864	χ^2=0.0002 0.990
Recessive GG vs. AA+AG	8/7 vs. 60/88	1.676 (0.577-4.868)	χ^2=0.917 0.338	χ^2=0.466 0.495
Heterozygous AG vs. AA	24/39 vs. 36/49	0.838 (0.430-1.630)	χ^2=0.272 0.602	χ^2=0.124 0.725
Female population				
Dominant GG+AG vs. AA	29/36 vs. 11/40	2.929 (1.281-6.700)	χ^2=6.719 **0.010**	χ^2=5.737 **0.017**
Recessive GG vs. AA+AG	4/6 vs. 36/70	1.296 (0.344-4.889)	χ^2=0.147 0.701	χ^2=0.001 0.971
Heterozygous AG vs. AA	25/30 vs. 11/40	3.030 (1.292-7.108)	χ^2=6.732 **0.009**	χ^2=5.709 **0.017**

* p value with Yates correction
χ^2 = chi-square statistics; OR = odds ratio; CI = confidence interval

(PROMO, ALGGEN server) (Figure 2).[32,33] This indicated that different polymorphic alleles bind different proteins, which could in turn affect splicing or gene expression. Studies, performed on breast cancer patients, confirmed the significant association of this polymorphism with cancer risk[15]; however, we did not find any studies regarding the effect of rs151658 on gastric cancer risk. In our study, we identified the association of G/G genotype with intestinal type of gastric cancer, while C/G genotype was significantly increased in cases with diffuse type of gastric cancer. Interestingly, comparison of genotype distribution for rs151658 between Slovenian patients with gastric cancer and European population showed that C/C genotype was over-represented in patients with gastric cancer. The significance of this finding is not clear and further analyses are needed on larger cohorts of patients in order to determine its usefulness in clinical setting.

To assess if the above mentioned genotypes perhaps had an effect on TTK protein levels, immunoblotting was performed. While the results regarding the effect of genotypes on protein abundance remain inconclusive, it should be noted that polymorphisms usually exert low-penetrance effects, which could more profoundly affect the pathogenesis of gastric cancer in early stages; however, when the disease progresses, the mutation load and aberrant expression of other genes mask their effects. We did, however, confirm higher abundance of TTK in tumour tissues, which is in accordance with several other studies and points out the deregulation of cell cycle homeostasis, higher proliferative trend of tumour cells and weakened spindle assembly checkpoint leading to increased genome instability and aneuploidy.[37,38] Furthermore, this study showed a trend of increased TTK abundance associated with the spread of cancer cells to regional lymph nodes indicating a possible link between TTK levels and metastatic potential of malignant gastric cells.

Homozygous mutations of critical spindle-assembly *BUB1B* are extremely rare and associated with the diseases such as mosaic variegated aneuploidy syndrome 1 (biallelic mutations) and premature chromatid separation trait, which are both characterized by aneuploidy and chromosomal instability.[39] *BUB1B* overexpression has been found in gastric cancers, although the results are often conflicting. In one study, the overexpression of *BUB1B* was associated with tumour proliferation[40], however, Enjoji *et al*. observed that patients with higher expression of *BUB1B* had improved relapse-free survival.[41] Furthermore, Ando *et al*. found that high expression of *BUB1B* correlated with invasion, lymph node metastasis, liver metastasis, and poor prognosis.[14] Bohers *et al*. confirmed that the function of *BUB1B* is dosage-dependent by gradual reduction of *BUB1B* expression by shRNA in cell lines.[42] In their experiment, residual levels of BUB1B protein below 50% of the normal level indicated premature chromatid separation and ane-

TABLE 6. Comparison of *TTK* rs151658, *BUB1B* rs1031963, and *BUB1B* rs1801376 genotypes between the European population and examined groups of Slovenian population

Population	N	Genotype counts	P
***TTK* rs151658**			
EUR	503	97 (C|C) / 246 (C|G) / 160 (G|G)	$\chi^2 = 8.391$; P = 0.015 [a] $\chi^2 = 11.143$; P = 0.004 [b] NS [c]
SI (total)[a]	283	80 (C|C) / 124 (C|G) / 79 (G|G)	
SI (cases)[b]	108	36 (C|C) / 48 (C|G) / 24 (G|G)	
SI (controls)[c]	175	44 (C|C) / 76 (C|G) / 55 (G|G)	
***BUB1B* rs1031963**			
EUR	503	125 (C|C) / 259 (C|T) / 119 (T|T)	NS [d] NS [e] $\chi^2 = 5.715$; P = 0.057 [f]
EUR - male	240	56 (C|C) / 124 (C|T) / 60 (T|T)	- [c] - [d] $\chi^2 = 6.348$; P = 0.042 [f]
SI (total)[d]	280	80 (C|C) / 143 (C|T) / 57 (T|T)	
SI (cases - female)[e]	36	6 (C|C) / 23 (C|T) / 7 (T|T)	
SI (cases - male)[f]	65	25 (C|C) / 29 (C|T) / 11 (T|T)	
***BUB1B* rs1801376**			
EUR	503	240 (A|A) / 217 (A|G) / 46 (G|G)	NS [g] F = 6.569; P = 0.034 [h] NS [i]
EUR - female	263	135 (A|A) / 109 (A|G) / 19 (G|G)	- [g] F = 8.277; P = 0.014 [h] - [i]
SI (total)[g]	279	136 (A|A) / 118 (A|G) / 25 (G|G)	
SI (cases - female)[h]	40	11 (A|A) / 25 (A|G) / 4 (G|G)	
SI (cases - male)[i]	68	36 (A|A) / 24 (A|G) / 8 (G|G)	

EUR = European population; F = Fisher statistics; SI (cases) = gastric cancer patients; SI (controls) = control population; SI (total) = combined populations of patients and controls; χ^2 = chi-square statistics; superscript letters indicate comparisons between European population and Slovenian populations

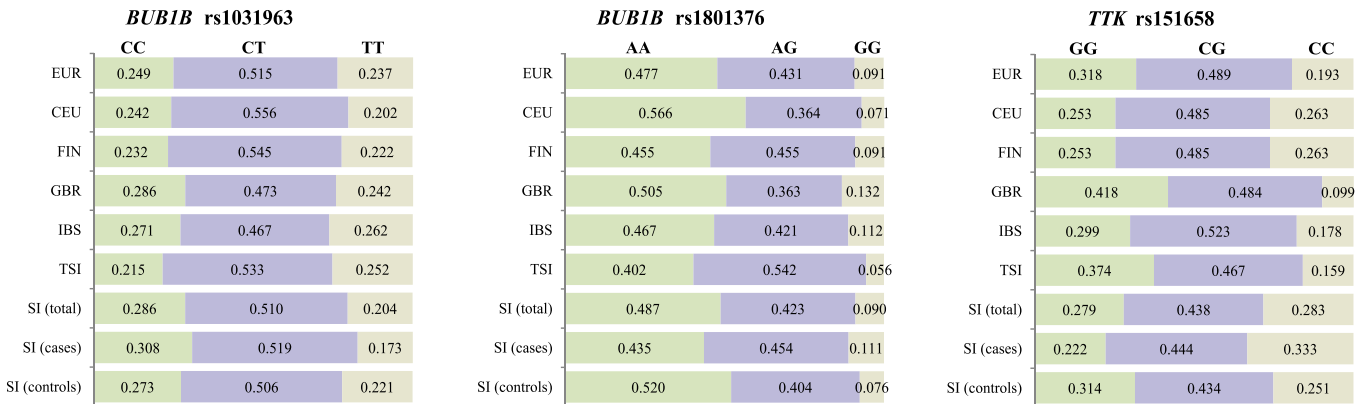

FIGURE 3. Distribution of genotype frequencies of polymorphisms rs151658, rs1031963, and rs1801376 between European populations and Slovenian population.

CEU = Utah Residents (CEPH) with Northern and Western European Ancestry; EUR = European population; FIN = Finnish in Finland; GBR = British in England and Scotland; IBS = Iberian Population in Spain; SI (cases) = gastric cancer patients; SI (controls) = control population; SI (total) = combined populations of patients with gastric cancer and healthy controls; TSI = Tuscany in Italy

uploidy. These conflicting effects of BUB1B could be mediated by different polymorphisms, present in the nucleotide sequence of the gene. *BUB1B* rs1031963 polymorphism is in 5'-promoter region, which harbours binding sites for C/EBPbeta, GR, HNF-4alpha, LEF-1, SRY, TCF-4E, and TCF4 if T allele is present (PROMO, ALGGEN server).[32,33] Interestingly, if C allele is present, the DNA sequence harbours no transcription factor motifs. In our study the T/T genotype was associated with well differentiated tumours in total population, whereas in female population, when analysed separately, it was associated with moderately differentiated tumours. Well differentiated adenocarcinomas tend to have a better prognosis than infiltrative poorly differentiated adenocarcinomas. Furthermore, T/T+C/T genotypes were nominally associated with reduced risk of gastric cancer in male population, whereas C/C genotype was more common in male patient population. Comparisons with European population showed similar results. *BUB1B* rs1801376 A/A genotype was significantly higher in female patients with diffuse gastric cancer. A/A genotype was also increased in samples, which were characterized by invasion of tumour cells into subserosa in male population, and was associated with tumours, growing throughout whole stomach tissue. The consequence of this functional polymorphism is amino acid substitution Q349R in conserved region KEN, which is the binding site for CDC20.[43] CDC20 is co-activator of anaphase promoting complex APC/C.[24] Impaired function of KEN region in *BUB1B* could thus affect the regulation of anaphase delay, which ensures genome stability by providing time for correct spindle assembly, chromosome alignment and segregation. In addition, A/G genotype showed significant association with gastric cancer risk in female population of gastric cancer patients compared to Slovenian control group and European population.

In conclusion, our study provides evidence that polymorphisms in mitotic kinases *TTK* rs151658, *BUB1B* rs1031963 and rs1801376 could have an effect on gastric tumorigenesis and risk of adenocarcinoma development. In addition, we observed differences in genotype distributions between certain clinic-pathological features in patient populations, which could be used as the diagnostic aid in clinical setting; however, a large scale evaluation of these polymorphisms and functional analyses of their effect on protein products are needed to confirm their role in gastric carcinogenesis.

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research article

Metastatic sebaceous cell carcinoma, review of the literature and use of electrochemotherapy as possible new treatment modality

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Background. Metastatic extraorbital sebaceous carcinoma is a rare event that could involve the head and neck. The treatment of choice for the initial stage of the disease is surgery and/or radiotherapy. The treatment of recurrent or advanced disease is still controversial.

Material and methods. Extensive literature search was done, and the treatment options are discussed.

Results. Results. The literature search found several treatment modalities in use for the treatment of metastatic extraorbital sebaceous carcinoma. Electrochemotherapy was not included in the reported treatments. We used this technique for a man of 85 years old with a recurrent and locally metastatic extraorbital sebaceous carcinoma of the scalp.During the period of 8 months, two sessions of electrochemotherapy were employed, which resulted in an objective response of the tumour and good quality of life.

Conclusions. Electrochemotherapy has shown to be a interesting tools for treatment of metastatic extraorbital sebaceous carcinoma when other radical options are not available or convenient.

Key words: electrochemotherapy; head and neck tumour; extraorbital tumour; sebaceous carcinoma

Introduction

The sebaceous carcinoma (SC) is a rare and potentially aggressive adnexial neoplasm of sebaceous gland. Its prevalence varies from 0.05% to 0.7% of all the skin cancers.[1] Approximately, the 25% of sebaceous carcinomas occur in extra-orbital sites, in 15% of these cases the torso and in 10% the extremities are affected.[2]

Extraorbital SC is considered as a less aggressive neoplasm when compared with its periorbital counterpart (a reduced tendency for regional metastasis, 1.4% for extraorbital *vs*. 4.4% for periorbital).[3] Despite this, highly aggressive extraorbital SC has been reported in the literature.[4]

The typical clinical presentation is indistinguishable from other more common dermatologic conditions. Usually, clinical features are a painless pink or yellow firm papule, gradually enlarging and ranging from skin-coloured to red papules, plaques, or nodules. This aspecific presentation delays the time of diagnosis. Histologically an unencapsulated, lobular, dermally based collections of sebaceous and undifferentiated cells that may contain lipid granules in the cytoplasm with a characteristic "frothy" appearance.[5]

SC requires vigilant follow-up after treatment due to its potentially aggressive nature. Local recurrence rate is 4%–28% for both periorbital and extraorbital lesions.[6]

Pathogenesis of SC is poorly understood, however associations with Muir-Torre syndrome[7] is described as well as irradiation[8], immunosuppression[9], familial retinoblastoma.[10] This neoplasm can occur on any sebaceous glands rich skin, particularly face, scalp, and neck. For unknown reasons SC is more prevalent in periorbital skin (39%), especially eyelid, and in extraorbital skin of the head and neck (41%). Some genitalis and extremities cases are reported.[11]

The management of skin cancer of the head and neck region is still challenging. Surgery is commonly considered the treatment of choice and significantly improves the locoregional control, if associated with adjuvant radiotherapy.[12] In the head and neck area, where it is difficult to obtain safety margins wide enough, radiotherapy can be the first treatment option. In particular some areas have been included in the last American Joint Committee on Cancer (AJCC) classification as more at risk of involved margins after surgery in squamous cell carcinoma.[13]

Methods

A systematic literature review for case reports of extra-ocular, cutaneous, multifocal metastasizing SC in the time between 1960 and 2015 was conducted. We therefore searched the literature for all cases mentioning a skin directed therapy and response results for metastatic sebaceous carcinoma utilizing PubMed's MEDLINE database.

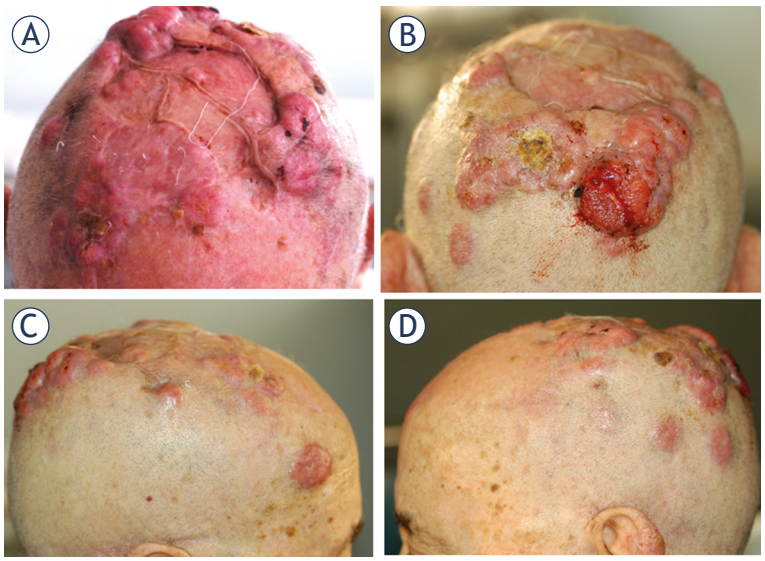

FIGURE 1. Metastatic sebaceous carcinoma, before electrochemotherapy.

Results

Local and distant cutaneous/subcutaneous metastases were described in the course of disease in 8 cases (Table 1).[14-21] Heterogeneity regarding managing of skin cutaneous metastases in head and neck is due to the variability in size, site and clinical comorbidities. Although a few reported cases have shown that skin directed therapy regimens help in palliative treatment, prospective studies have not been performed and their role has not yet been settled due to the rarity of this disease.

TABLE 1. Clinical cases of extra-ocular metastatic sebaceous carcinoma treated with skin directed therapies

Authors	Sex	Age	Localization / metastases	Treatment	Prognosis
Mellette *et al.* (1981) [14]	M	63	Nose / cutaneous and paratiroid metastases	Curettage and surgical excision Parotidectomy Radiotherapy	nod
Moreno *et al.* (2001) [16]	M	45	Left axilla / lymph node metastases	Surgery	6 months
Moura *et al.* (2002) [17]	M	71	Forehead / cutaneous, nodal and skeletal metastases	Surgery Local radiation Chemo and cryosurgery	4 years without recurrence
Khan *et al.* (2003) [18]	F	49	Vulva / subcutaneous and nodal metastases	Surgery Radiotherapy Palliative chemotherapy	nod
Murphy *et al.* (2004) [19]	M	71	Right nostril / left nostril metastases	Surgery	18 months without recurrence
Swick *et al.* (2009) [20]	M	83	Right flank / subcutaneous, nodal, pulmonary and hepatic metastases	Palliative radiotherapy	nod
Bhat *et al.* (2011) [21]	M	32	Left feet / cutaneous metastases	Surgery Chemotherapy	nod
Bolm *et al.* (2015) [15]	M	87	Left ear / nodal, pulmonary and cutaneous metastases	Surgery	18 months without recurrence
Our case	M	85	Scalp / cutaneous and subcutaneous metastases	Electrochemotherapy	2 months

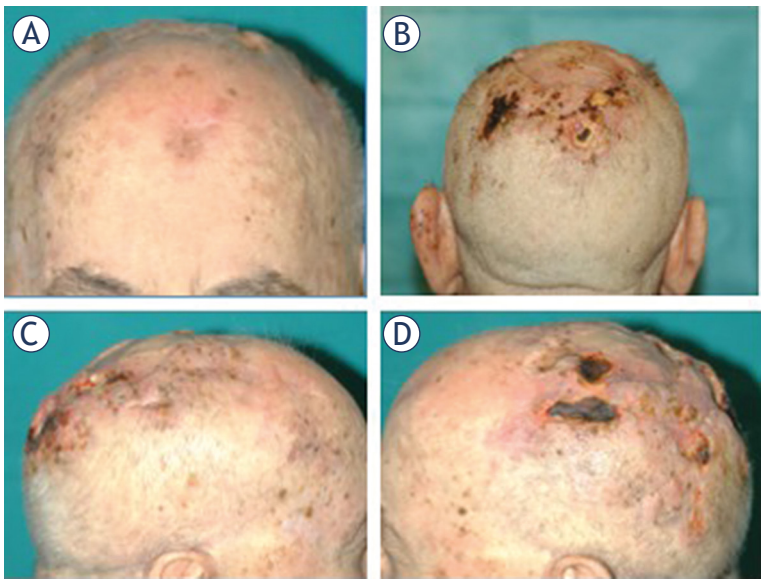

FIGURE 2. Response to treatment after two electrochemotherapy sessions (2 months).

Electrochemotherapy for sebaceous carcinoma

Herein we report a case of a 85-years old Caucasian male came to our attention for the first time presenting 9 papulo-nodular lesions on the scalp ranging from 1 to 3 cm in diameter (Figure 1 A–B). Moreover, some of them bled frequently. The clinical scenario was suggestive for locally metastatic cutaneous carcinoma but the eruptive emergence of skin lesions necessitated a better diagnostic determination. One year before, he had a prior surgical excision of a nodular lesion of the scalp carried out in another hospital treated with skin grafting and histologically diagnosed as cutaneous carcinoma.

Thus, we performed three skin biopsies on three different lesions with histopathological diagnosis of SC. The total body PET-CT scan confirmed the lack of visceral metastatic spreading.

A month after the first visit we observed the appearance of new lesions on the scalp and those previously described were increased in size. After collecting the informed consent for the off label procedure, we performed electrochemotherapy with a 30% reduced dose of the 15000 IU/m^2 intravenous bleomycin due to the patient impaired renal function using the Cliniporator TM device (IGEA Ltd, Carpi, Italy). The treatment of tumour area of 20 cm^2 was performed with hexagonal electrodes in 80 runs of electric pulse applications.

Neither post-treatment complications, nor cranial nerves injuries nor post-procedural pain were observed. Globally 16 lesions were treated with a complete bleeding control.

After one month the lesions treated were flat (Figure 1). Despite this other 4 new lesions appeared in the area on the previous skin graft. A second section of ECT was performed with the same parameters.

Two months after the second electrochemotherapy session the patients has not showed any relapse and was disease free (Figure 2).

Discussion

Herein we described the first case of Electrochemotherapy in the treatment of metastatic extraorbital SC. SC typically consists of roundish nests of tumour-cells with central necrosis, in proximity to normal sebaceous glands. The tumour cells of not well-differentiated SC may display high- grade features with a high frequency of mitotic figures. Angiolymphatic invasion is a consistent finding in SC, like an intraepidermal pagetoid tumour-spread.

The role of Immunohistochemistry in the diagnosis of SC is fundamental as it consistently expresses Epithelial Membrane Antigen (EMA) (which is absent in squamous cell carcinoma and basal cell carcinoma) and, in the centres of the tumour-nests, CD15. Surrounding sebaceous glands are used as a positive internal control for CD15. CD 10 is usually absent in SC. Ansai *et al*.[22] showed that positivity for adipophilin is most useful in the diagnosis of sebaceous neoplasms.

Because of the rarity of this tumour, no standard therapy exists specially in its metastatic onset. In the last years, electrochemotherapy has been proposed as a novel therapeutic option for the control of recurrent cutaneous, subcutaneous or mucosal neoplastic lesions of different histologies.[23-24] There is an increasing body of published clinical data on electrochemotherapy.[25] This procedure achieves rates of objective response between the 56% and the 100%, depending on the tumour size and histology.[26-27] Electrochemotherapy combines the administration of a poorly permeant cytotoxic agent, such as bleomycin with the local application of electric pulses that induce reversible electroporation, thus improving drug diffusion into cells.[28] Electrochemotherapy has demonstrated a high rate of efficacy and favorable toxicity profile in a European multicenter study on skin metastases from different tumour histotypes.[29] In this study, the objective response (OR) rate on treated tumour

nodules was 89.0% with complete regression in 73.3% of cases. A recently published meta-analysis including 47 prospective studies comparing five skin-directed therapies (electrochemotherapy, radiation, photodynamic therapy, intralesional therapy, and topical therapy), electrochemotherapy demonstrated an OR rate of 75.4% (complete response [CR] rate 47.5%) with a low toxicity profile (grade 3 in less than 6% of patients).[30] The main advantages of electrochemotherapy include:

- high success rate in local tumour control after a single session;
- no damage to healthy peripheral tissue (using low doses of chemotherapeutic agents electrochemotherapy is very specific for dividing tumour cells, sparing the surrounding normal tissue);
- no protein denaturation, so that tumour antigens are not destroyed and may elicit an immune response;
- excellent safety profile (in clinical use, no serious adverse events were reported in association with electrochemotherapy;
- advantageous cost/benefit ratio: the technology and the drugs used, in particular bleomycin, do not require large investments;
- improvement of patient's quality of life.

Reported studies showed clinical activity, positive impact on patients subjective clinical perception and low toxicity profile of electrochemotherapy and encourages us to propose to patients this technique with an palliative intent, after the failure of conventional treatment options, such as surgery, radiotherapy, and systemic therapies.[31] Especially in Kaposi Sarcoma, where skin lesions often cause pain and disfigurement and may lead to functional disability, electrochemotherapy has become the standard of care as first line treatment strategy.[32]

To the best of our knowledge no data on skin metastases from extracutaneous SC treated with electrochemotherapy are documented in the literature. Extraorbital SC has a high risk of local recurrence and it usually appears in elderly. Herein, we reported a CR at 2 months time from two sessions of electrochemotherapy for SC skin metastases. The choice of using electrochemotherapy in our patient was determined by the locally advanced pathology that would not permit a surgical resection and by the patient's refusal of radiotherapy. This case demonstrates that electrochemotherapy can be considered as an effective palliative treatment option for patients with recurrent or advanced-stage tumour, not suitable for conventional treatments.

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research article

The proliferation marker Ki67, but not neuroendocrine expression, is an independent factor in the prediction of prognosis of primary prostate cancer patients

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Background. Neuroendocrine markers, which could indicate for aggressive variants of prostate cancer and Ki67 (a well-known marker in oncology for defining tumor proliferation), have already been associated with clinical outcome in prostate cancer. The aim of this study was to investigate the prognostic value of those markers in primary prostate cancer patients.

Patients and methods. NSE (neuron specific enolase), ChrA (chromogranin A), Syp (Synaptophysin) and Ki67 staining were performed by immunohistochemistry. Then, the prognostic impact of their expression on overall survival was investigated in 166 primary prostate cancer patients by univariate and multivariate analyses.

Results. NSE, ChrA, Syp and Ki67 were positive in 50, 45, 54 and 146 out of 166 patients, respectively. In Kaplan-Meier analysis only diffuse NSE staining (negative *vs* diffuse, $p = 0.004$) and Ki67 (≤ 10% *vs* > 10%, $p < 0.0001$) were significantly associated with overall survival. Ki67 expression, but not NSE, resulted as an independent prognostic factor for overall survival in multivariate analysis.

Conclusions. A prognostic model incorporating Ki67 expression with clinical-pathological covariates could provide additional prognostic information. Ki67 may thus improve prediction of prostate cancer outcome based on standard clinical-pathological parameters improving prognosis and management of prostate cancer patients.

Key words: primary prostate cancer; prognosis; Ki67; NSE

Introduction

Conventional clinical parameters alone are inadequate for differentiating indolent and aggressive prostate cancer. Therefore, molecular biomarkers are needed to better define prognosis of prostate cancer patients.

Neuroendocrine markers could be used to detect particularly aggressive variants of prostate cancer. Typical markers used to identify neuroendocrine differentiation (NED) in tumor tissue are neuron specific enolase (NSE), chromogranin A (CgA) and synaptophysin (Syp).[1-3] Neuroendocrine differentiation, measured by one or more of those markers, has been associated with disease progression[4] or poor survival in prostate cancer[5], but up to now its prognostic value has not been clarified because of controversial results[6,7,8]. However, Epstein *et al*.[9]

have recently suggested using neuroendocrine markers to better characterize and classify NED in prostate cancer. They also outlined Ki67 ranges in those tumors, which usually have a high proliferative index.[4,9-13] Ki67 is a well-known marker in oncology for defining tumor proliferation. Expression of Ki67 detection by immunohistochemistry[14] is used as a prognostic marker for cell proliferation in many tumors, especially in breast carcinoma[15] and cervical cancer.[16] In prostate cancer it has also been associated with clinical outcome, irrespective of treatment.[4,17-27]

The aims of this study were to 1) investigate the immunohistochemical expression of neuroendocrine and Ki67 markers in primary prostate cancer patients in order to identify tumors characterized by biological aggressiveness and poor prognosis, 2) evaluate neuroendocrine expression with respect to Ki67 staining.

Patients and methods

Patients

Detailed histopathological and clinical data were retrospectively collected for 166 patients, who were diagnosed with primary prostate cancer in a single institution of the North-eastern Italy from January 1992 to December 1994, therefore associated to a long follow-up period. Inclusion criteria for this study were: a) diagnosis of prostate cancer and b) availability of formalin-fixed and paraffin-embedded tissues for immunohistochemical staining and molecular analyses. Only TURP (N = 122, 73.9%) and prostatectomy (N = 43, 26.1%) specimens were used (missing information for one patient). Fine needle biopsies were excluded because of the low amount of tissue. Patients did not receive any treatment before diagnosis. The use of formalin-fixed and paraffin-embedded prostate cancer tissues and their related clinical information were approved by the Ethical Committee of the University of Trieste (Report 23; 5.10.2009) before the beginning of the study.

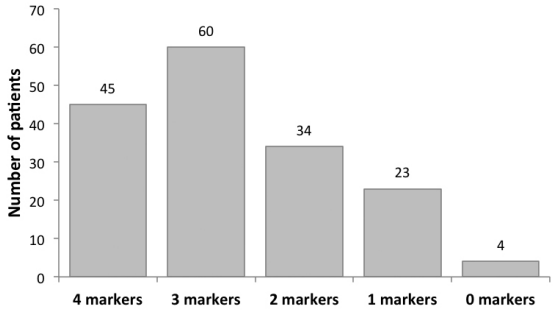

FIGURE 1. Determination of Ki67, NSE, CgA, SYP in primary prostate cancer patients. Number of concurrent biomarkers evaluated. The number of patients is reported above each column.

Tissue microarray and immunohistochemical staining

Representative multiple areas of the primary tumours were selected by two pathologists (G.S. and R.B.) for TMA construction. Tissue cores were chosen at the border of the primary tumour. Tissue cylinders of 1.0 mm in diameter were taken from the selected regions of the donor's paraffin block and were placed into a recipient paraffin block using a tissue-arrayer (Galileo TMA CK3500; Integrated Systems Engineering, Milano, Italy), as previously described.[28] Multiple cores were taken for cases as representative of heterogeneous histological areas. Neuroendocrine differentiation (NED) was evaluated using NSE, ChrA, Syp as neuroendocrine markers.

Immunostainings for Ki67 (clone MIB-1; DakoDenmark A/S, Glostrup, Denmark), 1:200 dilution; NSE (clone E27; NeoMarker; ThermoScientific, Waltham, MA, USA) 1:2500; ChrA (clone LK2H10; NeoMarker; ThermoScientific, Waltham, MA, USA), 1:500; Syp (clone SY 38; Thermo-Fisher; ThermoScientific, Waltham, MA, USA) 1:75 were performed in a Lab Vision Autostainer 480S (Thermo Scientific, Waltham, MA, USA) with the UltraVision LP Large Volume Detection System HRP Polymer (Lab Vision Corporation, Thermo Scientific) according to manufacturer's recommended protocol. For evaluation of the immunostaining, positively stained cells were counted across 3 high-power fields. Staining intensity was not taken into consideration. Due to technical issues related to the detachment of tissue cores it was not possible to analyze the four biomarkers in all samples (Figure 1). The percentage of the positively stained cells was reported for each specimen. Ki67 expression was dichotomized for assessing its prognostic value using a cut-off of 10%.[7,29]

Statistical analysis

The Kaplan-Meier method was used to generate overall survival (OS) curves, which was defined as the time between the date of diagnosis and the date of death or the last follow-up (FU) observation. Patients were censored if they were still alive or they were lost to FU. The log-rank test was used to evaluate differences between groups. Association

TABLE 1. Neuroendocrine marker staining in our cohort of primary prostate cancer patients

Staining	Neuroendocrine markers		
	NSE	*CgA*	*Syp*
Negative	28 (31.5)	49 (40.5)	44 (34.6)
Diffuse	50 (56.1)	45 (37.2)	54 (42.5)
Focal	3 (3.4)	19 (15.7)	17 (13.4)
Spotty	8 (9)	8 (6.6)	12 (9.5)
Total	89	121	127
missing	77	45	39

Data reported as N (%)

TABLE 2. Distribution of Ki67 positively stained cells in our cohort of primary prostate cancer patients

% Ki67-positive cells	Number of samples	
	N	*%*
0	14	9.6
≤ 5	70	47.9
> 5 and ≤ 10	24	16.5
> 10 and ≤ 20	18	12.3
> 20 and ≤ 30	13	8.9
> 30	7	4.8
Total	146	100%

of OS with each prognostic factor was evaluate in univariate and multivariate analyses by using the Cox proportional hazards model. All variables associated with univariate value of $p \leq 0.05$ were included in the multivariate model using a stepwise method. The proportional hazards assumptions were checked before applying the Cox regression model. The goodness of fit was assessed using a likelihood-ratio test. The discrimination ability was quantified by calculating the concordance index that ranges from 0.5 (no discrimination) to 1 (perfect discrimination). Possible correlations of the expression of Ki67 and neuroendocrine markers with the other prognostic variables were assessed by $\chi 2$ test or Wilcoxon rank sum. All statistical tests were performed using STATA software (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP) and p values ≤ 0.05 were considered as statistically significant.

Results

Neuroendocrine marker staining

NSE expression was assessable in 89 prostate cancer cases. Of those, 28 cases (31.5%) were completely negative, whereas 61 (68.5%) revealed a cytoplasmic positivity, referring to diffuse, focal and spotty staining (Table 1). Detachment of tissue cores did not allow analyzing NSE in some cases. ChrA was negative in 49 out of 121 cores (40.5%) and it was positive on the cytoplasmic level in 72 cores (59.5%). Positive cases had weakly to highly diffused, or focal or spotty reactivity (Table 1). Syp was analyzed in 127 cases of which 44 (34.6%) were negative and 83 (65.4%) showed a cytoplasmic positivity, from spotty to focal to diffused (Table 1). Only diffused expression was considered as posi-

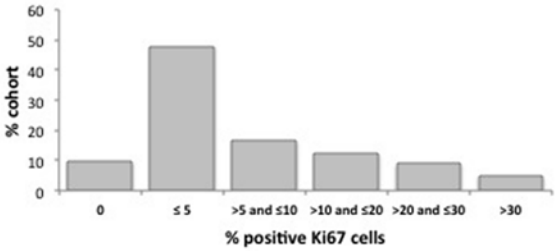

FIGURE 2. Distribution of Ki67 staining score. Positive Ki67 cells in TURP and prostatectomy specimens from a cohort of 166 primary prostate cancer patients.

TABLE 3. Association between Ki67 expression and clinicopathological variables (N = 146)

Variable	Ki67 expression			
	≤ 10%	*> 10%*	Tot	*P*-value
Nuclear grading				
1	22	1	23	
2	72	23	95	0.001*
3	14	14	28	
Gleason Score				
< 7	56	8	64	0.001*
≥ 7	51	30	81	
missing 1				
Age at diagnosis (median, years)				
≤ 71	53	19	72	0.922
> 71	55	19	74	
Age at diagnosis (continuous)	108	38	146	0.973
NSE expression				
Negative	21	4	25	0.073
Positive[a]	28	16	44	
missing 77				
CgA expression				
Negative	37	10	47	0.217
Positive[a]	24	12	36	
missing 40				
Syp expression				
Negative	31	8	39	0.326
Positive[a]	33	14	47	
missing 60				
Type of intervention				
TURP	78	33	111	0.081
Prostatectomy	29	5	34	
missing 1				
Total	108	38	146	

[a] Only diffuse expression was considered as positive staining; *Significant value

TABLE 4. Univariate analysis of prognostic factors in patients with primary prostate cancer based on Cox proportional hazards regression model

Variable	UNIVARIATE	
	HR (95% CI)	*P*-value
Nuclear grading		
2 vs 1	1.45 (0.90-2.34)	0.125
3 vs 1	3.49 (1.94-6.27)	< 0.001*
Gleason score		
≥ 7 vs < 7	2.97 (2.07-4.26)	< 0.001*
Ki67 expression		
> 10% vs ≤ 10%	2.75 (1.85-4.09)	< 0.001*
Age at diagnosis (continuous)	1.04 (1.02-1.07)	< 0.001*
NSE expression		
Positive[a] vs negative	1.99 (1.19-3.34)	0.009*
CgA expression		
Positive[a] vs negative	0.96 (0.62-1.47)	0.840
Syp expression		
Positive[a] vs negative	1.18 (0.77-1.81)	0.447
Type of intervention		
Prostatectomy vs TURP	0.45 (0.30-0.67)	< 0.001*

CI = confidence interval; HR = hazard ratio
[a] Only diffuse expression was considered as positive staining; *Significant value

TABLE 5. Multivariate analysis of prognostic factors in patients with primary prostate cancer based on Cox proportional hazards regression model stratified by type of intervention (N = 144)

Variable	MULTIVARIATE	
	Adjusted HR (95%CI)	Adjusted *P*-value
Nuclear grading		
2 vs 1	1.27 (0.75-2.13)	0.373
3 vs 1	1.93 (1.01-3.68)	0.045*
Gleason score		
≥ 7 vs < 7	2.41 (1.56-3.74)	< 0.001*
Ki67 expression		
> 10% vs ≤ 10%	2.14 (1.41-3.25)	< 0.001*
Age at diagnosis (continuous)	1.04 (1.02-1.07)	0.001*

CI = confidence interval; HR = hazard ratio; *Significant value

tive for each marker. Focal or spotty stainings were evaluated as negative.

No significant associations were found between neuroendocrine markers and clinicopathological variables. A slight association was revealed between NSE expression and Gleason score (p = 0.04). Moreover, a positive relationship was found between CgA and SYP expression (p = 0.01). The type of intervention was significantly associated with CgA (p = 0.009) and NSE (p = 0.001) expression.

Ki67 staining

Ki67 staining analysis was measured in 146 out of 166 prostatic cases. The median Ki67 staining score was 5% with an IQR of 9 whereas the mean value was 10.3% with a standard deviation of 14.2% (range of 0 – 90%). The percentage of the positively stained cells was recorded for each sample (Table 2, Figures 2,3).

Ki67 was scored and stratified into two groups (low ≤ 10%; high > 10%) as already reported.[23,24] No statistically significant difference was observed for Ki67 staining between TURP and prostatectomy specimens (p = 0.08). Ki67 expression was significantly associated with Gleason score and nuclear grading, but not with age at diagnosis or with neuroendocrine markers (Table 3).

Univariate analysis

In univariate analysis, diffuse expression of NSE, Ki67 expression > 10%, Gleason score ≥ 7 and nuclear grading ≥ 2 predicted for shorter OS (Table 4).

Considering neuroendocrine markers, only NSE staining was significantly associated with OS. Patients with diffuse NSE expression had a reduced survival time (median OS 2 years; 95% CI, 2–4) compared to patients with negative expression (median OS 7; 95% CI, 5–10; p = 0.004), showing nearly double-fold increased risk of death (Table 4, Figure 4). Additionally, NED measured as positive at least at one of the three markers did not result significant.

Kaplan-Meier survival curves showed a significant difference (p < 0.001) between low and high levels of Ki67 staining with median survival time of 6 years (95% CI, 5–9) and 2 years (95% CI, 1–2) for patients with ≤ 10% and > 10% of positive cells, respectively (Figure 5A). Moreover, splitting group four categories according to Ki67 staining were obtained: negative, 1–10%, 11–20%, > 20%. Negative patients showed a median overall survival (10 years, 95% CI: 4–9·) which was four years longer than in patients with Ki67 staining of 1–10% (6 years, 95% CI: 5–8; p < 0.001) (Figure 5B). An improved discrimination was also reached in the category > 10%. Patients with Ki67 staining > 20% were associated with a 2.5-fold increased risk of death, compared with patients showing Ki67 expression of 10–20% (the overall 2-year survival

rate of 50%–95% CI: 25–70 *vs* 20%, 95% CI: 6–39, p < 0.001). These data further support the potential role of Ki67 immunostaining in selecting patients according to proliferation rate, and thus to tumor aggressiveness. However, we decided to assess the prognostic value of Ki67 for overall survival of prostate cancer patients by using the previous binary variable because of harmonization with already published studies.[7,29]

Age at diagnosis and type of intervention significantly impacted on OS (p < 0.001). Thus, considering the relationship existing with other variables, they were included in multivariate analysis to take into account their possible confounding effect.

Multivariate analysis

Multivariate analysis was done by stratifying according to type of intervention, because of no proportional risks between TURP and prostatectomy groups (Test of proportional-hazards assumption, $\chi^2 = 8.86$, df = 1, p = 0.003). Ki67 expression, but not NSE, resulted as an independent prognostic factor for OS. In the final multivariable model the risk of death was higher for older patients (p = 0.001) with a nuclear grading of 3 (p = 0.04), a Gleason score ≥ 7 (p < 0.001) and Ki67 expression > 10% (p < 0.001) (Table 5). Unfortunately, our dataset did not include information on PSA before surgical intervention for all patients, because they were diagnosed many years ago before the routinely application of PSA screening.

Comparison of the multivariate model incorporating Ki67 expression with a base model including conventional variables only (nuclear grading, Gleason score, age at diagnosis stratified by type of intervention) showed an improved fit which suggested an enhanced prognostic ability over the models containing clinicopathological variables only ($\chi^2 = 11.33$, df = 1, p = 0.0006). These data indicated that in a multivariate analysis Ki67 is a relevant and independent prognostic factor for OS of primary prostate cancer patients undergoing TURP or radical prostatectomy. The concordance index (0.72) revealed a good accuracy of the model in predicting OS.

Discussion

This study shows that Ki67 expression, but not NED, is an independent prognostic factor for OS in primary prostate cancer patients who underwent TURP or prostatectomy.

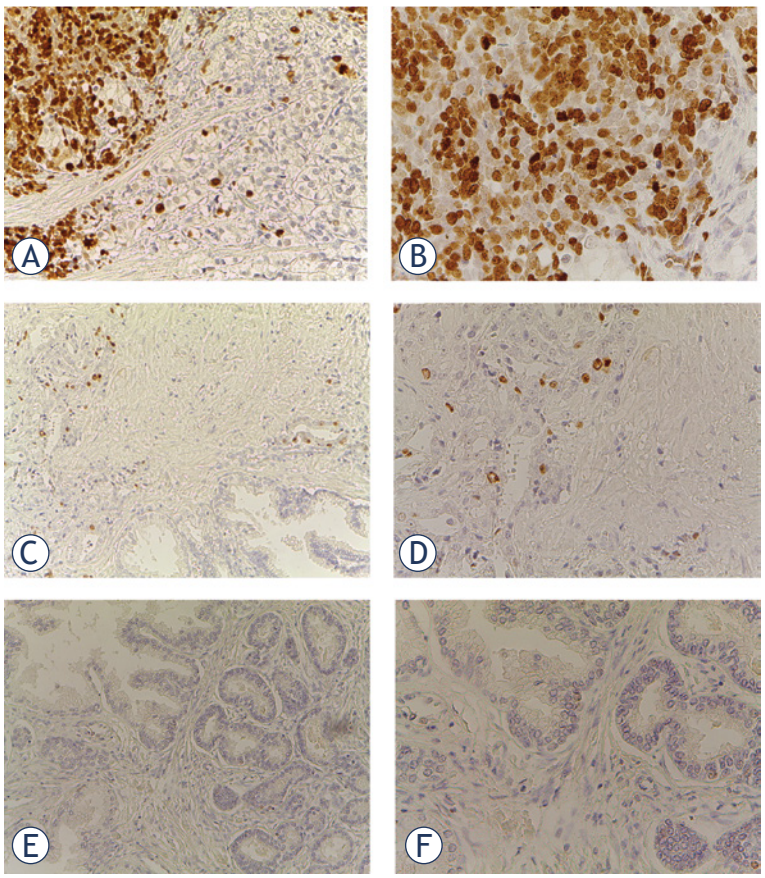

FIGURE 3. Representative immunohistochemical staining for Ki67. **(A)** prostate adenocarcinoma with Ki67 > 10% 20 x and 40 x **(B)** magnification; **(C)** prostate adenocarcinoma with Ki67 ≤ 10% 20 x and 40 x **(D)** magnification; **(E)** prostate adenocarcinoma negative for Ki67 20 x and 40 x **(F)** magnification.

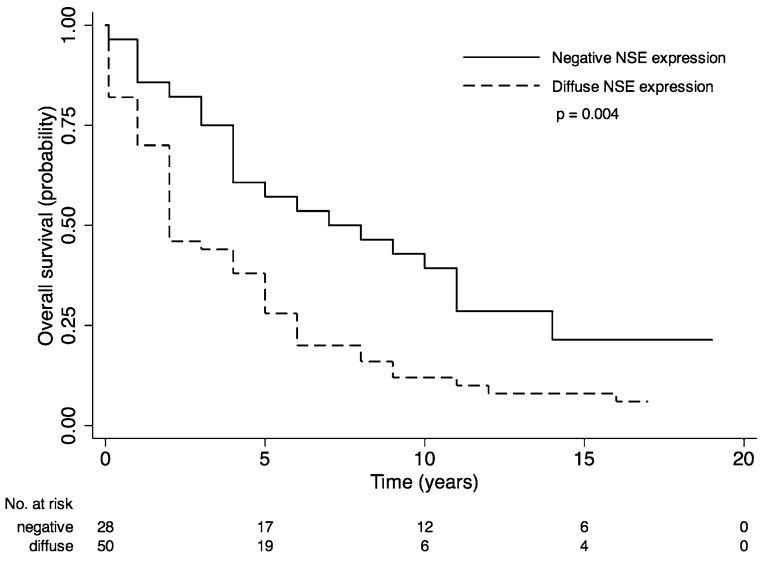

FIGURE 4. Survival curves by NSE staining in patients with primary prostate cancer. p-value from log-rank test is reported. Numbers of at risk (still alive) patients are indicated below the x-axis.

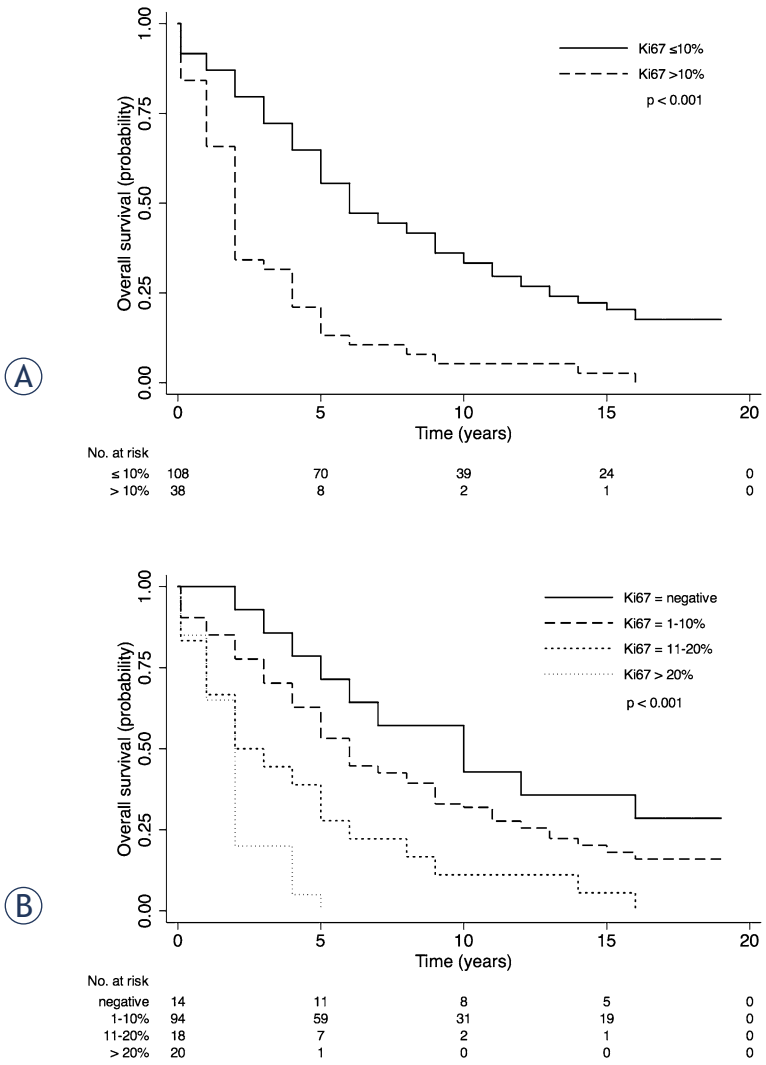

FIGURE 5. Survival curves by Ki67 expression in patients with primary prostate cancer. **(A)** Ki67 staining dichotomized in ≤ 10% and > 10%; **(B)** Ki67 staining divided into negative, 1–10%, 11%–20%, > 20%. p-value from log-rank test is reported. Numbers of at risk (still alive) patients are indicated below the x-axis.

Our data demonstrate that NED, as measured by NSE, CgA and SYP immunohistochemistry, is present at the time of diagnosis in a large proportion of our cohort (over 50%), but without influence on OS. NSE staining seems to influence OS, but it was not confirmed as an independent prognostic factor in the multivariate analysis. These results are consistent with current published literature where strong evidence of NSE as potential prognostic factor is lacking (reviewed in [6,8]), especially at early stages.[30] NED in PCa increases with higher histological grades[31] and disease progression, especially in response to androgen deprivation therapy.[32-34] It seems that androgen deprivation therapy may promote the transformation of prostate adenocarcinoma into a neuroendocrine cancer, defined as t-NEPC (transformed neuroendocrine prostate cancer), as a mechanism of resistance.[35] Ki67 expression has already been proposed as a candidate marker for gastroenteropancreatic[36] and lung neuroendocrine cancers.[37] An increased proliferation using Ki67 expression has been shown in prostate tumors with high NED compared to tumors with low or without NED.[4,10,11] In this study we did not find any association between NED and Ki67 expression, although a trend for a positive relationship between NSE staining and Ki67 expression (p = 0.08) has been observed.

Limitations of this study are: small sample size, missing data for neuroendocrine IHC, statistically significant results obtained by using the less specific marker for NED.[9] Another limitation is the analysis based on mixed sample population of prostatectomy and TURP which was partially solved using a stratified Cox model to investigate the risk difference in two groups. Of relevance, our cohort represents a long-term time series which allows investigating OS in cancers with a long life expectancy.

The prognostic significance of concurrent presence of NED features in prostate adenocarcinoma is currently very controversial.[6] Although NED may have an adverse effect on prognosis of newly diagnosed prostate cancer, other mechanisms probably influence the prognosis by favoring the selection of the neuroendocrine pattern transformation under a specific stimulus, such as the pressure by androgen deprivation therapy.[38] The mechanisms that are currently involved are not known.

Interestingly, we found that cell proliferation measured by Ki67 staining scored as a dichotomous variable with a 10% cut-off is an independent prognostic factor for OS in our cohort. Comparing prognostic models with and without Ki67 demonstrated that Ki67 expression could yield additional prognostic information to that provided by conventional clinicopathological parameters improving prognosis of prostate cancer patients. Furthermore, our data show that Ki67 expression correlates with Gleason score and nuclear grading, highlighting its association with prostate cancer aggressiveness, in agreement with others.[4,23,25,39,40] Several studies have shown that Ki67 is useful to predict prostate cancer prognosis either on TURP[19,23] or needle biopsy[21,23,25,40] specimens. Furthermore, it has been proposed as a candidate prognostic marker both for overall and specific survival endpoints[17-22] as well as disease progression.[4,17,18,20,22-27] Its prognostic rel-

evance does not seem to be influenced by therapy, as it has been reported to predict prognosis in patients treated by prostatectomy, alone[4,24,25,27] or with adjuvant therapy[26], or radiotherapy, alone[17,22,23] or with androgen deprivation therapy[17,18,20,22], or conservatively managed.[19,21]

Consistent with our results, Ki67 emerges as a powerful marker of biological aggressiveness that could provide supplemental prognostic information, concerning the cellular proliferation rate, in addition to that provided by currently used markers, which are related to tumor pattern and extension only. Therefore, Ki67 may improve prediction of prostate cancer outcome based on standard clinical parameters, and may help stratify and select patients for more aggressive treatments. The utility of Ki67 was also demonstrated in selecting candidates with clinically insignificant cancer suitable for active surveillance among patients with PSA < 4 ng/ml at diagnosis.[40] The validity of Ki67 expression as an indicator of prognosis for prostate cancer patients in different treatment cohorts, including both radical[4,17,18,20,22-27] and conservative therapies[19,21], and in many investigative materials, such as biopsy[21,23,25,40], TURP[19,23] or prostatectomy[4,24-27] specimens, further supports its implementation in clinical practice, as recently sustained also in a meta-analysis.[41] However, larger prospective studies are needed to validate its use in routine pathology.

Despite unresolved issues on cut-offs, we suggest the analysis of Ki67 in routine diagnostic practice as an additional factor for improving prognosis and management of prostate cancer patients.

Acknowledgments

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research article

Pancreaticoduodenectomy for ductal adenocarcinoma of the pancreatic head with venous resection

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Background. Recent reports have shown that patients with vascular tumour invasion who undergo concurrent vascular resection can achieve long-term survival rates equivalent to those without vascular involvement requiring pancreaticoduodenectomy alone. There is no consensus about which patients benefit from the portal-superior mesenteric vein resection and there is no consensus about the best surgical technique of vessel reconstruction (resection with or without graft reconstruction). As published series are small the aim of this study was to evaluate our experience in pancreatectomies with en bloc vascular resection and reconstruction of vessels.

Methods. Review of database at University Clinical Centre Maribor identified 133 patients (average age 65.4 ± 8.6 years, 69 female patients) who underwent pancreatoduodenectomy between January 2006 and August 2014. Clinical data, operative results, pathological findings and postoperative outcomes were collected prospectively and analyzed. Current literature and our experience in pancreatectomies with en bloc vascular resection and reconstruction of portal vein are reviewed.

Results. Twenty-two patients out of 133 (16.5%) had portal vein-superior mesenteric vein resection and portal vein reconstruction (PVR) during pancreaticoduodenectomy. In fourteen patients portal vein was reconstructed without the use of synthetic vascular graft. In these series two types of venous reconstruction were performed. When tumour involvement was limited to the superior mesenteric vein (SPV) or portal vein (PV) such that the splenic vein could be preserved, and vessels could be approximated without tension a primary end-to-end anastomosis was performed. When tumour involved the SMV-splenic vein confluence, splenic vein ligation was necessary. In the remaining eight procedures interposition graft was needed. Dacron grafts with 10 mm diameter were used. There was no infection after dacron grafting. One patient had portal vein thrombosis after surgery: it was thrombosis after primary reconstruction. There were no thromboses in patients with synthetic graft interposition. There were no significant differences in postoperative morbidity, mortality or grades of complication between groups of patients with or without a PVR. Median survival time in months was in a group with vein resection 16.13 months and in a group without vein resection 15.17 months. Five year survival in the group without vein resection was 19.5%. Comparison of survival curves showed equal hazard rates with log-rank p = 0.090.

Conclusions. Survival of patients with pancreatic cancer who undergo an R0 resection with reconstruction was comparable to those who have a standard pancreaticoduodenectomy with no added mortality or morbidity. Synthetic graft appeared to be an effective and safe option as an interposition graft for portomesenteric venous reconstruction after pancreaticoduodenectomy.

Key words: pancreaticoduodenectomy; pancreatic cancer; vein resection

Introduction

In cases of pancreatic cancer pancreaticoduodenectomy with complete resection offers the only chance for cure. Historically, involvement of regional vascular structures by pancreatic carcinoma has been considered a contraindication for reconstruction.[1] At the time of diagnosis more than three quarters of patients have locally advanced disease or distant metastasis that preclude radical surgery and 5-year survival after "curative" surgery ranges from 10 to 20% even in recent large series.[2] Advances in surgical techniques, perioperative care and the institution of tertiary specialized centres have been the key for a substantial improvement in mortality and morbidity rate. Venous resection (VR) is performed to achieve negative resection margins because the tumour involves the vessel or inflammatory adhesions preclude a safe separation of the vein. Another theoretical benefit of VR is to achieve clearance of surrounding perivascular and perineural tissue. Venous resections (VR) include excision of portal vein (PV), superior mesenteric vein (SMV) or the superior mesenteric-portal vein confluence (SMPV).[2]

Although the utility of aggressive vascular resection in pancreatic adenocarcinoma continues to be debated[3,4] several institutional series have demonstrated the feasibility of margin negative resection with acceptable morbidity rates comparable to those after isolated pancreaticoduodenectomy. Recent reports also have shown that patients with vascular tumour invasion who undergo concurrent vascular resection can achieve long-term survival rates equivalent to those without vascular involvement requiring PD alone.[5-9]

Reconstruction of the PV or SMV is a challenge for the vascular surgeon because of the lack of size-matched autogenous conduit. In addition, concerns about graft infection have restricted the use of prosthetic grafts during the intra-abdominal surgery.[9] Numerous techniques of VR have been described, ranging from partial excision of the lateral wall to major segmental resections.[10-12] The resultant defects can be repaired with either a primary anastomosis or a graft. A variety of different native vessels and synthetic grafts have been described to bridge the defect. Each method, however, has limitations and the optimal conduit and surgical methods remain a controversy.[13-15] As published series are small the aim of this study was to evaluate our experience in pancreatectomies for ductal adenocarcinoma with en bloc vascular resection and reconstruction of vessels.

Methods

Approval of the Research Council of Surgical Clinics was obtained to perform the audit of patients with pancreatic adenocarcinoma undergoing surgery between January 2006 and August 2014. Clinical data, operative results, pathological findings and postoperative outcomes were collected prospectively and analyzed.

Preoperative evaluation

All patients underwent contrast-enhanced CT as a routine preoperative work-up. Magnetic resonance imaging, endoscopic ultrasound scan, and laparoscopy were performed on an individual basis based on the multidisciplinary team discussion. The final operative decision lay with the surgeon at laparatomy. Only patients deemed respectable preoperatively were included. The criteria for en bloc resection where there was no evidence of metastatic disease were the following: tumour not involving the root of the small bowel mesentery; tumour not involving the superior mesenteric artery, celiac axis, or hepatic artery; and intention of obtaining R0 resection margin status. Patients with portal vein occlusion were not included.

The general condition of the patients was determined by American Society of Anesthesiologists (ASA) score.[16] For study purposes regarding preoperative level of bilirubin, Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) subgroups were formed.

Perioperative data

The operative approach was a median laparotomy until the 2007 later a bilateral subcostal laparotomy was preferred. Ultrasound examination of the pancreas was always used for evaluation of vascular involvement and for possible liver metastases.

Pancreatic head resections were done in a conventional manner.[17] In the last two years the artery first approach (posterior approach) was used whenever the infiltration and resection of superior mesenteric vein were planned.[18] Jejunum was exclusively used for the anastomosis to the pancreas (duct to mucosa type, two layers) and for bile reconstruction (on layer) successively. A separate Roux en Y loop for pancreatojejuno anastomosis was done only in 2 patients. All gastro/pyloro-jejuno anastomoses were placed above the colon. In all patients with pylorus-preserving pancreaticoduodenectomy (PPPD)/Whipple resection a small en-

teroentero anastomosis was added to connect the afferent end efferent loop of gastro/pyloro-jejuno anastomosis. In total and left pancreatectomy by rule the spleen and the splenic vessels were resected "en block", however in three 3 total pancratectomies with additional intraductal papillary mucinous neoplasia of the left pancreas the spleen was preserved. The pancreatic stump was almost exclusively closed with sutures. At the end of operation abdominal drains were always placed. In 3 cases spleen could be preserved, however in others ligation of splenic artery at the origin and splenic vein at the confluence was done. For prevention of pancreatic fistula in cases with the soft texture of the pancreas somatostatin 0.6 mg daily for 5 to 8 days was administrated.[19]

Vascular resections were carried out as primary closure of the vein, end to end anastomosis, or a segmental resection and reconstruction with interposition graft. Dacron grafts with 10 mm diameter were used.

Postoperative follow up

After surgery, the patients were followed up to detect complications, local recurrence, distant metastasis and survival rate. The surgical complications were noted and classified.[20] Laboratory tests and control of the tumour markers CEA and CA 19-9 as well as ultrasound and/or CT scans were obtained at three to four month intervals within 2 years after the operation and then later at six month intervals. The samples of fluid on drains were regularly examined for amylase on the day 4 and anytime in the course if the volume on drains was more than 50ml to rule out the possible pancreatic fistula (PF).[21]

Adjuvant chemotherapy was given according to final patohistological stage (pTNM) and was gemcitabine based on the majority of cases.

Hospital stay was defined as time from operation to final dismissal from the hospital.

30 and 60 day mortality was defined as any postoperative death within 30 or 60 days after the operation.

All resected specimens were sent to standardized pathohistological work up to the Department of pathology in Maribor.

Statistical analysis

Perioperative and clinicopathological parameters were evaluated and further compared between the two groups of patients. Categorical data were compared using x^2 test or Fischer`s exact test.

TABLE 1. Selected clinical characteristics and preoperative data in patients undergoing pancreaticoduodenectomy with or without vein resection (VR). There is no statistically important differences between both groups

Variable	Without VR (n = 111)	With VR (n = 22)	P-value test
Demographics			
Age (average years)	65.6 ± 7.7	63.95 ± 9.5	P = 0.45; t test
Sex (male:female)	53:58	9:13	P = 0.64; Chi square
ASA score			
1	34	7	
2	61	12	
3	16	2	
4	0	0	P = 0.83; Chi square
Bilirubin level			
Below 100mmol/l	77	18	
Above 100mmol/l	34	4	P = 0.306; Chi square
CEA (ng/l)			
Increased (> 5ng/l)	30	5	P = 0.795; Chi square
CA 19-9 (IU/l)			
Increased (> 30IU/l)	78	16	P = 1.0; Chi square

ASA = American Society of Anesthesiologists; CA 19-9 = carbohydrate antigen 19-9 ; CEA = carcinoembryonic antigen

Comparison of two different means was done by t-test. Survival curves were computed according to the Kaplan-Meier method. SPSS version 20 software (SPSS, IBM Corp, Armonk, NY, USA) was used to collect data and perform statistical analyses.

Results

Review of database at University Clinical Centre Maribor identified 133 patients (average age 65.4 ± 8.6 years, 69 female patients) who underwent pancreaticoduodenectomy for ductal adenocarcinoma of the pancreatic head between January 2006 and August 2014.

In the first group there were one 111 patients (83.5%), (53 male, 58 female patients, average 65.6 ± 7.7 years) with a standard pancreaticoduodenectomy without portal vein resection (PD - VR). In the second group there were 22 patients out of 133 (16.5%) (10 male, 12 female patients, average 63.95 ± 9.5 years) who had portal vein - superior mesenteric vein resection and portal vein reconstruction (PD + VR) during pancreaticoduodenectomy. There was no statistically important difference in preoperative patient characteristics between the PD - VR and PD + VR groups (Table 1). The dif-

TABLE 2. TNM stage in 111 patients who underwent pancreaticoduodenectomy without vein resection in comparison with vein resection (VR) group. There is no statistically important difference in N0 and N1 stage between both groups (P = 0.432, Fischer`s exact test, no statistical significance).

TNM stage	Without VR (n = 111)	With VR (n = 22)
T0	0	0
T1	10	0
T2	26	0
T3	75	22
T4	0	0
N0	32	4
N1	79	18

TABLE 3. List of surgical complications developed after pancreatoduodenectomy. Comparison between group without vein resection (PD [pacreaticoduodenectomy] – VR [vein resection]) and group with vein resection (PD + VR)

Type of surgical complication	Without VR (n = 111)	With VR (n = 22)
Pancreatic fistula	5 (4.5%)	1 (4.5%)
Bile leak	3 (2.7%)	1 (4.5%)
Intraperitoneal bleeding	6 (5.4%)	0
Abdominal abscess	5 (4.5%)	0
Gastric emptying syndrome	1 (0.9%)	1 (4.5%)
Rupture of the laparatomy	4 (3.6%)	0
Necrosing pancreatitis	1 (0.9%)	0
Ileus of Roux-Y	1 (0.9%)	0
Critical ischemia of the colon	1 (0.9%)	0
Poral vein thrombosis	0	1 (4.5%)
60 day mortality	5 (4.5%)	1 (4.5%)

ference between groups in TNM staging is shown in Table 2. All patients in PD + VR group are in stage T3, however, there was no difference in N and M classification (P = 0.432, Fischer`s exact test, no statistical significance) (Table 2). Surgical complications are listed in Table 3. The occurrence of surgical complications in the second group was to low for valid statistical comparison. There was no statistically important difference in histology (extension) of resection margins between groups (Table 4), (Chi-square 2.79, p = 0.247). There was one early death in vein reconstruction group (4.5%) and 5 deaths in another group (4.5%). There was no statistically significant difference between both

TABLE 4. Resection margins of extirpated tumors. There is no statistically important difference bewteen both groups (with or without venous resection [VR]) (Chi-square 2.79, p = 0.247)

Resection margin	Without VR (n = 111)	With VR (n = 22)
R0	102	18
R0,1	5	3
R1	4	1

TABLE 5. Cause of intrahospital deaths between both groups. (P = 1.0, Fischer`s exact test, no statistical significance in death rate)

Cause of death	Without VR	With VR
Massive pulmonary embolia	1	0
Cerebrovascular insult	1	0
Myocardial infarction	2	1
Bronchopneumonia	1	0

VR = vein resection

groups (P = 1.0, Fischer`s exact test, no statistical significance in death rate). Causes of intrahospital deaths are listed in Table 5.

Adjuvant chemotherapy didn't impact the long term survival. In fourteen patients portal vein was reconstructed without the use of synthetic vascular graft. In these series two types of venous reconstruction were performed. When tumour involvement was limited to the superior mesenteric vein (SPV) or portal vein (PV) such that the splenic vein could be preserved, and vessels could be approximated without tension a primary end-to-end anastomosis was performed. When tumour involved the SMV - splenic vein confluence, splenic vein ligation was necessary (Figure 1). In the remaining eight procedures interposition graft was needed. Dacron grafts with 10 mm diameter were used. There was no infection after dacron grafting. One patient had portal vein thrombosis after surgery: it was thrombosis after primary reconstruction. There were no thromboses in patients with synthetic graft interposition.

Survival analysis

Median survival time in months was in a group with vein resection (PD + VR) 16.1 months and in a group without vein resection (PD - VR) 15.2 months. Five year survival in the group without

vein resection was 19.5%. Comparison of survival curves showed equal hazard rates with log-rank p = 0.090 (z = 1.659 at 5% C; C = 1.96) (Figure 2 and Figure 3).

Discussion

Pancreatic cancer is the 4th most common cause of cancer death in the Western world.[22] The mortality rate closely approximates the incidence, but surgical resection is generally accepted as having a beneficial effect on survival.[1,2] However, due to the presence of metastatic disease or invasion of local structures, most patients are not operative candidates at presentation. Historically, involvement of regional vasculature by pancreatic carcinoma has been considered a contraindication to resection.[1] Advances in surgical technique, intensive care and neoadjuvant chemotherapy have increased the rate of resectability, particularly for patients whose pancreatic cancer involves the portal vein (PV) and superior mesenteric vein (SMV). Vascular resection has become routine for locally advanced pancreatic tumours. Venous resections are supported only when an R0 resection is achieved. Many recent studies have shown that venous resection does not alter overall mortality and is therefore not a contraindication to extended tumour resection.[1,15] However the resection and reconstruction of the PV is a technically challenging procedure and the number of patients undergoing this type of operation in any given series is small.[14,15] Currently, venous resection has been reported in up to 20% of pancreaticoduodenectomies at high-volume pancreatic surgery centers.[12,22] It has been suggested that pancreatic head resection (PHR) with venous resection (VR) might be associated with a higher complication rate when compared with pancreatic head resection alone.[14] In our study as in some other studies the morbidity of PHR combined with VR was similar to PHR alone.[22] In the meta-analysis by Zhou et al., of 19 studies that reported on mortality, no difference was observed between PHR with VR and PHR alone.[23]

Arterial resection is more rarely performed; they can include the celiac trunk, superior mesenteric and hepatic arteries, but usually arterial involvement is regarded as a contraindication to surgery as it carries a higher postoperative mortality, lack of survival benefit and are more likely associated to R1 resections.[12]

Some studies show a greater proportion of R1 resections in pancreatectomies with vein resection

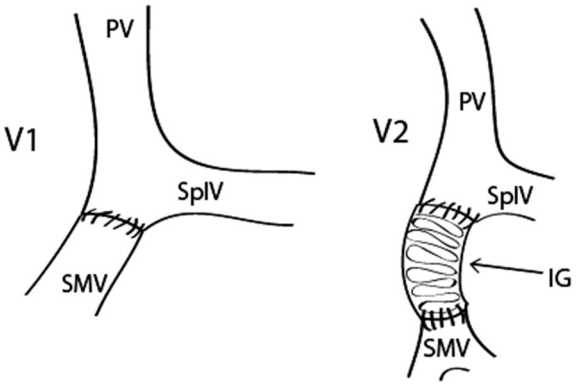

FIGURE 1. In presented series basically two types of venous reconstruction were performed. When tumour involvement was limited to the superior mesenteric vein (SMV) or portal vein (PV) such that the splenic vein (SplV) could be preserved, and vessels could be approximated without tension a primary end-to-end anastomosis was performed (V1). In the remaining cases interposition graft (IG) was needed (V2).

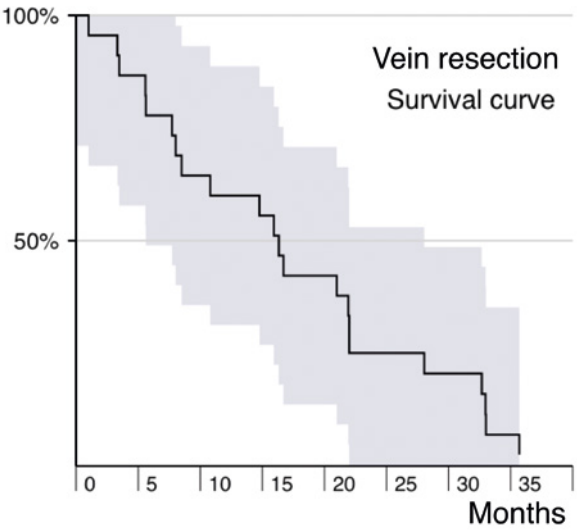

FIGURE 2. Kaplan-Meier survival plot for patients with vein reconstruction (pacreaticoduodenectomy [PD]+ vein resection [VR]). Median survival time in months was in this group 16.1 months.

than in pancreatectomies alone.[22] However, the greater proportion of R1 resections in PHR with VR group might be connected with differences in histopahtological reporting. The nature of tissue sampling of the circumferential resection margins differs between institutions. As a result, R1 rates vary considerably in the literature ranging from 37% to 75%.[22,24] Additionally, some studies have shown that R1 resections have had no adverse effect on survival.[12,22] In contrast, the ESPAC-1 trial suggested that resection margin status was a nega-

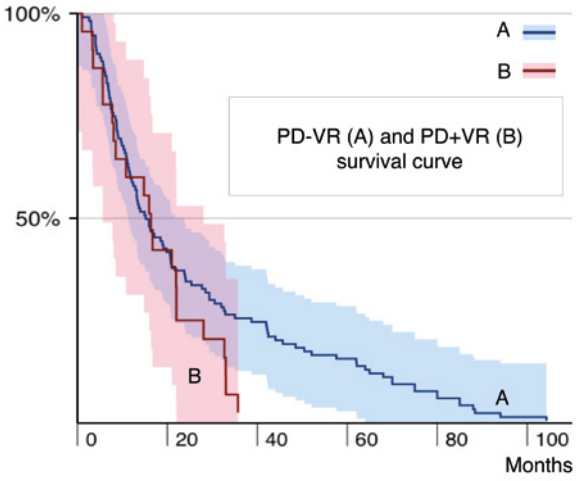

FIGURE 3. Comparison of Kaplan-Meier survival plots for both groups. Median survival time in months was in group with vein resection (pacreaticoduodenectomy [PD]+ vein resection [VR]) 16.1 months **(line B)** and in group without vein resection (PD - VR) 15.2 months **(line A)**. Five year survival in group without vein resection (line B) was 19.5%. Comparison of survival curves showed equal hazard rates with log-rank p = 0.090 (z = 1.659 at 5% C; C = 1.96).

tive predictor of survival.[25] In our study R1 status had no adverse effect on survival. However, with such discrepancies in the literature with regard to the resection margin status it could be postulated that until histopathologic reporting is more standardized universally its role as a prognostic indicator remains equivocal.[22] The opponents of the PHR with VR also argue that these tumours are larger with worse prognosis because of vessel-wall invasion and higher potential of developing liver metastases.[26] Several studies have shown that true histologic venous invasion has no impact on survival rates. Yekebas *et al.* found no statistically significant impact of tumour size, resection margin status and histologic vascular wall invasion on life expectancy.[27] Tseng and colleagues found no difference in median survival between patients with who did and who did not have histopahtologic evidence of vein invasion.[12] In our study life expectancy of PHR combined with VR was similar to PHR alone.

Few studies have analyzed the durability of the venous reconstruction or reported on the morbidity associated with graft thrombosis.[11,12] In these series two types of venous reconstruction were performed. When tumour involvement was limited to the superior mesenteric vein (SPV) or portal vein (PV) such that the splenic vein could be preserved, and vessels could be approximated without tension a primary end-to-end anastomosis was performed. When tumour involved the SMV-splenic vein confluence, splenic vein ligation was necessary. In the remaining eight procedures interposition graft was needed. Dacron grafts with 10 mm diameter were used. There was no infection after dacron grafting. Of the 6 thromboses observed all were in the acute setting (less than 30 days), however, none of these six patients died secondary to acute thrombosis.

The literature documenting portal vein graft thrombosis rates is sparse.[1,12,28,29] DiPerna *et al.* observed patency rates of 93% and 90% at 12 and 24 months, respectively. However, in this series, there were only eight portal vein resections with reconstruction.[29] Tseng *et al.* noted occlusion in 6.9% of portal vein grafts, but specific timing and morbidity were not discussed.[12] The thrombosis rate in this series was lower than in those previously reported (4.5%).

Recommendations for anticoagulation following major venous reconstruction for malignancy have varied.[1,30] No difference was observed in thrombosis rates when comparing patients receiving therapy and those who did not.[1] Currently, our approach to patients with SMV-PV involvement is similar to other published series.[1] Primary end-to-end anastomosis is performed in those patients requiring segmental resection if it can be accomplished without tension. In those patients who cannot be reconstructed with primary end-to-end anastomosis, an interposition graft is used, with the synthetic dacron graft being our first preference due to its acceptable results in portal decompression surgery.[31]

Generally, the use of a synthetic graft such as dacron or polytetrafluoroethylene (PTFE) is discouraged because of fear from infection or anastomosis disruption from pancreatic juices, and just a few small reports exist.[9,15] When portomesenteric vein resection is necessary during PD, primary anastomosis of the portomesenteric veins is always the first choice for reconstruction. However portal vein thrombosis was observed frequently after primary vein anastomosis, for several reasons. The most important is probably the anastomotic tension that may go unrecognized when intestines are returned to their original position after pancreaticoduodenectomy.[15] Some centres use vein interposition graft harvested from the jugular or renal location. However, additional resection of vein is connected with potentially higher morbidity. Additionally the need for vein resection is often not known until the last stage of resection. Because PV clamping time should be kept to a minimum, the suitability and ready availability of synthetic grafts make

them a desirable conduit for PVR. Synthetic graft provides the necessary length to bridge any gap between the mesenteric vessels and the PV, thus avoiding tension.[15] The potential risk of infection has restricted the use of synthetic grafts in PVR. Another disadvantage in this scenario is the potential risk of anastomosis disruption following a pancreatic leak. There were no graft infections or anastomotic leaks in this series. It is interesting to note that ligation of the splenic vein, not only in presenting series wasn't presented with long term complications.[12]

Similar survival times after surgical resection in both groups raises once again the question about which factors independently influence the long term outcome in patients with pancreatic cancer.[32] Survival after surgical resection is related to several factors: most important seem to be the extent of local invasion of the primary tumour, lymph node involvement, vascular invasion, perineural invasion, cellular differentiation, and uninvolved surgical margins. El Ghazzawy *et al.* reviewed experience in the US Department of Veterans affairs hospitals from 1987–1991. In the group that underwent surgical resection, perineural invasion, microlymphatic invasion, vascular invasion, or tumour differentiation did not independently influence survival when tumours were controlled for stage.[33] Exactly which factors are truly independent remains controversial.[22,32]

Conclusions

Survival of patients with pancreatic cancer who undergo a resection with reconstruction was comparable to those who have a standard pancreaticoduodenectomy with no added mortality or morbidity. Synthetic graft appeared to be an effective and safe option as an interposition graft for portomesenteric venous reconstruction after pancreaticoduodenectomy.

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research article

Interdisciplinary consensus statement on indication and application of a hydrogel spacer for prostate radiotherapy based on experience in more than 250 patients

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Background. The aim of the study was to reach a consensus on indication and application of a hydrogel spacer based on multicentre experience and give new users important information to shorten the learning curve for this innovative technique.

Methods. The interdisciplinary meeting was attended by radiation oncologists and urologists, each with experience of 23 – 138 hydrogel injections (SpaceOAR®) in prostate cancer patients before dose-escalated radiotherapy. User experience was discussed and questions were defined to comprise practical information relevant for successful hydrogel injection and treatment. Answers to the defined key questions were generated. Hydrogel-associated side effects were collected to estimate the percentage, treatment and prognosis of potential risks.

Results. The main indication for hydrogel application was dose-escalated radiotherapy for histologically confirmed low or intermediate risk prostate cancer. It was not recommended in locally advanced prostate cancer. The injection or implantation was performed under transrectal ultrasound guidance *via* the transperineal approach after prior hydrodissection. The rate of injection-related G2-toxicity was 2% (n = 5) in a total of 258 hydrogel applications. The most frequent complication (n = 4) was rectal wall penetration, diagnosed at different intervals after hydrogel injection and treated conservatively.

Conclusions. A consensus was reached on the application of a hydrogel spacer. Current experience demonstrated feasibility, which could promote initiation of this method in more centres to reduce radiation-related gastrointestinal toxicity of dose-escalated IGRT. However, a very low rate of a potential serious adverse event could not be excluded. Therefore, the application should carefully be discussed with the patient and be balanced against potential benefits.

Key words: prostate cancer; hydrogel spacer; dose-escalated radiotherapy; proctitis; toxicity

Background

Dose escalated intensity-modulated radiation treatment (IMRT with radiation doses ≥ 76 Gy) is a highly effective, curative treatment option for localized prostate cancer. Biochemical control is directly related to radiation dose with a dose effect per each additional Gy.[1] For example, escalation from 70 to 80 Gy is connected with a 15% increase in PSA control. This dose effect is described for all risk groups. However, an increased radiation dose is also associated with rising levels of grade ≥ 2 acute and chronic toxicity.[1] Lower gastrointestinal toxicity rates can result from smaller posterior safety margins or even no safety margins[2], potentially compromising local tumour control.

A novel method to reduce rectal toxicity during dose-escalated IMRT is the insertion of a hydrogel spacer between the Denonvilliers' fascia and anterior rectal wall to separate these structures.[3] The created space generates a distance of 10 – 15 mm between both organs.[4-6] Recent studies unequivocally demonstrated a significant reduction in high-dose areas on the anterior rectal wall.[4,5,7,8] As expected, better rectal sparing from higher radiation doses was associated with only mild toxicity from the dose-escalated treatment.[4,9,10]

The application technique[3], dosimetric studies[4,8] and some early toxicity data[4,10], as mentioned above, were all published within the last two years. However, despite rising numbers of hydrogel injections, reports on practical aspects or pitfalls of hydrogel application as well as frequency and management of side effects of the administration were not or were only provided for single cases.[11] Therefore, the first consensus meeting was held in July 2013 to discuss this practical issue and to generate answers for users on the indication, application and management of side effects of a hydrogel spacer for dose-escalated radiotherapy. Thereafter, toxicity data of the injection technique was collected from > 250 patients of four centres to better balance the benefit and potential risks of this new method.

The aim of this consensus report is to offer new users of this technique easy access to relevant information on practical application and patient management to shorten the learning curve[7] and to carefully balance potential benefits against potential risks of this technique.

Patients and methods

The interdisciplinary meeting was attended by radiation oncologists and urologists, each with experience of 23 – 138 hydrogel injections (SpaceOAR®) in prostate cancer patients before dose-escalated IMRT. In the first part of the meeting, user experiences were discussed and questions were defined to comprise practical information relevant for successful gel injection and treatment. In the second part, answers to the defined key questions were developed. Prospective data from the multi-institutional clinical trial[10], prospective mono-institutional data (German Clinical Trials Register DRKS00003273)[4] and data collected retrospectively from patient files were considered in this interdisciplinary process to evaluate hydrogel application in current practice. The prospective studies were approved by each institution's ethics committee. With regard to the participating centres approvals were given by the University of Aachen[10], the University of Heidelberg[10] and the University of Tübingen.[4] All of these patients (n = 62) gave their written informed consent to participate in these studies.[4,10] After discussing the intended analysis of retrospectively collected data (n = 196) the institutional review board (Ethics Committee of the University of Tübingen) had no objections (266/2015BO2). Patients gave informed consent to standardized data documentation and evaluation of treatment related toxicity.

After the meeting, participants were asked to state the incidence of side effects to better balance risks and beneficial effects. Finally, the statement was revised and consented. Recommendations derived from prospective studies were indicated as level of evidence (LOE) 2a (evidence obtained from at least one well-designed controlled study without randomisation). Consensus statements based on expert opinions were indicated as LOE 4.

The SpaceOAR® System (resulting in 10 mL hydrogel) is FDA cleared and CE Mark approved, and commercially available in the US and most countries of Western Europe.

Results

The following key questions were developed with regard to practical aspects of hydrogel application and patient management:

1. Indication: what criteria are required to recommend the injection of a hydrogel spacer in an individual patient?
2. Injection technique: how should the injection be optimally applied?
3. Potential toxicity: which side effects could theoretically occur?

4. Prophylaxis: are prophylactic procedures reasonable?
5. Actual toxicity: what is the current grade 2 or higher toxicity rate of hydrogel injection measured according to CTC v 4.0.[12]
6. Treatment of side effects: how should side effects be treated?
7. Absolute exclusion criteria: what are absolute exclusion criteria for the injection?
8. Relative exclusion criteria: what are relative exclusion criteria for the injection?
9. Special aspects of radiation treatment planning: Which aspects of radiation treatment planning should be considered?

The following key answers were developed:

Indication

A hydrogel spacer can be considered for dose-escalated radiotherapy (radiation doses ≥ 76 Gy in conventional 1.8 – 2.0 Gy fractions) for histologically confirmed low or intermediate risk prostate cancer (LOE 2a).

A hydrogel spacer can be considered for dose-escalated radiotherapy (radiation doses ≥ 76 Gy in conventional 1.8 – 2.0 Gy fractions) for histologically confirmed prostate cancer with any localized disease (LOE 4). The risk of a microscopic T3 stage with risk of adhesions potentially impairing the hydrodissection should be considered.

Following hydrogel injection, other forms of dose-escalated radiotherapy as hypofractionated radiotherapy, particle beam radiotherapy or brachytherapy were also carried out.[13-17]

Injection technique

Hydrogel injection can be performed under local (possibly additional sedation), spinal or general anaesthesia. Additional procedures that are planned at the same time (*i.e.* brachytherapy, marker implantation *etc.*) determine the selected anaesthesia and should be performed in advance or a few days later since hydrogel injection might worsen visibility by air contamination. For preparation of the patient see also 3. Prophylaxis. Generally, the patient is placed in the lithotomy position. The injection is performed transperineally under transrectal ultrasound (TRUS) guidance using a linear side-fire TRUS probe and a stand-off balloon to optimize visibility. A stepper unit stabilizes the probe, so that both hands are free for the procedure.[3,18] The transperineal route is well known for procedures such as prostate biopsies, fiducial placement or prostate brachytherapy.[19]

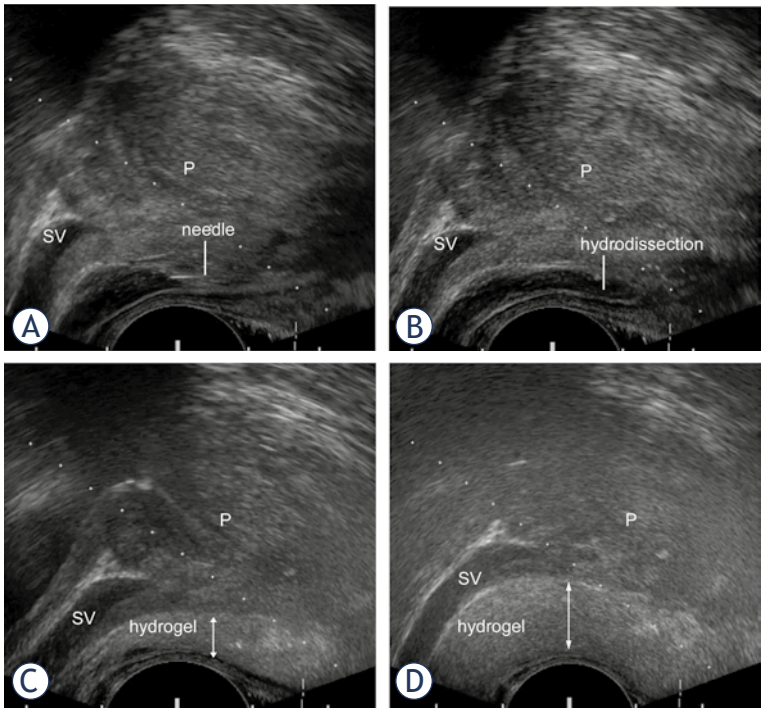

FIGURE 1. Hydrogel injection. Sagittal transrectal ultrasound images showing **(A)** the needle placed at the Denonvillier's fascia at the start of hydrodissection, after complete hydrodissection **(B)**, at the start **(C)** and after successful hydrogel injection **(D)**. Air contamination after hydrogel injection worsens visibility **(D)**.

P = prostate; SV = seminal vesicles

All centres involved in this consensus used the hydrodissection technique before spacer injection to separate Denonvilliers' fascia and the anterior rectal wall. This fluid-mediated tissue separation technique is also used in other settings like cataract surgery and carpal tunnel syndrome treatment.[20,21] In short, an 18 gauge needle is inserted 1 – 2 cm above the patient's anus through his perineum. The needle is advanced either parallel to the probe or slightly angled towards the prostate apex. The correct needle position is below the prostatic apex in midaxial and midsagital position of prostate (so called midgland position). Lowering the probe before hydrodissection might facilitate the procedure. Hydrodissection is performed with 10 – 20 ml of saline or lidocaine (as local anaesthesia) diluted in saline. A slow injection of the fluid is necessary to ensure later a symmetric distribution of the spacer. Only in case of a successful hydrodissection, the hydrogel can be applied.

Hydrogel is formed during the simultaneous injection *i.e.* mixing of the precursor (polyethylene glycol powder) and accelerator solutions (diluent). The solutions are mixed as they pass through a Y-connector prior to passing through the injection needle. Both solutions polymerise to a soft PEG-based gel within 10 seconds. An injection of 10 mL hydrogel results in a separation of about 9 – 10 mm between the prostate and rectal wall (Figure 1).[6] The injection procedure can be completed within a few minutes.

Potential toxicity

Depending on the type of and experience with anaesthesia, patients might experience pain and discomfort during needle insertion and hydrogel injection. After spacer injection, patients may feel discomfort and rectal tenesmus. Data on pain frequency and pain intensity after injection was not routinely collected. Therefore, only retrospective data on pain management indicating the use of ampyrone sulfonate analgesics (metamizole) for the day of the procedure and sometimes afterwards was available. During spacer injection, there might be a risk of the needle and hydrogel penetrating the rectal wall, urethra, bladder or prostate. Bleeding, necrosis or ulceration of the bladder or rectal wall may follow. Lower urinary tract symptoms or even urinary retention could result from pressure on the prostate or the bladder from the spacing gel. Local inflammation or infection is possible, as with every invasive procedure. Air or hydrogel might be potentially injected into vessels.

Prophylaxis

Anticoagulants should be discontinued. Antibiotic prophylaxis is applied in some centres with fluoroquinolones or cephalosporines. However, no infections have been diagnosed up to now, even in centres with > 100 hydrogel injections without antibiotics. A rectal enema might be used to optimize TRUS conditions during the procedure. Constipation and hard stools need to be avoided during treatment to decrease pressure on the rectal wall and a low residue diet and/or laxatives may be indicated.

Actual toxicity

Experience from all centres were participating in this consensus statement included 258 cases of hydrogel application before external beam radiotherapy for localized prostate cancer. All patients were treated with photons.

Hydrogel associated complications, defined as grade 2 or higher toxicity, were experienced by 5 patients (2%). Hydrogel was injected intraprostatically in one single case. In 4 cases, rectum penetration was diagnosed at different intervals following injection. An injection into the rectal wall was observed in a single patient shortly after injection and radiotherapy was therefore started several weeks later. Two rectum penetrations were diagnosed during an external beam photon treatment after reports of passing mucous discharge. The patients were treated conservatively and radiotherapy was interrupted in one case. One patient reported increased bowel urgency 3 – 4 weeks after the end of radiotherapy before the diagnosis of a rectum penetration on proctoscopy.

All patients with the mentioned complications were followed-up with proctoscopies and/or pelvic MRI (magnetic resonance imaging). Rectal wall defects healed in all patients completely after several weeks.

Observation and treatment of side effects

Post-injection care comprises usually the first day with examination of potential urological side effects (bleeding, obstruction, pain) including the removal of a urinary catheter (if present). Side effects must be treated symptomatically. Urinary catheterization is needed in cases of urinary obstruction. Hydrogel (PEG) is not toxic or allergenic and all known injections into the prostate, bladder or rectal wall resolved without further sequelae.[6] Patience is required as the hydrogel remains stable for three months and subsequently liquefies within 6 months. This was documented in 98% of patients (n = 43/44) in the multi-center study.[10] Antibiotic treatment is indicated in cases of penetration, perforation or ulceration of the rectal wall and depending on the extent, patients could be kept on parenteral nutrition or a low residue diet.

Radiotherapy should not be started during an infection or after inadvertent injection into the bladder or rectal wall before the healing process of a defect is complete.

Absolute exclusion criteria

(complication risk exceeds potential benefits)

locally advanced prostate cancer (space cannot be effectively created, tumour cell dissemination cannot be excluded)

active bleeding disorder or clinically significant coagulopathy

Relative exclusion criteria

anticoagulants (discontinuation usually possible)

active inflammatory or infectious disease in the perineum or injection area (prostatitis, anorectal inflammatory disease with increased risk of ulceration, fistula or bleeding such as ulcerative colitis or Crohn's disease)

previous treatment of prostate with high risk of adhesions (high-intensity focused ultrasound, cryotherapy, radiotherapy).

Presently, very limited experience exists in hydrogel application after previous radiotherapy or high-intensity focused ultrasound.[16,22] Hydrogel injection was performed without problems; however adhesions can make an injection difficult or impossible.

9. Special aspects of radiation treatment planning

Radiation treatment planning CT should start approximately five days after hydrogel injection to allow for decreasing of post-procedural swelling (and not to overestimate prostate volume).[22] An post-injection MRI (T2-sequence sufficient without contrast media) fused to the planning CT could help to better identify the spacer (because the hydrogel is sometimes not distinguishable from the rectal wall due to same density in CT). An additional advantage of an MRI is the capability to evaluate the properness of injection. Circumferential CTV-PTV-margins depend on the verification strategy (with IGRT usually 7–10 mm, posterior if necessary less). Monitoring of the spacer volume is not necessary during radiation treatment. Stability over 3 months after injection was shown for the gel in the multicenter study.[10]

Discussion

The most relevant practical aspects of hydrogel injection after 258 applications were summarized in this consensus statement. A detailed description of indications, prophylaxis and management of side effects should provide new users with a fast and comprehensive introduction to the successful application of this new method. After a short learning period, the procedure can be performed to a high standard, ensuring low toxicity. Most data used are derived from well-defined controlled but not randomized studies or prospective investigations, leading to Level IIA evidence for indication and application of the hydrogel spacer.

In the multi-institutional phase II trial (52 patients recruited, 49 patients after successful spacer injection), patients were informed of higher probability of grade 2+ toxicity, as no experience existed. With a carefully estimated probability of 6 – 20%, it included an injection into the rectal wall, bladder wall and urethra, ulceration and necrosis of the rectal wall, bleeding and urinary retention. Three patients who were initially treated within this study experienced procedure-related events after hydrogel injection including focal rectal necrosis due to inadvertent injection of hydrogel into the rectal wall, bladder piercing during injection with hydrogel leak into the bladder, urinary retention and a device-related proctitis.[6] All of these events occurred during the initial experience (learning curve in the first patient cohort) and resolved completely. Adaptations of the injection procedure (side-fire TRUS probe, stepper, stand-off balloon) were conducted which facilitated handling of the needle and hydrogel insertion. A learning curve has been reported for the application and treatment with a hydrogel, again stressing several technical aspects to achieve homogenous hydrogel distribution. This report summarizes important issues that need to be considered to achieve satisfactory spacer distribution.

Radiotherapy planning should not include the usual objectives for the dose to the rectal wall. A dose of 70Gy can be allowed for 20% of the rectal wall volume according to RTOG (Radiation Therapy Oncology Group) recommendations.[23] With a prescription dose of 76–78Gy, mean rectum volumes within the 70Gy isodose can range by about 1% with good spacer placement and adequate treatment planning.[7]

However, the findings of this multi-institutional evaluation of spacer-related toxicity (no G3+ event) were based on conventional fractionated dose-escalated IGRT and cannot be simply adopted to other radiation treatment schedules (hypofractionation) or treatment with other ionizing radiation sources. In a study with hypofractionation using particle beam therapy (without CT-image guidance) two cases (2/92; 2%) of G3-toxicity (colostomy) occurred, a relation to the hydrogel spacer injection cannot be excluded.[24]

Therefore, it is extremely important that patients are closely followed up at their centre after hydrogel injection. As the hydrogel is not tissue-toxic or allergenic, conservative management in case of inaccurate injection should be initiated as described above. Patience is required in case of inadvertent injection to the rectum or bladder wall, or in case

of rectal wall penetration or ulceration. All cases in this analysis where this occurred healed without long-term sequelae. A currently published randomized trial demonstrated well toleration of spacer application (10% mild transient procedural perineal discomfort) in 149 patients suggesting safety of this method with conventional fractionated dose-escalated IGRT, too.[25]

For optimized injection results, one expert in each centre was trained by another expert. The procedure was performed by only one or two experts at each centre, guaranteeing a high degree of experience. Last but not least, correct patient selection is essential. The optimal patient for this new method is at low risk of adhesions (inflammation, tumour spread due to locally advanced disease) and has a low risk of bleeding. The risk of tumour displacement by hydrodissection is very small, since prostatectomy series with limited pT3 stages reported in less than one fifth of patients an invasion and in no case a progression through the full thickness of the Denonvilliers' fascia.[26]

After successful injection, the benefit for the patients was measured by acute toxicity scores and by radiation planning parameters (dose-volume histograms). In brief, the theoretical benefit of an additional space between prostate and rectum translated into improved radiation treatment plans with approximately 10% reduction in relevant high-dose areas (dose level from 40–70Gy).[8] These improved radiation treatment plans with lower rectal doses converted into reduced acute toxicity rates. Grade 2 proctitis resulting from radiotherapy was a rare event compared to standard conformal or intensity-modulated radiotherapy, for example 12.5% acute toxicity in the multicenter phase II trial[10] in comparison to occasionally 50% or more in studies without a spacer.[27,28] The prevention of acute proctitis with this procedure is a benefit for the patient. Further benefits for the patients are conceivable. Consequential late side effects derive from persisting acute toxicity[29] and reduced acute toxicity will usually be associated with a lower risk of late toxicity. However, the evaluation of this potential long-term benefit needs longer follow-up. Another beneficial effect of improved rectum protection is the facilitation of dose escalation to the prostate. Since increased radiation doses improve outcome in the range of approximately 1.5% better biochemical control per Gy after a mean follow-up of five years[1], these dosimetric changes are relevant for improved tumour control with a lower risk of toxicity.

This spacer consensus focuses on the use of Polyethylene-glycol (PEG) hydrogel spacers in dose-escalated radiotherapy of prostate cancer. However, at least four different bio-resorbable spacer materials (PEG-hydrogel, balloon of copolymer of polylactic acid or similar poly (α-hydroxy acids), hyaluronic acid and collagen) are currently evaluated. PEG hydrogel spacers and bio-resorbable balloons have demonstrated an excellent biocompatibility profile in humans compared to other spacers made of hyaluronic acid or collagen.[30] Direct comparison of PEG hydrogel spacer and bio-resorbable balloon demonstrated the following. PEG spacers were less invasive (smaller needle diameter with 1.3 *vs*. 2 – 3mm). The balloon spacer was superior in reducing rectum dose (-28%), but exhibited an average volume loss of > 50% during the full course of treatment (37–40 fractions), while the volume of gel spacers remained fairly constant.[31]

Displacement of radiosensitive organs by spacers is not limited to primary prostate cancer alone. Further applications being investigated include treatment of recurrent prostate cancers[32], gynecological malignancies[33] and esophageal gel-shifting facilitating treatment of mediastinal nodes.[34] The principle to displace radiosensitive organs from high dose areas is also used in case of adhesions of small intestinum and radiation targets. For such special situations are invasive surgical techniques available like laparoscopic mesh placement.[35]

We conclude that hydrogel injection can be considered for dose-escalated radiotherapy. Well trained physicians, correct patient selection and knowledge of the management of potential side effects are essential for optimal application. The benefit for the patient is improved protection of the rectal wall, which is associated with low radiation related proctitis rates. This allows dose-escalation associated with improved tumour control. However, a very low rate of a potential serious adverse event cannot be excluded and should carefully be discussed with the patient and be balanced against potential benefits. The evaluation of this potential long-term benefit needs longer follow-up.

Disclosures

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research article

Excellent outcomes after radiotherapy alone for malignant spinal cord compression from myeloma

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Background. Uncertainty exists whether patients with spinal cord compression (SCC) from a highly radiosensitive tumor require decompressive spinal surgery in addition to radiotherapy (RT). This study addressed the question by evaluating patients receiving RT alone for SCC from myeloma.

Patients and methods. Data of 238 patients were retrospectively analyzed for response to RT and local control of SCC. In addition, the effect of RT on motor function (improvement, no further progression, deterioration) was evaluated. Overall response was defined as improvement or no further progression of motor dysfunction. Prior to RT, patients were presented to a neurosurgeon for evaluation whether upfront decompressive surgery was indicated (*e.g.* vertebral fracture or unstable spine).

Results. In the entire cohort, the overall response rate was 97% (53% improvement plus 44% no further progression). Following RT, 88% of the patients were able to walk. Of the 69 non-ambulatory patients 44 patients (64%) regained the ability to walk. Local control rates at 1, 2 and 3 years were 93%, 82% and 82%, respectively. A trend towards better local control was observed for patients who were ambulatory before starting RT ($p = 0.08$) and those with a more favorable performance status ($p = 0.07$).

Conclusions. RT alone provided excellent response rates, functional outcomes and local control in patients with SCC from myeloma. These results should be confirmed in a prospective randomized trial.

Key words: myeloma; spinal cord compression; radiotherapy alone; overall response; local control

Introduction

Myeloma patients account for about ten percent of patients presenting with malignant spinal cord compression (SCC).[1] Radiotherapy (RT) alone is the most frequently used treatment for these patients worldwide. Ten years ago, a small randomized trial of 101 patients was published that compared RT alone to decompressive surgery plus stabilization followed by RT in highly selected patients.[2] In that trial the combined approach resulted in significantly better functional outcome (ambulation) and survival than irradiation alone. Therefore, upfront neurosurgery has become significantly more popular in several countries. Although patients with highly radiosensitive tumors such as myeloma,

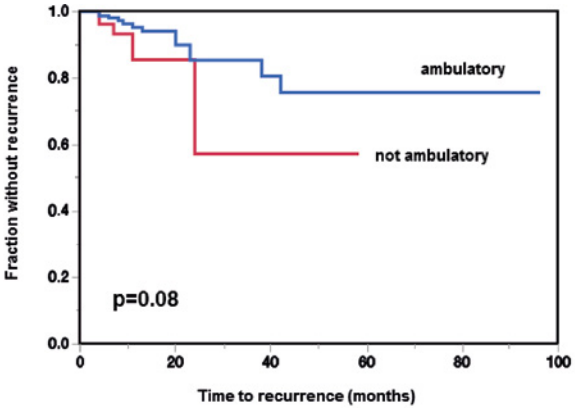

FIGURE 1. Kaplan-Meier curves of patients who were ambulatory prior to RT and of those patients who were not ambulatory.

lymphoma and germ cell tumors were excluded from the randomized trial of 101 patients, many neurosurgeons extrapolated from these findings and perform decompressive surgery in myeloma patients.[2] The question remains whether RT alone is sufficient or needs to be supplemented by upfront decompressive surgery in malignant SCC from a highly radiosensitive tumor. This study aims to contribute to this open question by investigating overall response and local control of SCC in patients treated with RT alone for SCC from myeloma.

Patients and methods

Data of 238 patients presenting with motor deficits of the lower extremities in consequence of SCC from vertebral body myeloma were retrospectively analyzed. Prior to the start of RT, the patients were presented to a neurosurgeon for evaluation whether upfront decompressive surgery was indicated, *e.g.* in case of vertebral body fracture, unstable spine or sphincter dysfunction. Patient who did not require surgery were included in this study.

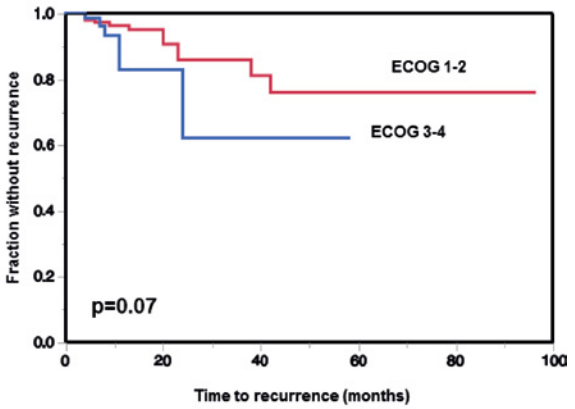

FIGURE 2. Kaplan-Meier curves of patients with an ECOG performance score of 1–2 and those patients with an ECOG performance score of 3–4.

RT was performed with 6–18 MV photon beams from a linear accelerator (mostly after 3D-treatment planning), and target volumes included the vertebrae affected by SCC plus on additional vertebra on either side. The study has been approved by the local ethics committee. For this retrospective study, specific written informed consent was not required.

The primary endpoint local control was defined as freedom from a symptomatic in-field recurrence of SCC in the irradiated parts of the vertebral column. In addition, the effect of radiation treatment on motor function (improvement, no further progression, deterioration) was measured. Improvement and deterioration of motor function were defined as a change of one point on a five-point scale (0 = normal strength; 1 = ambulation without aid; 2 = ambulation with aid; 3 = no ambulation; 4 = complete paraplegia).[3] Patients with complete paraplegia who did not improve after RT were rated as deteriorated. Motor function at about one month (three to six weeks) following RT was compared to motor function at baseline (*i.e.* before the start of RT Overall response to RT was defined as either improvement or no further progression of motor dysfunction.

RT was administered without upfront neurosurgery and performed either as short-course RT (1 x 8Gy, 5 x 4Gy) or longer-course RT (10 x 3Gy, 15 x 2.5Gy, 20 x 2Gy). The RT regimen plus ten other factors were analyzed for local control of SCC. The other factors included age at the time of RT (≤ 64 years *vs.* ≥ 65 years, median age: 64 years), gender, myeloma subtype (IgG subtype *vs.* other subtypes), time from first diagnosis of myeloma to SCC (≤ 15 *vs.* > 15 months), presence of extra-osseous lesions before RT (no *vs.* yes), further osseous lesions before RT (no *vs.* yes), gender, time developing motor deficits before RT (faster: ≤ 14 *vs.* slower: > 14 days), gait function before the start of RT (ambulatory *vs.* not ambulatory), number of vertebrae involved by SCC (1–2 *vs.* ≥ 3) and performance status (Eastern Cooperative Oncology Group (ECOG) performance score 1–2 *vs.* 3–4). The univariate analyses of local control of SCC were done with the Kaplan-Meier method[4], and the Kaplan-Meier curves were compared with the log-rank test. Those factors being significant or showing a trend ($p < 0.09$) for local control were additionally analyzed in a multivariate manner with the Cox proportional hazards model.

TABLE 1. Impact of the eleven factors on local control of SCC (univariate analysis)

	At 1 year (%)	At 2 years (%)	At 3 years (%)	p-value
Age				
≤ 64 years (n = 125)	94	86	86	
≥ 65 years (n = 113)	92	75	75	0.81
Gender				
Female (n = 88)	90	78	78	
Male (n = 150)	94	85	85	0.47
Myeloma subtype				
IgG subtype (n = 153)	96	84	84	
Other subtypes (n = 85)	88	78	78	0.14
Time from myeloma diagnosis to SCC				
≤ 15 months (n = 128)	95	81	81	
> 15 months (n = 110)	91	85	85	0.83
Extra-osseous lesions				
No (n = 218)	94	82	82	
Yes (n = 20)	67	n.a.	n.a.	0.19
Further osseous lesions				
No (n = 91)	93	86	86	
Yes (n = 147)	93	76	76	0.73
Time developing motor deficits				
Faster (≤ 14 days) (n = 112)	93	81	81	
Slower (> 14 days = (n = 126)	93	83	83	0.79
Gait function before the start of RT				
Ambulatory (n = 169)	95	85	85	
Not ambulatory (n = 69)	85	57	57	0.08
Number of vertebrae involved by SCC				
1-2 (n = 112)	95	87	87	
≥ 3 (n = 126)	92	77	77	0.17
ECOG performance score				
1-2 (n = 150)	96	86	86	
3-4 (n = 88)	83	62	62	0.07
Radiotherapy regimen				
Short-course RT (n = 84)	94	69	69	
Longer-course RT (n = 154)	93	90	90	0.29
Entire cohort (n = 238)	93	82	82	

n.a. = not available

Results

Two-hundred-and-thirty-seven patients were available for evaluation of response to RT. The overall response rate at one month was 97% (230 of 237 patients); 53% of patients (n = 126) showed improvement and 44% (n = 104) no further progression. Following RT, 88% of the patients (209 of 237) were able to walk. Of 69 non-ambulatory patients 44 patients (64%) regained the ability to walk after RT.

In the entire cohort of 238 patients, the local control rates at 1, 2 and 3 years following RT of SCC were 93%, 82% and 82%, respectively. In the univariate analysis, no factor was significantly associated with local control of SCC. A trend towards better local control was observed for patients who were ambulatory before RT was started (p = 0.08, Figure 1) and for patients with a more favorable performance status (p = 0.07, Figure 2). These two factors were additionally evaluated in the multivariate analysis, where both pre-RT gait function (risk ratio: 2.34; 95%-confidence interval: 0.80–6.10; p = 0.11) and performance status (risk ratio: 2.36; 95%-confidence interval: 0.85– 6.09; p = 0.09) did not reach significance (significance = defined as p < 0.05).

Discussion

Malignant SCC represents a serious complication for patients with a malignant disease.[1,5] A rapid start of treatment is required. Until 2005, radiotherapy alone has been considered the unquestioned standard treatment for SCC. In 2005, a randomized trial of 101 selected patients with SCC from different primaries, who had a good performance status and a relatively good survival prognosis, suggested that the results of RT alone can be improved by upfront decompressive surgery.[2] In that trial, 84% (42 of 50) of patients were able to walk after surgery plus RT compared to 57% (29 of 51) of patients

after RT alone (p = 0.001). The results were supported by a meta-analysis including 24 surgical series (n = 999) and four radiotherapy series (n = 543), mostly uncontrolled cohort studies.[6] These data have led to a fundamental change of practice. In several countries, neurosurgery proceeding RT has become very popular. In some centers, the majority patients with SCC receive the combined treatment rather than RT alone. This new trend includes also patients with highly radiosensitive tumors such as myeloma, although these patients were excluded from the previously mentioned randomized trial.[2] The question is whether these patients really need surgery in addition to RT? One should bear in mind that spinal surgery is associated with significant risks and complications such as severe wound infections. A second surgery, extensive bleeding, postoperative pneumonia, and major thromboembolic events occurred in more than 10% of patients.[2,7,8] In addition, iatrogenic neurologic complications were reported for 9% of patients receiving surgery of the lumbar spine.[9]

In the current study, functional outcomes were excellent with a post-RT overall ambulation rate of 88% and a rate of regaining ambulatory status of 64%. Furthermore, local control of SCC achieved with RT alone was long lasting. At 3 years following RT, local control was still 82%. These excellent local control rates were achieved irrespectively of patient characteristics. None of the eleven investigated characteristics was significantly associated with local control of SCC. However, pre-RT gait function and performance status showed a trend. Three-year local control rates were 57% in initially non-ambulatory patients and 62% in patients with an ECOG performance score of 3–4, respectively. The question whether these patients would benefit from the addition of upfront decompressive surgery to RT can be properly answered only in a prospective trial. Clear indications for neurosurgery also for very radiosensitive tumors include vertebral fractures, unstable spine, sphincter dysfunction, and impairment of the spinal cord by bony fragments. According to a recent retrospective study focusing on surgery for vertebral involvement of myeloma, the probability of receiving surgery was about 40%.[10] No differences in disability and quality of life were observed between patients receiving RT alone and those receiving RT plus upfront surgery. For highly selected patients, stereotactic body radiation surgery (SBRT) may also be an option.[11,12] However, it has been recommended to use SBRT for malignant SCC only within clinical trials.[12]

In summary, in patients with malignant SCC from myeloma, RT alone provides excellent response rates, functional outcomes such as post-RT ambulation, and local control of SCC. These results should be confirmed in a prospective randomized trial.

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research article

The role of neoadjuvant chemotherapy in patients with advanced (stage IIIC) epithelial ovarian cancer

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Background. Primary treatment of patients with advanced epithelial ovarian cancer consists of chemotherapy either before (neoadjuvant chemotherapy, NACT) or after primary surgery (adjuvant chemotherapy). The goal of primary treatment is no residual disease after surgery (R0 resection) what is associated with an improvement in survival of patients. There is, however, no evidence of survival benefits in patients with R0 resections after prior NACT.

Methods. We retrospectively reviewed the records of patients who were treated with diagnosis of epithelial ovarian cancer at Institute of Oncology Ljubljana in the years 2005–2007. The differences in the rates of R0 resections, progression free survival (PFS), overall survival (OS) and in five-year and eight-year survival rates between patients treated with NACT and patients who had primary surgery were compared.

Results. Overall 160 patients had stage IIIC epithelial ovarian cancer. Eighty patients had NACT and eighty patients had primary surgery. Patients in NACT group had higher rates of R0 resection (42% *vs.* 20%; p = 0.011) than patients after primary surgery. PFS was 14.1 months in NACT group and 17.7 months after primary surgery (p = 0.213). OS was 24.8 months in NACT group and 31.6 months after primary surgery (p = 0.012). In patients with R0 resections five-year and eight-year survival rates were 20.6% and 17.6% in NACT group compared to 62.5% and 62.5% after primary surgery (p < 0.0001), respectively.

Conclusions. Despite higher rates of R0 resections achieved by NACT, survival of patients treated with NACT was inferior to survival of patients who underwent primary surgery. NACT should only be offered to patients with advanced epithelial cancer who are not candidates for primary surgery.

Key words: advanced ovarian cancer; neoadjuvant chemotherapy; primary surgery

Introduction

The standard treatment of patients with advanced epithelial ovarian cancer is a combination of primary surgery followed by chemotherapy. In the recent years it became clear that the goal of surgery is to achieve no macroscopic residual disease, since the survival of patients with no residual disease is superior to survival of patients with visible residual disease.[1] To achieve this goal, several aggressive surgical techniques have been proposed. Often multivisceral resections are performed (diaphragm resection, splenectomy, colon resection, extensive peritonectomy, etc.)[2], which increase morbidity of patients.

Not all patients are candidates for primary surgery, either due to extend of their disease (unlikely to achieve no residual disease) or due to poor general condition (too ill to undergo an extensive operation). In this situation patients are treated with initial (neoadjuvant) chemotherapy, typically a combination of a platinum-based drug and a tax-

ane.[3] Patients, who are treated with neoadjuvant chemotherapy, are more likely to undergo surgery with no residual disease than patients with primary surgery.[4] Therefore many authors believe that neoadjuvant chemotherapy is justified in order to have best chance to achieve a status of no residual disease.[4,5]

However, treatment with neoadjuvant chemotherapy has not yet been shown to provide better survival than treatment with primary surgery.[4,6] It has been shown that survival of patients with stage III or IV who have no residual disease after primary surgery can be up to 50% at 10 years.[7] It is not known, if patients who achieve a status of no residual disease with neoadjuvant chemotherapy, have the same or equally good prognosis. There are data from one randomized trial which showed similar survival of patients treated with neoadjuvant chemotherapy to those treated with primary surgery, but, this study was criticized due to poor survival rates in both groups.[4]

We compared the differences in the rates of no residual disease (R0 resection) after surgery and the differences in five-year and eight-year survival rates in patients treated with neoadjuvant chemotherapy or primary surgery in correlation to the extent of residual disease post-surgery.

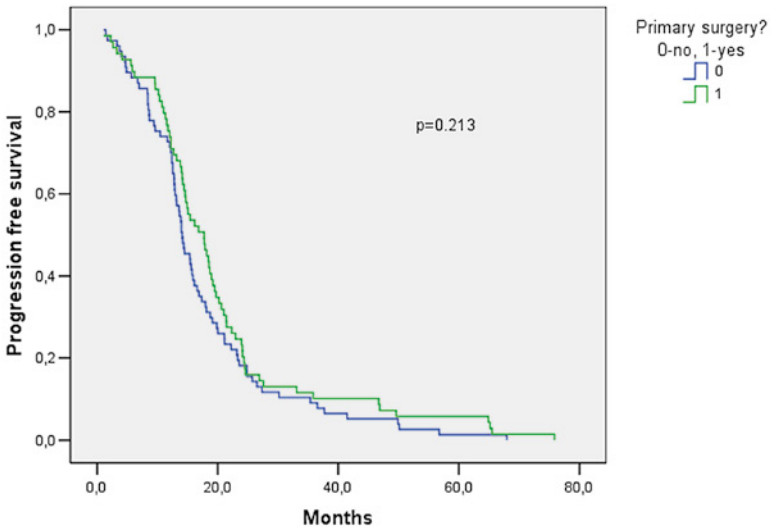

FIGURE 1. Progression free survival of patients after primary surgery and neoadjuvant chemotherapy (NACT).

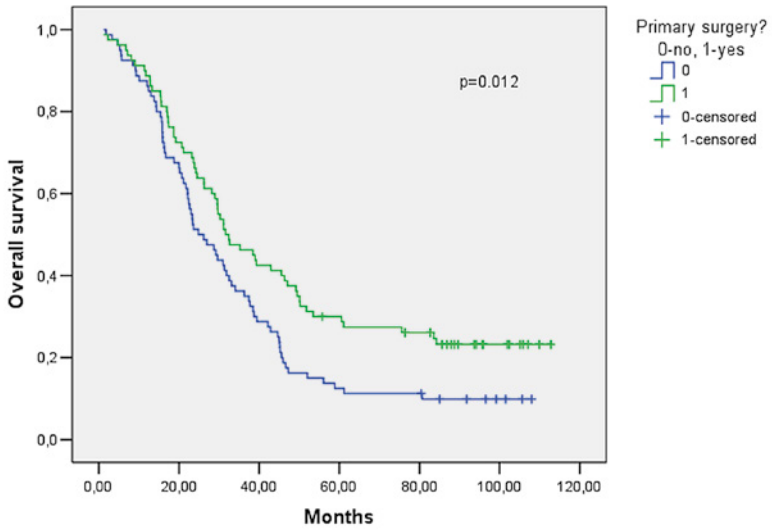

FIGURE 2. Overall survival of patients after primary surgery and neoadjuvant chemotherapy (NACT).

Methods

Patients

We retrospectively reviewed the records of patients who were treated with diagnosis of epithelial ovarian cancer at Institute of Oncology Ljubljana in the period from 1st of January 2005 until 31st of December 2007. During this period we identified 346 patients who were treated for epithelial ovarian cancer. Of these 160 patients had stage FIGO IIIC disease – they were eligible for analysis. Of the 160 eligible patients, 80 patients had neoadjuvant chemotherapy (NACT) and 80 patients had primary surgery.

Patients had primary surgery in seven different hospitals performed by many gynecologic surgeons. In all patients, hysterectomy, bilateral oophorectomy, infracolic omentectomy with limited peritonectomy was performed. In only few of the patients multivisceral resections (diaphragm resection, splenectomy, colon resection, extensive peritonectomy) were performed – therefore no comparison between different surgical techniques outcomes was carried out.

In the NACT group 40% of patients received platinum and taxane therapy (paclitaxel 175mg in 3h *i.v.* infusion and carboplatin AUC 6 *i.v.* infusion) and 55% of patients received carboplatin (AUC 6) monotherapy before surgery. Post-surgery all patients received 3 additional cycles of chemotherapy - the same regimen as in neoadjuvant setting. Median number of cycles in NACT group was 7 (range 1–13).

Of the 80 patients, who had primary surgery, 82% of them received platinum and taxane therapy (paclitaxel 175mg in 3h *i.v.* infusion and carboplatin AUC 6 *i.v.* infusion), 16% of patients received carboplatin (AUC 6) monotherapy. Median number of cycles of chemotherapy was 6 (range 1–9). All patients were treated with chemotherapy at

Institute of Oncology Ljubljana by medical oncologist specialized for gynecologic oncology.

Reasons given for NACT included extent of disease (50%), co-morbidities (10%), and poor performance (40%). For patients who had NACT, stage was established by combination of clinical examination, imaging (US, CT of abdomen), cytology and biopsy. For patients who were treated with primary surgery, stage was established by intraoperative examination and review pf pathology reports. The extent of residual disease was based on the diameter of the single largest lesion. Patients without macroscopic evident residual lesions had R0 resection, patients with less than 10 mm residual lesions had R1 resection, patients with residual lesions of 10 mm or more had R2 resection.

Our retrospective study was approved by the Institutional Review Board and Ethics Committee.

Analysis

We compared patients who had NACT with those who had primary surgery for a range of clinical variables. The extent of residual disease post-surgery was measured. We observed the differences in the rates of no residual disease after surgery, differences in progression free survival (PFS), overall survival (OS) and in five-year and eight-year survival rates in patients after various treatments. Patients were followed from the date of diagnosis until death from ovarian cancer, death from another cause or lost from follow-up.

The primary endpoints were OS, five-year and eight-year survival rates. The secondary end points were R0 resection rates and PFS.

Survival curves were calculated by Kaplan-Meier's method. Univariate and multivariate analyses using log-rank test and Cox's regression model were used for the assessment of the factors associated with OS and for comparison of factors between patients who had NACT with those who had primary surgery. Quantitative variables were compared using Student or the Wilcoxon test. Categorical variables were compared using Chi-square test. The differences were considered statistically significant if the p values were less than 0.05. Software package SPSS 15.0 for Windows was used.

Results

We analyzed 160 patients with stage IIIC epithelial ovarian cancer who were treated at Institute of Oncology Ljubljana in the period from 1st of January 2005 until 31st of December 2007. Median follow-up of patients was 8.4 years (range 6.7–10 years). Of 160 patients, 80 patients had primary surgery and 80 patients had NACT.

TABLE 1. The characteristics of patients and surgical outcomes

	Primary surgery N = 80	NACT N = 80	p
Age (years)	60.2	64.8	**0.006**
High grade serous	43 (54%)	53 (66%)	0.307
Performance			
0 or 1	66 (83%)	32 (40%)	**< 0.0001**
>1	14 (17%)	48 (60%)	
R0 resection	16 (20%)	34 (42%)	**0.011**
R1 resection	18 (23%)	18 (22%)	1.0
R2 resection	46 (57%)	11 (14%)	**< 0.0001**
Inoperable	-	17 (21%)	-
PFS (months)	17.7	14.1	0.213
OS (months)	31.6	24.8	**0.012**

NACT = neoadjuvant chemotherapy; OS = overall survival; PFS = progression-free survival; R0 = no macroscopic residual disease; R1= <1 cm residual disease; R2= >1 cm residual disease

TABLE 2. Five-year and eight-year survival of patients after different surgical outcomes

	Five-year survival	Eight year survival	p
Surgery - R0 resection	62.5%	62.5%	**P < 0.0001**
NACT - R0 resection	20.6%	17.1%	
Surgery - R1 resection	38.9%	27.8%	**P < 0.0001**
NACT - R1 resection	16.7%	11.1%	
Surgery - R2 resection	15.5%	0%	**P < 0.0001**
NACT - R2 resection	0%	0%	
Inoperable disease	0%	0%	-

NACT = neoadjuvant chemotherapy; R0 = no macroscopic residual disease; R1= <1 cm residual disease; R2= >1 cm residual disease

The characteristics of patients and surgical outcomes of patients who had primary surgery or NACT are shown in Table 1. Patients treated with primary surgery were younger (60.2 *vs*. 64.8 years; $p < 0.001$). Patients treated with primary surgery also had a better performance status according to WHO classification ($p < 0.001$).

In patients treated with NACT higher rates of R0 resection were observed (42% *vs*. 20%; $p < 0.001$) and lower rates of R2 resection rates (14% *vs*. 57%; $p < 0.001$) compared to patients who had primary surgery. After NACT in 21% of patients disease remained to be inoperable.

Median PFS of patients was 14.1 months after NACT and 17.7 months after primary surgery (Figure 1). The difference was not statistically sig-

TABLE 3. Factors correlated with survival on univariate analysis

	p
Age (years)	
< 60	0.008
≥ 60	
Histology	
High grade serous	0.066
Other histology	
Performance status	
0 or 1	
> 1	< 0.0001
Extent of residual disease	
R0	
R1	< 0.0001
R2	
Therapy	
Primary surgery	**0.012**
NACT	

NACT = neoadjuvant chemotherapy

TABLE 4. Factors correlated with survival on multivariate analysis

	p
Age (years)	
< 60	0.775
≥ 60	
Histology	
High grade serous	0.370
Other histology	
Performance status	
0 or 1	
> 1	**0.003**
Extent of residual disease	
R0	
R1	**< 0.0001**
R2	
Therapy	
Primary surgery	**0.038**
NACT	

NACT = neoadjuvant chemotherapy

nificant (p = 0.213). Median OS of patients was 24.8 months after NACT and 31.6 months after primary surgery (Figure 2). The difference was statistically significant (p = 0.012).

Five-year and eight-year survival rates of patients after different surgical outcomes are shown in Table 2. Patients treated with primary surgery had superior five-year and eight-year survival rates in all types of surgical outcomes compared to patients treated with NACT. Five-year and eight year survival rates were 62.5% and 62.5% *vs*. 20.6% and 17.1% after R0 resection (p < 0.001), 38 .9% and 27.8% *vs*. 16.7% and 11.1% after R1 resection (p < 0.001) and 15.5% and 0% *vs*. 0% and 0% after R2 resection (p < 0.0001), respectively. Survival curves of patients after different surgical outcomes are shown in Figure 3.

Univariate analysis showed that factors associated with survival were: performance status according to WHO (p < 0.0001), extent of residual disease (p < 0.0001), primary surgery (p = 0.012) and age (p = 0.008) (Table 3).

We conducted multivariate survival analysis using Cox's regression model. The following prognostic factors in the multivariable model: patient age at diagnosis (< 60 *vs*. ≥ 60 years); extent of residual disease (R0, R1, R2); chemotherapy (NACT *vs*. primary surgery); performance status according to WHO (0 or 1 *vs*. > 1) and histopathological subtype (high grade serous *vs*. other) were entered. Patients treated with primary surgery had better survival also on multivariate survival analysis. Besides that, other independent predictors of survival were extent of residual disease and performance status (Table 4).

Discussion

Our results show that treatment with NACT doubles the chance to have no visible residual disease (R0 resection) post-surgery compared to primary surgery in patients with stage IIIC epithelial ovarian cancer (42% *vs*. 20%). This is in concordance with already published studies.[4,5] Despite higher rates of R0 resections achieved by NACT, survival of patients treated with NACT was inferior to survival of patients who underwent primary surgery by almost 7 months (24.8 months *vs*. 31.6 months). Even in patients who had R0 resection post-surgery, there was a huge difference in probability to be five-year or eight-year survivor in favor of primary surgery. Patients with R0 resection at primary surgery had three-fold higher rates of five-year and eight-year survival rates than patients with R0 resection after NACT (62.5% *vs*. 20.6% and 62.5% *vs*. 17.1%, respectively). Our results are in concordance with many authors who reported similar results with inferior overall survival of patients after NACT despite higher rates of optimal debulking surgery.[5-8]

There have been published results of a randomized trial which showed similar survival of patients treated with NACT to those treated with primary surgery, but, this study was criticized due to poor survival rates in both groups.[4]

The limitation of our study was, that this was not a randomized trial, therefore at least to some extent the difference in the outcomes for the different groups might be the result of an imbalance in the baseline characteristics. Patients who received

NACT were more likely to have more extensive disease at diagnosis than patients who had primary surgery, and therefore it was expected to do relatively worse. Patients who received NACT were almost five years older (64.8 years *vs*. 60.2 years), had more co-morbidities and had worse performance than patients who had primary surgery. Half of the patients who received NACT did not receive combination of taxane and carboplatin chemotherapy due to poor performance or co-morbidities, whereas vast majority of patients received combination of taxane and carboplatin after primary surgery.

Multivariate analysis showed that independent predictors of survival were primary surgery, performance status and extent of residual disease. Therefore, a profound differences in survival among patients after R0 resection (and also R1 resection) between primary surgery and NACT can be at least to some extend explained by factors, other than disease itself.

Authors believe that it is improper to compare survival of patients who had R0 resection at primary surgery to patients who had R0 resection after prior NACT if the patients are not balanced regarding performance status, age, comorbidities, etc. It is like comparing apples with oranges.

It is not just the extent of the disease at diagnosis that is important for prognosis. The biology of the disease may also play an important role. At the moment there are no biologic markers that would help us to choose the best treatment strategy for patients with advanced epithelial ovarian cancer. It is known that the most common histologic type of epithelial ovarian cancer, high grade serous adenocarcinoma, is sensitive to chemotherapy, while other histologic types such as clear cell and mucinous ovarian cancer are resistant to chemotherapy. In the last years much effort has been done in discovering of predictive and prognostic molecular markers with molecular profiling of epithelial ovarian cancer. Recently published data have shown that at least three molecular subtypes of high-grade serous ovarian cancer exist, which may have different predictive and prognostic values in systemic treatment of patients with advanced epithelial ovarian cancer.[9]

At the moment there is still no consensus regarding the use of NACT. The lack of consensus on who are candidates for NACT was reflected at the fourth Gynecologic Cancer InterGroup (GCIG) consensus conference.[10] While the majority of attendees felt that NACT was a standard option for all patients with advanced epithelial ovarian cancer, others felt that NACT should be offered to a more clearly defined subgroup of women in whom

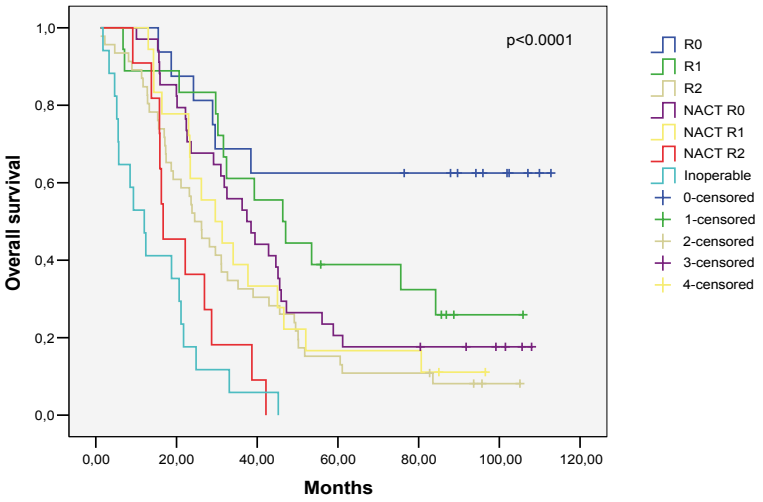

FIGURE 3. Survival curves of patients after different surgical outcomes.

NACT = neoadjuvant chemotherapy; R0 = no macroscopic residual disease; R1= <1 cm residual disease; R2= >1 cm residual disease

upfront surgery is contraindicated. The decision on whether to treat with NACT is based on the clinical status of the patient and whether or not disease is resectable at the time of presentation. Therefore, all patients require clinical staging. There are widely accepted criteria for unresectability.[11] It is often difficult to preoperatively assess whether patients with advanced epithelial ovarian cancer can be optimally cytoreduced at the time of primary surgery.[12] Therefore, many authors perform a staged surgical assessment for these patients and perform a diagnostic laparoscopy to further evaluate for resectability. If the surgeon conducting the assessment feels disease is resectable, primary surgery should be performed. If complete resectability is unlikely, NACT can be administered.

Despite better overall survival of our patients who had primary surgery, two-thirds of patients in this group had R2 resection (> 1 cm residual disease) which is considered as a sub-optimal resection. These patients had five-year survival rates of only 15.5% with none being alive at eight years post-surgery. In NACT group there were only 14 % of patients with R2 resection. We believe that the main reason for high rate of R2 resections at primary surgery was that primary surgery was performed in different hospitals mainly by surgeons not skilled with principles of oncology surgery, whereas all patients who had NACT underwent surgery provided by surgeon experienced in oncology. There are convincing data showing that surgical expertise plays a major role in outcome of patients with advanced epithelial ovarian cancer. In

multidisciplinary cancer centers with advanced expertise in gynecologic oncology, optimal debulking rates in excess of 70% have been reported even for patients with bulky stage IIIC disease.[7] Therefore, it is strongly recommended that a gynecologic oncologist must be involved in surgical decision making and treatment in these circumstances.

We believe there is enough evidence that in operable disease primary surgery with aim of no residual disease should be performed, since this offers best possible survival of patients with stage IIIC epithelial ovarian cancer.[1] Of course patients must be fit enough for surgery, in which often multivisceral resections are needed with intention to achieve no residual disease post-surgery, which increase morbidity of patients.[2] The NACT should only be offered to patients who are not candidates for primary surgery for whatever reason (advanced disease, poor performance, comorbidities, etc.). The aim of NACT should be to convert inoperable advanced disease to operable disease with goal to achieve optimal debulking (R0 resection). We believe that in patients with advanced epithelial ovarian cancer the aim of treatment should not only be the survival benefit but also the improvement in quality of life. Latter can be achieved by direct effect of chemotherapy on downsizing tumor burden, which offers higher chance for improvement in performance by lessening of the disease symptoms, offers higher chance to achieve optimal debulking at surgery, with less postoperative complications.[4,5,13] Since this was a retrospective study, a comparison of the quality of life between patients treated with NACT or primary surgery was not performed.

One of the remarks towards NACT was that patients who started their treatment with NACT never had the chance for complete or optimal cytoreductive surgery. Since 48% of patients who received NACT in our study population were not candidates for radical or ultraradical surgical procedures as a result of poor performance status or comorbidities, they should probably be excluded from comparison with patients who were capable to undergo primary surgery. Perhaps the survival of patients who had NACT, but were otherwise fit for primary surgery, should be compared to survival of patients who had suboptimal primary surgery (R2 resection), thus comparing different treatments for similar burden of the disease. If we compare these two groups of patients we can see that five-years survival rates in NACT group with R0 or R1 post-surgery (NACT-R0 and NACT-R1) were somewhat higher compared to patients who had R2 resection at primary surgery (20.6% after NACT-R0 surgery, 16.7% after NACT-R1 surgery and 15.5% after R2 primary surgery), the difference was not statistically significant.

To conclude, our results show that treatment with NACT doubles the chance to have no visible residual disease (R0 resection) post-surgery compared to primary surgery in patients with advanced (stage IIIC) epithelial ovarian cancer. Despite higher rates of R0 resections achieved by NACT, survival of patients treated with NACT was inferior to survival of patients who underwent primary surgery by almost 7 months. Therefore we strongly believe that NACT should only be offered to patients with advanced epithelial cancer who are not candidates for primary cytoreductive surgery, or when the disease is unresectable after staged surgical assessment performed by oncology surgeon.

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Pozitronska emisijska tomografija z ^{18}F-FDG in ^{18}F-flumazenilom pri bolnikih z neodzivno epilepsijo

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Izhodišča. Epilepsija je nevrološka motnja, za katero so značilni epileptični napadi, ki so posledica prekomerne nevronske aktivnosti v možganih. Približno 65 milijonov ljudi po svetu trpi zaradi epilepsije; 20–40 % se jih na terapijo z zdravili ne odziva. Zgodnje odkrivanje bolezni je ključnega pomena pri zdravljenju bolnikov z epilepsijo, saj pravilna lokalizacija mesta epileptogenega žarišča izboljša obravnavo teh bolnikov. Sodobne neinvazivne tehnike, ki jih uporabljajmo za strukturno in funkcionalno lokalizacijo žarišča, so elektroencefalografija (EEG), slikanje z magnetno resonanco (MRI), nuklearnomedicinska tomografija v kombinaciji z računalniško tomografijo (SPECT/CT) in pozitronska emisijska tomografija s CT ali MRI (PET/CT oz. PET/MRI). V zadnjih letih številne raziskave opisujejo, da lahko s pomočjo PET/CT napovemo izhod kirurškega zdravljenja bolnikov z neodzivno epilepsijo. Namen članka je sistematično preučiti vlogo dveh PET/CT radiofarmakov: ^{18}F-fluorodeoksiglukoze (^{18}F-FDG), ki jo pri bolnikih z neodzivno epilepsijo uporabljamo rutinsko, in ^{18}F-flumazenila (^{18}F-FMZ), ki ga uporabljamo le v kliničnih študijah.

Zaključki. Informacije o delovanju, ki jih dobimo s pomočjo PET in informacije o morfologiji, ki jih dobimo s CT ali MRI, so bistvenega pomena za predkirurško oceno bolnika z epilepsijo. ^{18}F-FDG PET/CT je danes rutinska metoda slikanja za določitev mesta epileptogenega žarišča pri bolnikih z neodzivno epilepsijo. Na žalost ^{18}F-FDG PET/CT ni idealna metoda: področja z zmanjšanim metabolizmom glukoze se ne ujemajo natančno s histopatološko ali MRI dokazano stopnjo sprememb skleroze hipokampusa. Nova obetavna nuklearnomedicinska metoda je prikaz epileptogenega žarišča z gostoto benzodiazepinskih receptorjev. Zaradi boljše občutljivosti in anatomske ločljivosti bi bil lahko ^{18}F-FMZ pomemben radiofarmak pri bolnikih z neodzivno epilepsijo.

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Razlike pri vrisovanju tarčnih volumnov v radioterapiji. Kako pomembne so in kaj lahko storimo?

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Izhodišča. Moderne obsevalne tehnike omogočajo obsevanje tarčnega volumna z visoko dozo ob upoštevanju doznih omejitev za rizične organe, kar omogoča boljšo lokalno kontrolo ob ohranjevanju kakovosti življenja. Neujemanje med vrisovalci pri vrisovanju tarčnih volumnov je opisano za različne tumorske lokalizacije. Nekatere raziskave kažejo, da so razlike pri vrisovanju večje kot napake v vseh ostalih korakih načrtovanja in izvajanja obsevanja. Namen članka je povzeti nivo razlik pri vrisovanju tarčnih volumnov opisanem v literaturi in oceniti učinkovitost strategij za njihovo zmanjševanje.

Zaključki. Pregled je potrdil pomembne razlike pri vrisovanju tarčnih volumnov za večino tumorskih lokalizacij, kar bi lahko vplivalo na lokalno kontrolo pri posameznih bolnikih. Kljub obetavnim rezultatom raziskav glede uporabe različnih anatomskih in funkcionalnih slikovnih metod pri vrisovanju tarčnih volumnov, bodo potrebne dodatne raziskave za opredelitev optimalne kombinacije le-teh. Dosledna uporaba priporočil za vrisovanje zmanjša neujemanje med vrisovalci. Njihova uporaba je priporočljiva tako v vsakdanji klinični praksi kot v sklopu kliničnih raziskav, saj je interpretacija rezultatov raziskav ob obstoječi stopnji razlik med vrisovalci lahko vprašljiva. Pomanjkanje znanja pri interpretaciji različnih slikovnih metod je pogost vzrok za neujemanje med vrisovalci, kar kaže, da sedanji obseg izobraževanja v sklopu specializacije radioterapije in onkologije ter v rednem kliničnem delu ni zadosten.

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Vloga nativne računalniške tomografije v diagnostiki tromboze možganskih venskih sinusov

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Izhodišča. Namen raziskave je bil preučiti senzitivnost in specifičnost nativne računalniške tomografije glave (CT) v diagnostiki tromboze možganskih venskih sinusov.

Metode. Pregledali smo klinične podatke in radiološke preiskave 53 bolnikov, ki so bili obravnavani v urgentni nevrološki ambulanti zaradi suma na trombozo možganskih venskih sinusov. Dva neodvisna ocenjevalca sta pregledala nativne CT preiskave bolnikov in ocenila prisotnost znakov tromboze možganskih venskih sinusov. Za referenčno preiskavo smo upoštevali CT venografijo ali magnetnoresonančno venografijo. Strinjanje med ocenjevalcema smo ocenili s pomočjo Kappa statistike. Dodatno smo izmerili atenuacijske vrednosti znotraj venskih sinusov ter jih primerjali med skupino bolnikov s potrjeno trombozo možganskih venskih sinusov in kontrolno skupino.

Rezultati. Trombozo možganskih venskih sinusov smo potrdili pri 13 bolnikih. Senzitivnost in specifičnost nativne CT preiskave sta bili 100 % in 83 %. Vrednost Kappa je bila 0,72 (dobro strinjanje med ocenjevalcema). Atenuacijske vrednosti znotraj možganskih venskih sinusov so bile pri bolnikih s trombozo značilno višje (73,4 ± 14,12 HU) kot v kontrolni skupini (58,1 ± 7,58 HU; p = 0,000). S pomočjo analize ROC smo določili pražno vrednost 64 HU, pri kateri sta bili senzitivnost in specifičnost metode 85 % in 87 %.

Zaključki. Nativni CT glave je učinkovita prva preiskava pri urgentni obravnavi bolnikov s sumom na trombozo možganskih venskih sinusov. Merjenje atenuacijskih vrednosti znotraj sinusov lahko pripomore k večji diagnostični vrednosti preiskave.

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Ponovitev bolezni v področnih bezgavkah pri 737 bolnikih s folikularno neoplazmo ali neoplazmo Hürthlejevih celic

Vogrin A, Bešić H, Bešić N, Marolt Mušič M

Izhodišča. Predoperativno ultrazvočno (UZ) preiskavo osrednjega in stranskega področja vratu priporočamo pri vseh bolnikih pred tiroidektomijo, kjer smo že dokazali malignom ali postavili citološki oziroma molekularni sum na malignost. Cilj raziskave je bil ugotoviti, kako pogosto se je rak ponovil v področnih bezgavkah pri bolnikih s folikularno neoplazmo ali neoplazmo Hürthlejevih celic in oceniti uporabnost predoperativne UZ preiskave pri bolnikih z neoplazmo.

Bolniki in metode. Zaradi folikularne neoplazme ali neoplazme Hürthlejevih celic smo od leta 1995 do leta 2014 na Onkološkem inštitutu operirali 737 bolnikov. Med njimi je imelo raka ščitnice 207 bolnikov (163 žensk, 44 moških, povprečna starost 52 let).

Rezultati. Med bolniki s folikularno neoplazmo smo rak diagnosticirali v 143/428 primerih, med bolniki z neoplazmo Hürthlejevih celic pa v 64/309 primerih. Ponovitev bolezni v področne bezgavke smo ugotovili pri 12/207 bolnikih (6 %) po srednji vrednosti spremljanja 55 mesecev. Med bolniki, ki so imeli raka, se je le-ta ponovil v regionalnih bezgavkah pri 2% bolnikov s folikularno neoplazmo in pri 14% bolnikov z neoplazmo Hürthlevih celic (p = 0,002). Po zdravljenju smo ponovno ugotovili rak v področnih bezgavkah v 3/428 (0,7 %) primerih bolnikov s folikularno neoplazmo in v 9/309 (3%) primerih bolnikov z neoplazmo Hürthlejevih celic.

Zaključki. Ponovitev bolezni v bezgavkah smo diagnosticirali pri 0,7 % bolnikov s predoperativno diagnozo folikularne neoplazme in pri 3 % bolnikih z neoplazmo Hürthlejevih celic. Ponovitev v področnih bezgavkah je redka tudi pri bolnikih z rakom, ki so imeli predoperativno diagnozo folikularna neoplazma. Predoperativna preiskava vratnih bezgavk pri bolnikih s folikularno neoplazmo verjetno ni uporabna, pri bolnikih z neoplazmo Hürthlejevih celic pa bi lahko bila koristna.

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Elektrokemoterapija z bleomicinom je učinkovita na BRAF mutiranih melanomskih celicah in ima potencirano delovanje z inhibitorji BRAF

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Izhodišča. Namen raziskave je bil ugotoviti učinkovitost elektrokemoterapije (ECT) med zdravljenjem bolnikov z melanomom z inhibitorji BRAF. Testirali smo učinkovitost ECT na BRAF mutiranih in nemutiranih melanomskih celicah *in vitro* in v kombinaciji z inhibitorjem BRAF.

Materiali in metode. ECT z bleomicinom smo izvedli na dveh humanih melanomskih celičnih linijah, eni z BRAF V600E mutacijo (SK-MEL-28) in eni brez BRAF mutacije (CHL-1). Učinkovitost ECT in ECT v kombinaciji z inhibitorjem BRAF vemurafenibom smo ovrednotili s preživetjem celic, ki smo ga določevali s testom klonogenosti.

Rezultati. Preživetje melanomskih celic z BRAF V600E mutacijo je bilo po ECT manjše kot pri celicah brez mutacije, kar kaže na to, da je ECT učinkovita ne glede na mutacijski status melanomskih celic. Dokazali smo tudi sinergistično delovanje ECT z bleomicinom in vemurafeniba v BRAF V600E mutiranih melanomskih celicah.

Zaključki. Učinkovitost ECT v BRAF mutiranih melanomskih celicah in njena potencirana učinkovitost v kombinaciji z vemurafenibom *in vitro* kaže na klinično uporabnost ECT pri bolnikih z melanomom z mutacijo BRAF in/ali med zdravljenjem z inhibitorji BRAF.

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Odkritje 'klik' 1,2,3-triazolijevih soli kot potencialnih antitumorskih učinkovin

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Izhodišča. Da bi povečali učinkovitost zdravljenja raka, smo pripravili in testirali nove potencialno antitumorsko aktivne spojine. Poročamo o testiranju novega tipa spojin, 1-(2-pikolil)-, 4-(2-pikolil)-, 1-(2-piridil)- in 4-(2-piridil)-3-metil-1,2,3-triazolijevih soli ter njihovih 1,2,3-triazolskih prekurzorjev.

Metode. Citotoksičnost novih spojin smo določali s spektrometričnim testom 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolijev bromid (MTT) na več tumorskih in eni normalni celični liniji. Učinek vezave izbranih spojin na dvojno vijačnico DNA (ds DNA) smo ugotavljali s testiranjem vpliva na termično stabilnost DNA telečjega timusa, medtem ko smo vpliv na celični ciklus določevali s pretočno citometrično analizo. Tvorbo reaktivnih kisikovih spojin (ROS) smo določevali z dodatkom specifičnega substrata, 5-(in-6)-klorometil-2',7'-diklorodihidrofluorescein diacetata, acetil estra (CM-H_2DCFDA).

Rezultati. Osnovni triazoli na splošno niso aktivni, medtem ko so triazolijeve soli zelo citotoksične na celice HeLa. Triazolijeve soli so pokazale veliko specifično citotoksičnost na različne tumorske celice. Ena od spojin, 3-metil-4-(4-metoksifenil)-1-(2--pikolil)-1H-1,2,3-triazolijev heksafluorofosfat(V) (2b), je bila bistveno bolj citotoksična na tumorske kot pa na normalne celice, z visokim terapevtskih indeksom 7,69 za celice pljučnega raka H460. Ta spojina je bila podobno aktivna na osnovne celice raka grla HEp-2 in na njihovo na zdravila odporno podlinijo 7T, kar kaže na njen potencial v zdravljenju na zdravila odpornih rakov. Spojina 2b je ustavila celice v G1 fazi celičnega cikla. Ni vezala ds DNA, temveč je inducirala ROS v obdelovanih celicah, kar je sprožilo celično smrt.

Zaključki. Naši rezultati nakazujejo, da je smiselno nadaljevati raziskave 'klik' triazolijevih soli kot antitumorskih učinkovin.

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Funkcionalni polimorfizmi antioksidativnih genov pri neoplazmi Huerthlejevih celic ščitnice - povezava med polimorfizmom gena *GPX1* in ponovitvijo raka Huerthlejevih celic ščitnice

Krhin B, Goričar K, Gazić B, Dolžan V, Bešić N

Izhodišča. Za Huerthlejeve celice ščitnice je značilno veliko število mitohondrijev in oksidativnih encimov. Ker povečan oksidativni metabolizem lahko vodi v povečan oksidativni stres oziroma ga lahko povezujemo z večjo verjetnostjo razvoja raka, smo v naši raziskavi preverjali, ali obstaja povezava med funkcionalnimi polimorfizmi antioksidativnih genov (*SOD2*, *CAT*, *GPX*, *GSTP1*, *GSTM1* in *GSTT1*) in nastankom ali kliničnim potekom raka Huerthlejevih celic ščitnice (HCTC).

Bolniki in metode. Retrospektivno raziskavo smo izvedli pri 139 bolnikih, pri katerih smo zaradi suma na neoplazmo Huerthlejevih celic ščitnice opravili operacijo ščitnice. Diagnozo HCTC, adenoma Huerthlejevih celic ščitnice (HCTA) ali gomolja Huerthlejevih celic ščitnice (HCTN) smo postavili s histopatomorfološko analizo. DNA smo izolirali iz stebričkov histološko potrjenega zdravega dela ščitnice, pridobljenega iz arhiviranih parafinskih blokov tumorjev, fiksiranih v formalinu. S postopki genotipizacije smo določali prisotnost polimorfizmov v antioksidativnih genih. Z logistično regresijo pa smo primerjali porazdelitve posameznih genotipov med različnimi skupinami bolnikov.

Rezultati. HCTC smo ugotovili pri 53, HCTA pri 47 in HCTN pri 21 bolnikih. Pri 20 bolnikih s HCTC smo ugotovili prisotnost zasevkov, pri 16 pa ponovitev bolezni. Pri skupinah bolnikov s HCTC, HCTA in HCTN frekvence genotipov in alelov preučevanih polimorfizmov niso odstopale od Hardy-Weinbergovega ravnotežja. Dominantni genetski model ni pokazal povezave med porazdelitvijo frekvenc genotipov preučevanih polimorfizmov in prisotnostjo HCTC v primerjavi s HCTA in HCTN, prav tako ni bilo povezave s prisotnostjo zasevkov pri HCTC. Ugotovili pa smo povezavo med polimorfizmom *GPX1* in ponovitvijo HCTC (p = 0,040).

Zaključki. Polimorfizem *GPX1* lahko vpliva na možnost ponovitve HCTC.

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Vpliv polimorfizmov v segregacijskih genih BUB1B in TTK na dovzetnost za razvoj želodčnega raka

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Izhodišča. Maligna preobrazba normalnih želodčnih celic je zapleten večstopenjski proces, ki vodi v nastanek heterogenih tumorjev. Na razvoj želodčnega raka vplivajo poleg dejavnikov okolja tudi genetsko ozadje in genetske spremembe. Polimorfizmi enega baznega para (*angl. Single nucleotide polymorphisms*, SNP) v mitotskih segregacijskih genih bi lahko bili odgovorni za počasno kopičenje genetskih sprememb, ki vodijo v genomsko nestabilnost.

Bolniki in metode. V raziskavi primerov s kontrolami smo opredelili vpliv polimorfizmov rs151658 v mitotski kinazi *TTK* in rs1031963 ter rs1801376 v kinazi *BUB1B* na razvoj želodčnega raka. Z metodo imunskega odtisa smo določili količino TTK v rakavih tkivih bolnikov.

Rezultati. Odkrili smo, da genotipa C/G in G/G polimorfizma rs151658 značilno vplivata na dovzetnost za razvoj difuzne oziroma intestinalne oblike želodčnega raka (p = 0,049). Genotip A/A polimorfizma rs1801376 je bil značilno povezan z višjim tveganjem za razvoj želodčnega raka pri bolnicah (0,007), medtem ko se je pri moških z želodčnim rakom pogosteje pojavljal le pri preiskovancih, pri katerih so tumorske celice preraščale v subserozo (0,009). Pri nosilcih genotipa T/T polimorfizma rs1031963 so se pogosteje razvili dobro diferencirani tumorji (0,035). V dominantnem modelu sta bila genotipa TT+CT polimorfizma rs1031963 (razmerje obetov [OR] = 2,929, 95 % interval zaupanja [CI]: 1,281–6,700; p = 0,017) in genotipa GG+AG polimorfizma rs1801376 (OR = 0,364, 95 % CI: 0,192–0,691; p = 0,003) značilno povezana z višjim tveganjem za razvoj bolezni.

Zaključki. Rezultati raziskave kažejo, da polimorfizmi v mitotskih kinazah *TTK* in *BUB1B* v naši skupini preiskovancev statistično značilno prispevajo k povišanemu tveganju za razvoj želodčnega raka in mogoče vplivajo na potek razvoja tumorjev. Za opredelitev njihove klinične uporabnosti so potrebne nadaljnje raziskave v večjih skupinah bolnikov z želodčnim rakom različnih ras.

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doi:10.1515/raon-2016-0039

Metastatski sebacijski rak. Pregled literature in elektrokemoterapija kot nova možna oblika zdravljenja

Ribero S, Sportoletti Baduel E, Brizio M, Picciotto F, Dika E, Fierro MT, Macripò G, Quaglino P

Izhodišča. Metastatski sebacijski rak je redka bolezen glave in vratu. V začetni stopnji razvoja je terapija izbora kirurgija in/ali radioterapija. Zdravljenje ponovljene ali napredovale bolezni pa je še vedno različno.

Metode. Naredili smo izčrpno poizvedbo objavljene literature, ki je obravnavala terapevtske možnosti te redke bolezni.

Rezultati. V literature je opisanih več oblik zdravljenja metastatskega sebacijskega raka. Elektrokemoterapija do sedaj še ni bila opisna kot možen način zdravljenja. Prikažemo 85 let starega bolnika s ponovljeno, lokalno metastatsko boleznijo na temenu, ki smo ga zdravili z elektrokemoterapijo. To smo aplicirali dvakrat v obdobju 8. mesecev. Dosegli smo delni odgovor tumorja in dobro kakovost življenja bolnika.

Zaključki. Pregled literature nazorno nakazuje potrebo po novih načinih zdravljenja metastatskega sebacijskega raka. Na osnovi naše prve in pozitivne izkušnje predlagamo nadaljnje raziskave, ki bi uporabile elektrokemoterapijo za zdravljenje te redke entitete tumorja in bi jo nato uporabile kot terapijo izbora v kliničnih situacijah, kjer je potrebna lokalna kontrola tumorjev ali pa radikalni posegi niso možni ali zaželeni.

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doi:10.1515/raon-2016-0033

Proliferacijski faktor Ki67, vendar ne neuroendokrina ekspresija, je neodvisni napovedni dejavnik za primarni rak prostate

Pascale M, Aversa C, Barbazza R, Marongiu B, Siracusano S, Stoffel F, Sulfaro S, Roggero E, Bonin S, Stanta G

Izhodišča. Neuroendokrine označevalce in proliferacijski faktor Ki67 so že povezovali s potekom bolezni primarnega raka prostate. Namen raziskave je bil raziskati napovedno vrednost teh označevalcev pri bolnikih s primarnim rakom prostate.

Bolniki in metode. Neuron specifično enolazo (NSE), kromagranin A (ChrA), sinaptofizin (Syp) in Ki67 smo določevali imunohistokemično. S pomočjo univariantne in multivariantne analize smo ovrednotili njihovo izražanje in povezanost s celokupnim preživetjem pri 166 bolnikih s primarnim rakom prostate.

Rezultati. NSE, ChrA, Syp in Ki67 so bili pozitivni pri 50, 45, 54 in 146 od skupno 166 bolnikov. S Kaplan-Meier analizo smo dokazali, da sta samo difuzno barvanje NSE (negativni proti difuzno barvani vzorci $p = 0,004$) in proliferacijski označevalec Ki67 (< 10 % vs. > 10 %, $p < 0,0001$) povezana s celokupnim preživetjem bolnikov. V multivariatni analizi se je izražanje Ki67 pokazalo kot neodvisni napovedni dejavnik celokupnega preživetja ne pa izražanje NSE.

Zaključki. Napovedni model, ki vključuje izražanje Ki67 ob upoštevanju klinično patoloških parametrov, lahko predstavlja dodatno napovedno informacijo oz. lahko izboljša napoved izhoda bolezni raka prostate ter obravnavo bolnikov z rakom prostate.

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doi:10.1515/raon-2015-0017

Cefalna pankreatektomija z resekcijo ven pri duktalnem raku trebušne slinavke

Flis V, Potrč S, Kobilica N, Ivanecz A

Izhodišča. Nekatere raziskave kažejo, da imajo bolniki z rakom trebušne slinavke, pri katerih se rakava rašča širi v portalni venski sistem, po kirurški odstranitvi tumorja podobno preživetje kot bolniki, kjer ni razraščanja v vene. Mnenja o tem so deljena, katerim bolnikom z vraščanjem tumorja v venski sistem bi kirurška odstranitev tumorja koristila. Prav tako ni soglasja o tem, kateri kirurški postopek poprave odstranjenih ven je ustreznejši (rekonstrukcija z žilno protezo ali brez nje). Ker so so dosedanje raziskave vsebovale majhno število bolnikov, je bil namen pričujoče raziskave predstavitev izkušenj posamične ustanove pri cefalni duodenopankreatektomiji s hkratno resekcijo ven.

Bolniki in metode. Pregled računalniške podatkovne zbirke Univerzitetnega kliničnega centra v Mariboru za obdobje od januarja 2006 do avgusta 2014 je pokazal, da smo v omenjenem obdobju zaradi raka trebušne slinavke s cefalno duodenopankreatektomijo operirali 133 bolnikov (poprečna starost 65,4 ± 8,6 let; 69 žensk). Razčlenili smo njihove demografske, klinične in biokemijske podatke, histološke izvide in pooperativni izhod. Primerjali smo podatke bolnikov, kjer smo hkrati z duodenopankreatektomijo opravili tudi resekcijo ven, z bolniki, kjer resekcije ven nismo naredili.

Rezultati. Izmed 133 bolnikov je bilo 22 (16,5 %) takih, kjer smo hkrati s cefalno duodenopankreatektomijo opravili tudi resekcijo ven in njihovo rekonstrukcijo. Tako smo opredelili skupino 111 bolnikov (poprečna starost 65,6 ± 7, let; 58 žensk), kjer ven nismo resecirali in skupino 22 bolnikov (63,95 ± 9.5 let; 13 žensk), kjer smo resekcijo ven naredili. V slednji skupini smo pri 14 bolnikih portalno veno popravili brez uporabe umetnega žilnega vsadka, pri osmih pa smo vstavili umetni žilni vsadek iz dakrona. Pri bolnikih, pri katerih uporaba vsadka ni bila potrebna, smo vedno uporabili anastomozo konec s koncem, če smo jo lahko varno naredili brez tenzije na anastomozni črti. Kadar smo operirali področje na stičišču zgornje mezenterične in vranične vene, smo vranično veno podvezali. Pri osmih bolnikih smo uporabili umetni žilni vsadek iz dakrona s premerom 10 mm. Pri njih nismo videli zapletov na anastomozi. V skupini z direktnim šivom vene smo ugotovili eno trombozo vene. Neposredna primerjava obeh skupin (vsi bolniki z resekcijo ven v primerjavi z bolniki brez resekcije) je pokazala, da ni bilo statistično pomembnih razlik med skupinama v pooperativni obolevnosti in umrljivosti. Srednji čas preživetja je bil v skupini z resekcijo 16,13 mesecev in v skupini brez resekcije 15,17 mesecev. V skupini brez resekcije je bilo celokupno petletno preživetje 19,5 %. Primerjava krivulj preživetja med skupinama ni pokazala pomembnih statističnih razlik (test *log-rank* p = 0,090).

Zaključki. Preživetje bolnikov z rakom trebušne slinavke, kjer je bila poleg cefalne pankreatektomije opravljena tudi resekcija ven, je bilo primerljivo s skupino, kjer resekcija ven ni bila potrebna. Dodaten poseg na venah ni vplival na pooperativno obolevnost in umrljivost. Uporaba umetnega žilnega vsadka iz dakrona je bila varna alternativa neposredni venski popravi.

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doi:10.1515/raon-2016-0036

Interdisciplinarna soglasna izjava o indikaciji in uporabi hidrogelnega vmesnika pri radioterapiji prostate. Izkušnje pri več kot 250 bolnikih

Müller AC, Mischinger J, Klotz T, Gagel B, Habl G, Hatiboglu G, Pinkawa M

Izhodišča. Namen raziskave je bil doseči soglasje o indikaciji in uporabi hidrogelnega vmesnika na podlagi multicentričnih izkušenj ter posredovati uporabnikom pomembno informacijo, s katero bi skrajšali učenje te inovativne tehnike.

Metode. Interdisciplinarnega srečanja so se udeležili radioterapevti in urologi, ki so opravili 23–138 aplikacij hidrogela (SpaceOAR®) pri bolnikih z rakom prostate pred radioterapijo, kjer smo zviševali dozo. Želeli smo pridobiti praktične informacije, ki so pomembne za zaporedno injiciranje hidrogela in zdravljenje, zato smo obravnavali izkušnje uporabnikov in odgovorili na zastavljena ključna vprašanja. Pregledani smo stranske učinke, povezane s hidrogelom in ocenili delež, zdravljenje in napoved možnih tveganj.

Rezultati. Najpomembnejša indikacija za aplikacijo hidrogela je bila radioterapija z naraščajočo dozo pri histološko potrjenem raku prostate z nizkim ali vmesnim tveganjem. Priporočili smo jo pri lokalno napredovalem raku prostate. Injiciranje ali implantacijo smo opravljali pod nadzorom transrektalnega ultrazvoka in s transperinealnim pristopom po predhodni hidrodisekciji. Pri skupno 258 aplikacijah hidrogela je bil delež toksičnosti stopnje 2, ki je bila povezana z injiciranjem 2 % (n = 5). Najpogostejši zaplet (n = 4) je bilo predrtje stene rektuma, ki smo ga ugotovili ob različnih časovnih intervalih po injiciranju hidrogela in smo ga zdravili konzervativno.

Zaključki. Dosegli smo soglasje o aplikaciji hidrogelnega vmesnika. Dosedanje izkušnje kažejo, da je metoda izvedljiva, kar lahko spodbudi njeno uvedbo v več centrih. Na ta način bi lahko znižali z obsevanjem povezano gastrointestinalno toksičnost slikovno vodene radioterapije z naraščajočo dozo. Še vedno pa je možen zelo nizek delež resnih neželenih dogodkov. Zato bi morali skrbno proučiti aplikacijo in bolnika ter upoštevati možne prednosti takšnega zdravljenja.

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doi:10.1515/raon-2016-0029

Odlični rezultati zdravljenja kompresije hrbtenjače pri mielomu z radioterapijo

Rades D, Conde-Moreno AJ, Cacicedo J, Šegedin B, Rudat V, Schild SE

Izhodišča. Ni znano, ali bolniki s kompresijo hrbtenjače in radiosezibilnimi tumorji poleg radioterapije potrebujejo tudi kirurško dekompresijo hrtenjače. Zato smo v raziskavi analizirali potek bolezni pri bolnikih, ki so zboleli zaradi mielomoma in pri katerih smo ugotovili kompresijo hrbtenjače ter smo jih zdravili samo z obsevanjem.

Bolniki in metode. Retrospektivno smo analizirali podatke 238 bolnikov glede odgovora na RT in lokalno kontrolo kompresije hrbtenjače. Ocenili smo učinek radioterapije na motorično funkcijo (izboljšanje, brez nadaljnega slabšanja, poslabšanje). Odgovor smo opredelili kot izoboljšanje ali odsotnost nadaljnega slabšanja motorične okvare. Pred radioterapijo smo bolnike predstavili nevrokirurgu, da je ocenil, ali je indicirana takojšnja operacija z dekompresijo (zaradi zloma vretenca, nestabilne hrbtenice).

Rezultati. V celotni kohorti je bil odgovor na radioterapijo 97 % (izboljšanje 53 %, brez nadaljnega slabšanja 44 %). Po radioterapiji je lahko hodilo 88 % bolnikov. Izmed 69 hospitaliziranih bolnikov je shodilo 44 bolnikov (64 %). Lokalna kontrola po 1, 2 in 3 letih je bila 93 %, 82 % in 82 %. Trend izboljšanja lokalne kontrole smo opazili pri bolnikih, ki smo jih obravnavali pred začetkom radioterapije oambulantno (p = 0.08), in pri tistih z boljšim stanjem zmogljivosti (p = 0.07).

Zaključki. Pri bolnikih z mielomom in kompresijo hrbtenjače zagotavlja radioterapija odličen odgovor na zdravljenje, funkcionalne rezltate in lokalno kontrolo. Rezultate bo potrebno potrditi s prospektivno randomizirano raziskavo.

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doi:10.1515/raon-2016-0034

Vloga neoadjuvantne kemoterapije pri bolnicah z napredovalim epitelijskim rakom jajčnika (stadij IIIC)

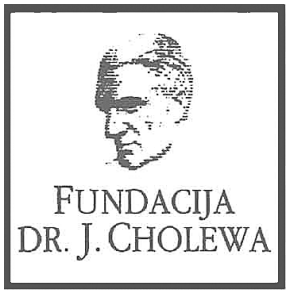
Škof E, Merlo S, Pilko G, Kobal B

Izhodišča. Primarno zdravljenje bolnic z napredovalim epitelijskim rakom jajčnika vključuje kemoterapijo pred (neoadjuvantna kemoterapija) ali po zamejitveni operaciji (adjuvantna kemoterapija). Cilj primarnega zdravljenja epitelijskega raka jajčnika je odstranitev vsega rakastega tkiva brez makroskopskega ostanka. Popolna resekcija (R0) po primarnem kirurškem zdravljenju predstavlja pomemben dejavnik v izboljšanju preživetja bolnic, medtem ko za enkrat še ni dokazov o enakem vplivu R0 resekcije, če primarno zdravljenje začnemo z neoadjuvantno kemoterapijo.

Bolnice in metode. V letih od 2005 do 2007 smo na Onkološkem inštitutu pregledali popise bolnic z diagnosticiranim epitelijskim rakom jajčnika. Med bolnicami, ki so zdravljenje začele z neoadjuvantno kemoterapijo in tistimi, ki so bile primarno operirane, smo primerjali stopnjo dosežene kirurške resekcije, čas do ponovitve bolezni, ter petletno in osemletno preživetje.

Rezultati. V raziskavo smo zajeli 160 bolnic s stadijem IIIC epitelijskega raka jajčnika. 80 bolnic smo pričeli zdraviti z neoadjuvantno kemoterapijo, pri preostalih 80-ih bolnicah pa je bilo primarno zdravljenje kirurško. Bolnice v skupini z neoadjuvantno kemoterapijo so imele višjo stopnjo resekcije R0 (42 % vs. 20 %; p = 0,011) kot bolnice s primarnim kirurškim zdravljenjem. Čas do ponovitve bolezni je bil v skupini z neoadjuvantno kemoterapijo 14,1 mesecev, po primarnem kirurškem zdravljenju pa 17,7 (p = 0,213). Celokupno preživetje je bilo 24,8 meseca v skupini z neoadjuvantno kemoterapijo in 31,6 meseca po primarni kirurgiji (p = 0,012). Pri bolnicah z doseženo resekcijo R0 sta bila petletno in osemletno preživetje v skupini z neoadjuvantno kemoterapijo 20,6 % in 17,6 % , v skupini s primarno kirurgijo pa 62,5 % in 62,5 % (p < 0,0001).

Zaključki. Kljub večjemu deležu resekcije R0, ki smo jo dosegli v skupini, ki je začela zdravljenje z neoadjuvantno kemoterapijo, je bilo preživetje teh bolnic nižje od preživetja bolnic s primarnim kirurškim zdravljenjem. Neoadjuvantna kemoterapija tako ostaja prednostna za bolnice z napredovalim epitelijskim rakom jajčnika, ki niso primerne za primarno radikalno kirurško zdravljenje.



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DIAGNOSTIKA IN ZDRAVLJENJE ZGODNJEGA RAKA

Simpozij bo potekal v Ljubljani, dne 7. oktobra 2016
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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the third quarter of 2016

Dr. Josip Cholewa Foundation for cancer research and education continues with its planned activities in the third quarter of 2016. 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. In parallel, it also makes efforts to provide financial and other support for the organisation of congresses, symposia and other forms of meetings to spread the knowledge about prevention and treatment of cancer, and finally about rehabilitation for cancer patients. In Foundation's strategy the spread of knowledge should not be restricted only to the professionals that treat cancer patients, but also to the patients themselves and to the general public.

The Foundation continues to provide support for »Radiology and Oncology«, a quarterly scientific magazine with a long tradition and with a respectable impact factor that publishes research and review articles about all aspects of cancer. The magazine is edited and published in Slovenia.

The Foundation will continue with its activities in the future, especially since the problems associated with cancer affect more and more people in Slovenia and elsewhere. Ever more successful treatment results in longer survival in many patients with previously incurable cancer conditions, thus adding many new dimensions in life of cancer survivors and their families.

Andrej Plesničar, M.D., M.Sc.
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Bolniki morajo dobiti navodilo za uporabo in opozorilno kartico za bolnika. *Preprečevanje skeletnih dogodkov pri odraslih s kostnimi metastazami solidnih tumorjev:* Priporočeni odmerek je 120 mg enkrat na 4 tedne v enkratni subkutani injekciji v stegno, trebuh ali nadlaket. *Gigantocelularni kostni tumor:* Priporočeni odmerek zdravila XGEVA® je 120 mg enkrat na 4 tedne v enkratni subkutani injekciji v stegno, trebuh ali nadlaket ter dodaten odmerek 120 mg 8. in 15. dan zdravljenja v prvem mesecu terapije. Bolnike z gigantocelularnim kostnim tumorjem je treba v rednih presledkih ocenjevati, da bi ugotovili, ali jim zdravljenje še koristi. Pri bolnikih, ki imajo bolezen z zdravilom XGEVA® obvladano, niso ocenili učinka prekinitve ali prenehanja zdravljenja, toda omejeni podatki pri teh bolnikih ne kažejo povratnega učinka po prenehanju zdravljenja. *Bolniki z okvaro ledvic:* Prilagoditev odmerka ni potrebna. *Bolniki z okvaro jeter:* Varnost in učinkovitost denosumaba nista raziskani. *Starejši bolniki (stari ≥ 65 let):* Prilagoditev odmerka ni potrebna. *Pediatrični bolniki:* Varnost in učinkovitost zdravila XGEVA® pri pediatričnih bolnikih (starih < 18 let) še nista bili dokazani z izjemo skeletno dozorelih mladostnikov z gigantocelularnim kostnim tumorjem. Zdravilo XGEVA® ni priporočljivo za pediatrične bolnike (stare < 18 let) z izjemo skeletno dozorelih mladostnikov z gigantocelularnim kostnim tumorjem. Odmerjanje za zdravljenje skeletno dozorelih mladostnikov z gigantocelularnim kostnim tumorjem, ki ni operabilen, ali pri katerem bi kirurška odstranitev verjetno povzročila hudo obolevnost, je enako kot za odrasle. Za subkutano uporabo. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. Huda, nezdravljena hipokalciemija. Nezaceljene lezije po zobnih ali ustnih kirurških posegih. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Vsi bolniki morajo prejemati dodatek kalcija in vitamina D, razen če ima bolnik hiperkalciemijo. Obstoječo hipokalciemijo je treba odpraviti še pred začetkom zdravljenja z zdravilom XGEVA®. Hipokalciemija se lahko pojavi kadarkoli med zdravljenjem z zdravilom XGEVA®. Kontrolo koncentracije kalcija je treba izvesti (i) pred prvim odmerkom zdravila XGEVA®, (ii) v dveh tednih po prvem odmerku, (iii) če se pojavijo simptomi, sumljivi za hipokalciemijo. O dodatnih kontrolah koncentracije kalcija med zdravljenjem je treba razmisliti pri bolnikih, ki imajo dejavnike tveganja za hipokalciemijo, ali če so takšne kontrole sicer umestne glede na bolnikovo klinično stanje. Bolnikom je treba naročiti, naj zdravnika obvestijo o simptomih, ki kažejo na hipokalciemijo. Če se med prejemanjem zdravila XGEVA® pojavi hipokalciemija, je lahko potrebno dodatno dodajanje kalcija in dodatne kontrole. Bolniki s hudo okvaro ledvic (očistek kreatinina < 30 ml/min) ali bolniki na dializi imajo večje tveganje za pojav hipokalciemije. Tveganje za pojav hipokalciemije in spremljajočega zvišanja paratiroidnega hormona se povečuje s povečano stopnjo okvare ledvic. Pri takšnih bolnikih so redne kontrole koncentracije kalcija posebej pomembne. Pri bolnikih, ki imajo nezaceljene lezije mehkih tkiv v ustih, je treba začetek zdravljenja/nov ciklus zdravljenja odložiti. Pred zdravljenjem z zdravilom XGEVA® je priporočljivo opraviti zobozdravstveni pregled in preventivno zobozdravstveno oskrbo ter individualno oceno koristi in tveganja. Pri ocenjevanju bolnikovega tveganja za pojav osteonekroze čeljustnice je treba upoštevati naslednje dejavnike tveganja: moč zdravila, ki zavira resorpcijo kosti (tveganje je večje z zelo močnimi spojinami), pot uporabe (tveganje je večje v primeru parenteralne uporabe) in kumulativni odmerek zdravila, uporabljenega za zdravljenje resorpcije kosti, rak, sočasne bolezni (npr. anemijo, koagulopatije, okužbo), kajenje, sočasna zdravljenja: kortikosteroide, kemoterapijo, zaviralce angiogeneze, radioterapijo glave in vratu, slabo ustno higieno, periodontalno bolezen, slabo prilegajoče se zobne proteze, že obstoječo zobno bolezen, invazivne zobozdravstvene posege, npr. ekstrakcije zob. Vsem bolnikom je treba naročiti, da morajo med zdravljenjem z zdravilom XGEVA® vzdrževati dobro ustno higieno, redno opravljati zobozdravniške preglede in nemudoma obvestiti zdravnika, če se pojavi kakršen koli simptom v ustih, na primer majanje zob, bolečina, oteklina, rana, ki se ne celi, ali izcedek. Med zdravljenjem je izvajanje invazivnih zobozdravniških posegov dovoljeno le po skrbnem razmisleku in se jim je treba izogniti v bližini termina za odmerjanje zdravila XGEVA®. Načrt vodenja bolnikov, ki se jim pojavi osteonekroza čeljustnice, je treba oblikovati na podlagi tesnega sodelovanja med lečečim zdravnikom in zobozdravnikom ali ustnim kirurgom, ki ima izkušnje z osteonekrozo čeljustnice. Razmisliti je treba o začasnem prenehanju zdravljenja z zdravilom XGEVA®, dokler se to stanje ne razreši in se sovpleteni dejavniki tveganja ublažijo, če je mogoče. Atipični zlomi stegnenice se lahko pojavijo že ob majhni poškodbi ali celo brez poškodbe, in sicer v subtrohanternem in diafiznem predelu stegnenice. Za te dogodke so značilni specifični radiografski izvidi. O njih so poročali tudi pri bolnikih z določenimi sočasnimi bolezenskimi stanji (npr. s pomanjkanjem vitamina D, revmatoidnim artritisom, hipofosfatazijo) in med uporabo določenih zdravil (npr. bisfosfonatov, glukokortikoidov, zaviralcev protonske črpalke). Ti dogodki so se pojavili tudi brez antiresorpcijskega zdravljenja. Podobni zlomi, opisani v zvezi z bisfosfonati, so pogosto obojestranski, zato je treba pri bolnikih, ki se zdravijo z denosumabom in so imeli zlom srednjega dela stegnenice, opraviti tudi pregled druge stegnenice. Pri bolnikih, pri katerih obstaja sum na atipičen zlom stegnenice, je treba razmisliti o prenehanju uporabe zdravila XGEVA® ob vrednotenju bolnika glede na individualno oceno koristi in tveganja. Bolnikom je treba naročiti, da morajo med zdravljenjem z zdravilom XGEVA® zdravniku poročati o novi ali nenavadni bolečini v stegnu, kolku ali dimljah. Bolnike s takšnimi simptomi je treba preiskati glede nepopolnega zloma stegnenice. Zdravilo XGEVA® ni priporočljivo pri bolnikih, ki se jim skelet še razvija. Bolniki, zdravljeni z zdravilom XGEVA®, sočasno ne smejo prejemati drugih zdravil, ki vsebujejo denosumab (za indikacije pri osteoporozi), in bisfosfonatov. Malignost pri gigantocelularnem kostnem tumorju ali napredovanje do metastatske bolezni je redek dogodek in je znano tveganje pri bolnikih z gigantocelularnim kostnim tumorjem. Bolnike je treba kontrolirati glede radioloških znakov malignosti, nove radiolucentnosti ali osteolize. Razpoložljivi klinični podatki ne kažejo povečanega tveganja za malignost pri bolnikih z gigantocelularnim kostnim tumorjem, zdravljenih z zdravilom XGEVA®. Zdravilo XGEVA® vsebuje sorbitol. Bolniki z redko prirojeno motnjo intolerance za fruktozo ne smejo uporabljati zdravila XGEVA®. Zdravilo vsebuje manj kot 1 mmol natrija (23 mg) na 120 mg, kar pomeni, da je praktično »brez natrija. **INTERAKCIJE:** Študij medsebojnega delovanja niso izvedli. Sočasna kemoterapija in/ali hormonsko zdravljenje ali predhodna intravenska izpostavljenost bisfosfonatom niso klinično pomembno spremenili najmanjše koncentracije denosumaba v serumu in farmakodinamike denosumaba (N-telopeptid v urinu, prilagojen na kreatinin, uNTx/Cr). **POVZETEK NEŽELENIH UČINKOV:** Zelo pogosti (≥ 1/10): dispneja, driska, mišično-skeletna bolečina. Pogosti (≥ 1/100 do < 1/10): hipokalciemija, hipofosfatemija, ekstrakcija zoba, hiperhidroza, osteonekroza čeljustnice. Redki (≥ 1/10.000 do < 1/1.000): preobčutljivost na zdravilo, anafilaktična reakcija, atipični zlom stegnenice. **FARMACEVTSKI PODATKI:** Shranjujte v hladilniku (2 °C - 8 °C). Ne zamrzujte. **NAČIN IN REŽIM PREDPISOVANJA TER IZDAJE ZDRAVILA:** Predpisovanje in izdaja zdravila je le na recept s posebnim režimom – ZZ. **IMETNIK DOVOLJENJA ZA PROMET:** Amgen Europe B.V., Minervum 7061, NL-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:** April 2016. **DATUM PRIPRAVE INFORMACIJE:** Julij 2016. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu. **LITERATURA:** 1. Lipton A, et al. Eur J Cancer. 2012; 48: 3082-3092.

SLHR-SU-P-163-0716-033827

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, eksfoliativni 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

ANGELINI

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

Iclusig- oglas- 01/jun- 2016

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 izognejo 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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See the difference

Individualizirano zdravljenje za bolnike z metastatskim kolorektalnim rakom

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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
www.Erbitux-international.com

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ZYDELIG je indiciran v kombinaciji z rituksimabom za zdravljenje odraslih bolnikov s kronično limfocitno levkemijo (KLL):[2]

- ki so se pred tem vsaj enkrat zdravili, ali
- za nadaljevanje zdravljenja pri bolnikih z delecijo 17p ali mutacijo TP53, pri katerih kemoimunoterapija ni primerna in so se že začeli zdraviti z zdravilom Zydelig kot zdravilom prve izbire.

ZYDELIG je indiciran kot monoterapija za zdravljenje odraslih bolnikov s folikularnim limfomom (FL), neodzivnim na dve predhodni obliki zdravljenja.[2]

Referenci: 1. Herman SEM, Gordon AL, Wagner AJ in sod. Phosphatidylinositol 3-kinase-δ inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood. 2010;116:2078-2088. **2.** Povzetek glavnih značilnosti zdravila Zydelig, junij 2016▼

Skrajšani povzetek glavnih značilnosti zdravila Zydelig

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

IME ZDRAVILA: Zydelig 100 mg filmsko obložene tablete ali Zydelig 150 mg filmsko obložene tablet. **KAKOVOSTNA IN KOLIČINSKA SESTAVA:** Ena filmsko obložena tableta vsebuje ali 100 mg ali 150 mg idelalizība. Za celoten seznam pomožnih snovi glejte SmPC za zdravilo Zydelig 100 ali 150 mg filmsko obložene tablete. **TERAPEVTSKE INDIKACIJE:** Zdravilo Zydelig je indicirano v kombinaciji z rituksimabom za zdravljenje odraslih bolnikov s kronično limfocitno levkemijo (KLL): ki so se pred tem vsaj enkrat zdravili, ali za nadaljevanje zdravljenja pri bolnikih z delecijo 17p ali mutacijo *TP53*, pri katerih kemoimunoterapija ni primerna in so se že začeli zdraviti z zdravilom Zydelig kot zdravilom prve izbire (glejte poglavje 4.4). Zdravilo Zydelig je indicirano kot monoterapija za zdravljenje odraslih bolnikov s folikularnim limfomom (FL), neodzivnim na dve predhodni obliki zdravljenja. **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje z zdravilom Zydelig mora voditi zdravnik z izkušnjami pri uporabi zdravil proti raku. Odmerjanje: Priporočeni odmerek zdravila Zydelig je 150 mg peroralno dvakrat na dan. Z zdravljenjem je treba nadaljevati do napredovanja bolezni ali nesprejemljive toksičnosti. Če bolnik izpusti odmerek zdravila Zydelig v roku 6 ur, ga bo običajno vzame, mora bolnik vzeti izpuščeni odmerek takoj, ko je mogoče, nato pa nadaljevati z običajnim režimom odmerjanja. Če bolnik izpusti odmerek za več kot 6 ur, naj bolnik ne nadomesti izpuščenega odmerka in nadaljuje z jemanjem po obstoječem režimu odmerjanja. *Prilagajanje odmerka: Povečanje jetrnih transaminaz* – Zdravljenje z zdravilom Zydelig je treba začasno ustaviti, če se pojavi povečanje aminotransferaze 3. ali 4. stopnje (alanin aminotransferaza [ALT]/aspartat aminotransferaza [AST] > 5 x zgornja meja normalne vrednosti [ULN, *upper limit of normal*]). Ko se vrednosti vrnejo na 1. ali nižjo stopnjo (ALT/AST ≤ 3 x ULN), se lahko zdravljenje nadaljuje z odmerkom 100 mg dvakrat na dan. Če se ta dogodek ne pojavi ponovno, se lahko odmerek po presoji lečečega zdravnika ponovno poveča na 150 mg dvakrat na dan. Če se dogodek ponovi, je treba zdravljenje z zdravilom Zydelig začasno ustaviti, dokler se vrednosti ne vrnejo na 1. ali nižjo stopnjo, nato naj se po presoji zdravnika nadaljuje z jemanjem 100 mg dvakrat na dan. *Driska/kolitis* - Zdravljenje z zdravilom Zydelig je treba začasno ustaviti, če se pojavi driska/kolitis 3. ali 4. stopnje. Ko se driska/kolitis vrne na 1. ali nižjo stopnjo, se lahko zdravljenje nadaljuje z odmerkom 100 mg dvakrat na dan. Če se driska/kolitis ne ponovi, se lahko odmerek po presoji lečečega zdravnika ponovno poveča na 150 mg dvakrat na dan. *Pnevmonitis* - Zdravljenje z zdravilom Zydelig je treba pri sumu na pnevmonitis začasno ustaviti. Ko je pnevmonitis minil in če je ponovno zdravljenje ustrezno, se lahko razmisli o nadaljevanju zdravljenja z odmerkom 100 mg dvakrat na dan. *Izpuščaj* - Zdravljenje z zdravilom Zydelig je treba začasno ustaviti, če se pojavi izpuščaj 3. ali 4. stopnje. Ko se izpuščaj vrne na 1. ali nižjo stopnjo, se lahko zdravljenje nadaljuje z odmerkom 100 mgdvakrat na dan. Če se izpuščaj ne ponovi, se lahko odmerek po presoji lečečega zdravnika ponovno poveča na 150 mg dvakrat na dan. *Nevtropenija*: Zdravljenje z zdravilom Zydelig je treba začasno ustaviti pri bolnikih z absolutnim številom nevtrofilcev (ANC) pod 500 na mm³, ANC je treba spremljati najmanj enkrat tedensko, dokler ni ANC s 500 na mm³, nato se zdravljenje lahko nadaljuje s 100 mg dvakrat dnevno (glejte poglavje 4.4). *Posebne populacije bolnikov: Starejši bolniki* - Pri starejših bolnikih (starost ≥ 65 let) odmerka ni treba posebej prilagajati. *Ledvična okvara* - Pri bolnikih z blago, zmerno ali hudo ledvično okvaro odmerka ni treba prilagajati. *Jetrna okvara* - Pri uvedbi zdravljenja z zdravilom Zydelig pri bolnikih z blago ali zmerno jetrno okvaro odmerka ni treba prilagajati, vendar se priporoča intenzivnejše spremljanje neželenih učinkov. Ni dovolj podatkov za priporočila pri bolnikih s hudo jetrno okvaro. Zato se pri dajanju zdravila Zydelig tej populaciji priporoča previdnost in intenzivnejše spremljanje neželenih učinkov. *Pediatrična populacija* - Varnost in učinkovitost zdravila Zydelig pri otrocih, starih do 18 let, se nista bili dokazani. Podatkov ni na voljo. Način uporabe: Zdravilo Zydelig je za peroralno uporabo. Bolnikom je treba naročiti, da naj pogoltnejo celo tableto. Filmsko obložene tablete se ne sme žvečiti ali zdrobiti. Filmsko obložena tableta se lahko jemlje s hrano ali brez nje. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. **POSEBNA OPOZORILA IN POSEBNI PREVIDNOSTNI UKREPI:** Resne okužbe: Zdravljenja z zdravilom Zydelig se ne sme uvesti pri bolnikih s kakršnimi koli znaki prisotne sistemske bakterijske, glivične ali virusne okužbe. Pri idelalizibu so se pojavile resne in smrtne okužbe, vključno z oportunističnimi okužbami, kot sta pljučnica, ki jo povzroča *Pneumocystis jirovecii* (PJP), in okužba s citomegalovirusom (CMV). Vse čas zdravljenja z idelalizibom je treba vsem bolnikom dajati profilakso za PJP. Pri bolnikih je treba ves čas zdravljenja spremljati respiratorne znake in simptome. Bolnike je treba opozoriti, da morajo nemudoma poročati o novih respiratornih simptomih. Treba je izvajati redne klinične in laboratorijske preglede za okužbo s CMV. Pri znakih okužbe ali viremije je treba zdravljenje z zdravilom Zydelig prekiniti. Nevtropenija: Pri bolnikih, ki so se zdravili z idelalizibom, se je med zdravljenjem pojavila nevtropenija 3. ali 4. stopnje, vključno s febrilno nevtropenijo. Pri vseh bolnikih je treba prvih 6 mesecev zdravljenja z idelalizibom najmanj vsaka 2 tedna spremljati krvno sliko, pri bolnikih z ANC pod 1,000 na mm³ pa najmanj enkrat tedensko (glejte poglavje 4.2). Povečana transaminaze - V kliničnih študijah z idelalizibom so opazili povečanja ravni ALT in AST 3. in 4. stopnje (> 5 x ULN). Ti laboratorijski izsledki, ki so jih običajno opazili v prvih 12 tednih zdravljenja, so bili večinoma asimptomatski in reverzibilni po prekinitvi odmerjanja. Večina bolnikov je z zdravljenjem nadaljevala z nižjim odmerkom brez ponovnega pojava. Pri vseh bolnikih je treba prve 3 mesece zdravljenja vsaka 2 tedna spremljati vrednosti ALT, AST in skupnega bilirubina, nato pa kot je klinično indicirano. Pri pojavu povečanja vrednosti ALT in/ali AST 2. ali višje stopnje je treba bolnike spremljati vsak teden, dokler se vrednosti ne vrnejo na 1. ali nižjo stopnjo. Driska/kolitis - Primeri hudega z zdravilom povezanega kolitisa so se pojavili relativno pozno (mesece) po začetku zdravljenja, včasih s hitrim poslabšanjem, vendar so minili v nekaj tednih po prekinitvi odmerjanja in dodatnem simptomatskem zdravljenju (npr. zdravila proti vnetju, kot je enterični budezonid). Izkušnje z zdravljenjem bolnikov z vnetno boleznijo črevesja v anamnezi so zelo omejene. Pnevmonitis - V kliničnih študijah z idelalizibom so poročali o primerih pnevmonitisa. Bolnike z resnimi pljučnimi dogodki, ki se ne odzivajo na običajno protimikrobno zdravljenje, je treba oceniti glede pnevmonitisa zaradi zdravila. Pri sumu na pnevmonitiso treba idelalizib prekiniti in bolnika ustrezno zdraviti. Zdravljenje je treba pri zmernem ali hudem simptomatskem pnevmonitisu ukiniti. Stevens-Johnsonov sindrom in toksična epidermalna nekroliza: Poročali so o Stevens-Johnsonovem sindromu (SJS) in toksični epidermalni nekrolizi (TEN) s smrtnimi izidi, ko so idelalizib dajali sočasno z drugimi zdravili, povezanimi s tema sindromoma. Pri sumu na SJS ali TEN je treba idelalizib nemudoma prekiniti in bolnika ustrezno zdraviti. Induktorji CYP3A - Izpostavljenost idelalizibu se lahko zmanjša pri sočasni uporabi z induktorji CYP3A, kot so rifampicin, fenitoin, šentjanževka (*Hypericum perforatum*) ali karbamazepin. Ker lahko zmanjšanje koncentracij idelalizība v plazmi zmanjša učinkovitost, se je treba sočasnemu dajanju zdravila Zydelig z zmernimi ali močnimi induktorji CYP3A izogniti. Substrati CYP3A – Primarni presnovek idelalizība, GS 563117, je močan zaviralec CYP3A4. Zato lahko idelalizib medsebojno deluje z zdravili, ki jih CYP3A presnavlja, kar lahko povzroči povečane koncentracije drugih zdravil v serumu. Pri sočasni uporabi idelalizība z drugimi zdravili je treba upoštevati povzetek glavnih značilnosti zdravila za druga zdravila glede priporočil o sočasni uporabi z zaviralci CYP3A4. Sočasnemu zdravljenju z idelalizibom in substrati CYP3A, ki povzroča resne ini/ali življenjsko ogrožajoče neželene učinke (npr. alfuzosin, amiodaron, cisaprid, pimozid, kinidin, ergotamin, dihidroergotamin, kvetiapin, lovastatin, simvastatin, sildenafil, midazolam, triazolam), se je treba izogniti in uporabiti alternativna zdravila, ki so manj občutljiva na zaviranje CYP3A4, če je mogoče. Jetrna okvara - Pri bolnikih z okvarjenim delovanjem jeter se priporoča intenzivnejše spremljanje neželenih učinkov, saj je pričakovati, da bo izpostavljenost pri teh bolnikih povečana, zlesti pri bolnikih s hudo jetrno okvaro. Bolniki s hudo jetrno okvaro niso bili vključeni v klinične študije idelalizība. Pri dajanju zdravila Zydelig tej populaciji se priporoča previdnost. Kronični hepatitis - Idelalizība niso preučili pri bolnikih s kroničnim aktivnim hepatitisom, vključno z virusnim hepatitisom. Pri dajanju zdravila Zydelig bolnikom z aktivnim hepatitisom je potrebna previdnost. Ženske v rodni dobi - Ženske v rodni dobi morajo med jemanjem idelalizība in še 1 mesec po prenehanju zdravljenja uporabljati zelo učinkovito kontracepcijo. Ženske, ki uporabljajo hormonske kontraceptive, morajo dodati pregradno metodo kot drugo obliko kontracepcije, saj trenutno ni znano, ali lahko idelalizib zmanjša učinkovitost hormonskih kontraceptivov.Pomožne snovi: Zdravilo Zydelig vsebuje azobarvilo sončno rumeno FCF (E110), ki lahko povzroči alergijske reakcije.

MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ: Idelalizib se primarno presnavlja s pomočjo aldehidne oksidaze in v manjšem obsegu s pomočjo CYP3A in glukuronidacije (UGT1A4). Njegov primarni presnovek je GS 563117, ki ni farmakološko aktiven. Idelalizib in GS 563117 sta substrata P-gp in BCRP. Učinek drugih zdravil na farmakokinetiko idelalizība (*Induktorji CYP3A* – V kliničnih študiji medsebojnega delovanja zdravil so ugotovili, da je sočasno dajanje enega odmerka 150 mg idelalizība z rifampicinom (močan induktor CYP3A) povzročilo ~75 odstotno zmanjšanje $AUC_{0\infty}$ idelalizība. Sočasnemu dajanju zdravila Zydelig z zmernimi ali močnimi induktorji CYP3A, kot so rifampicin, fenitoin, šentjanževka ali karbamazepin, se je treba izogniti, saj lahko to zmanjša učinkovitost. *Zaviralci CYP3A/P-gp* – V kliničnih študiji medsebojnega delovanja zdravil so ugotovili, da je sočasno dajanje enega odmerka 400 mg idelalizība s ketokonazolom 400 mg enkrat na dan (močan zaviralec CYP3A, P-gp in BCRP) povzročilo 26 odstotno zmanjšanje C_{max} in 79 odstotno povečanje $AUC_{0\infty}$ idelalizība. Smatrajo, da prilagoditev zatetnega odmerka idelalizība pri sočasnem dajanju z zaviralci CYP3A/P-gp ni potrebna, vendar se priporoča intenzivnejše spremljanje neželenih učinkov. Učinek idelalizība na farmakokinetiko drugih zdravil: *Substrati CYP3A* - Primarni presnovek idelalizība, GS-563117, je močan zaviralec CYP3A. V kliničnih študiji medsebojnega delovanja zdravil so ugotovili, da je sočasno dajanje idelalizība z midazolamom (obtudilv substrat CYP3A) povzročilo ~140 odstotno povečanje C_{max} in ~440 odstotno povečanje AUC_{∞} midazolama, ker GS-563117 zavira CYP3A. Sočasno dajanje idelalizība s substrati CYP3A lahko poveča njihovo sistemsko izpostavljenost in poveča ali podaljša njihovo terapevtsko aktivnost in neželene učinke. *In vitro* je bilo zaviranje CYP3A4 ireverzibilno, zato se pričakuje, da bo vrnitev na normalno encimsko aktivnost trajala več dni po prenehanju dajanja idelalizība. Substrati CYP2C8: *in vitro* je idelalizib zaviral in induciral CYP2C8, vendar ni znano, ali to velja tudi za učinek *in vivo* na substrate CYP2C8. Previdnost se svetuje pri uporabi zdravila Zydelig skupaj z zdravili z ozkim terapevtskim indeksom, ki so substrati CYP2C8 (paklitaksel). Substrati inducibilnih encimov (npr. CYP2C9, CYP2C19, CYP2B6 in UGT: *In vitro* je bil idelalizib induktor številnih encimov, tveganja za zmanjšanje izpostavljenosti in s tem manjše učinkovitosti substratov inducibilnih encimov, kot so CYP2C9, CYP2C19, CYP2B6 in UGT, pa ni mogoče izključiti. Previdnost se svetuje pri uporabi zdravila Zydelig skupaj z zdravili z ozkim terapevtskim indeksom, ki so substrati teh encimov (varfarin, fenitoin, S mefenitoin). Substrati BCRP, OATP1B1, OATP1B3 in P-gp: Sočasno dajanje več odmerkov idelalizība 150 mg dvakrat na dan zdravim osebam je povzročilo primerljive izpostavljenosti za rosuvastatin (AUC 90 % IZ: 87, 121) in digoksin (AUC 90 % IZ: 98, 111), kar kaže, da naj idelalizib ne bi klinično pomembno zaviral BCRP, OATP1B1/1B3 ali sistemskega P-gp.Tveganja za zaviranje P-gp v prebavilih, ki bi lahko povzročile večjo izpostavljenost substratov, občutljivih za črevesni P-gp, kot je dabigatran eteksilat, ni mogoče izključiti. Pediatrična populacija: Študije medsebojnega delovanja so izvedli le pri odraslih. Za več informacij glejte SmPC za zdravilo Zydelig 100 ali 150 mg filmsko obložene tablete. **PLODNOST, NOSEČNOST IN DOJENJE:** Ženske v rodni dobi - Na podlagi izsledkov pri živalih lahko idelalizib škoduje plodu. Ženske med jemanjem zdravila Zydelig in še 1 mesec po prenehanju zdravljenja ne smejo zanositi. Zato morajo ženske v rodni dobi med jemanjem zdravila Zydelig in še 1 mesec po prenehanju zdravljenja uporabljati zelo učinkovito kontracepcijo. Trenutno ni znano, ali lahko idelalizib zmanjša učinkovitost hormonskih kontraceptivov, zato morajo ženske, ki uporabljajo hormonske kontraceptive, dodati pregradno metodo kot drugo obliko kontracepcije. Nosečnost - Podatkov o uporabi idelalizība pri nosečnicah ni oziroma so omejeni. Študije na živalih so pokazale vpliv na sposobnost razmnoževanja. Zdravila Zydelig ne uporabljajte pri nosečnicah in pri ženskah v rodni dobi, ki ne uporabljajo učinkovite kontracepcije. Dojenje - Ni znano, ali se idelalizib in njegovi presnovki izločajo v materino mleko. Tveganja za dojenega novorojenca/otroka ne moremo izključiti. Med zdravljenjem z zdravilom Zydelig je treba prenehati z dojenjem. Plodnost - Podatki o vplivu idelalizība na plodnost pri ljudeh niso na voljo. Študije na živalih kažejo na možne škodljive učinke idelalizība na plodnost in razvoj zarodka. **NEŽELENI UČINKI:** Neželeni učinki zdravila, o katerih so med zdravljenjem samo z idelalizibom ali v kombinaciji idelalizība z monoklonskimi protitelesi proti CD20 najpogosteje poročali, so: zelo pogosti (≥ 1/10) neželeni učinki: okužbe, nevtropenija, driska/kolitis, povečanje transaminaze. Izpuščaj, pireksija in povečanje triglyceridov so poročali kot zelo pogosti pri kateri koli stopnji, a pri ≥ 3. stopnji navedeni kot pogosti. Pogosti (≥ 1/100 do < 1/10) neželeni učinki: pnevmonitis. Redki (≥ 1/10,000 do < 1/1,000): Stevens-Johnsonov sindrom/toksična epidermalna nekroliza, 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 zastopnike, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si*

IMETNIK DOVOLJENJA ZA PROMET Z ZDRAVILOM: Gilead Sciences International Ltd., Cambridge, CB21 6GT, Velika Britanija
Način in režim predpisovanja ter izdaje zdravila: Rp/Spec - Predpisovanje in izdaja zdravila je le na recept zdravnika specialista usteznega področja medicine ali od njega pooblaščenega zdravnika.

Datum zadnje revizije besedila: 6/2016
Za vse morebitne nadaljnje informacije o tem zdravilu se lahko obrnete na predstavništvo imetnika dovoljenja za promet z zdravilom: Medicopharmacia, d.o.o., Verovškova 72, 1000 Ljubljana, Slovenija, telefon: 01 5886 860.

PRED UPORABO ZDRAVILA ZYDELIG 100 ALI 150 MG FILMSKO OBLOŽENE TABLETE NATANČNO PREBERITE CELOTEN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA.

Datum priprave gradiva: junij 2016

SAMO ZA STROKOVNO JAVNOST.

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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

macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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Vsak dan šteje

za bolnike z napredovalim karcinomom ledvičnih celic

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BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

SSUTENT 12,5 mg, 25 mg, 37,5 mg, 50 mg trde kapsule
Sestava in oblika zdravila: Ena kapsula vsebuje 12,5 mg, 25 mg, 37,5 mg ali 50 mg sunitiniba (v obliki sunitinibijevega malata). **Indikacije:** Zdravljenje neizrezljivega in/ali metastatskega malignega gastrointestinalnega stromalnega tumorja (GIST) pri odraslih, če zdravljenje z imatinibom zaradi odpornosti ali neprenašanja ni bilo uspešno. Zdravljenje napredovalega/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

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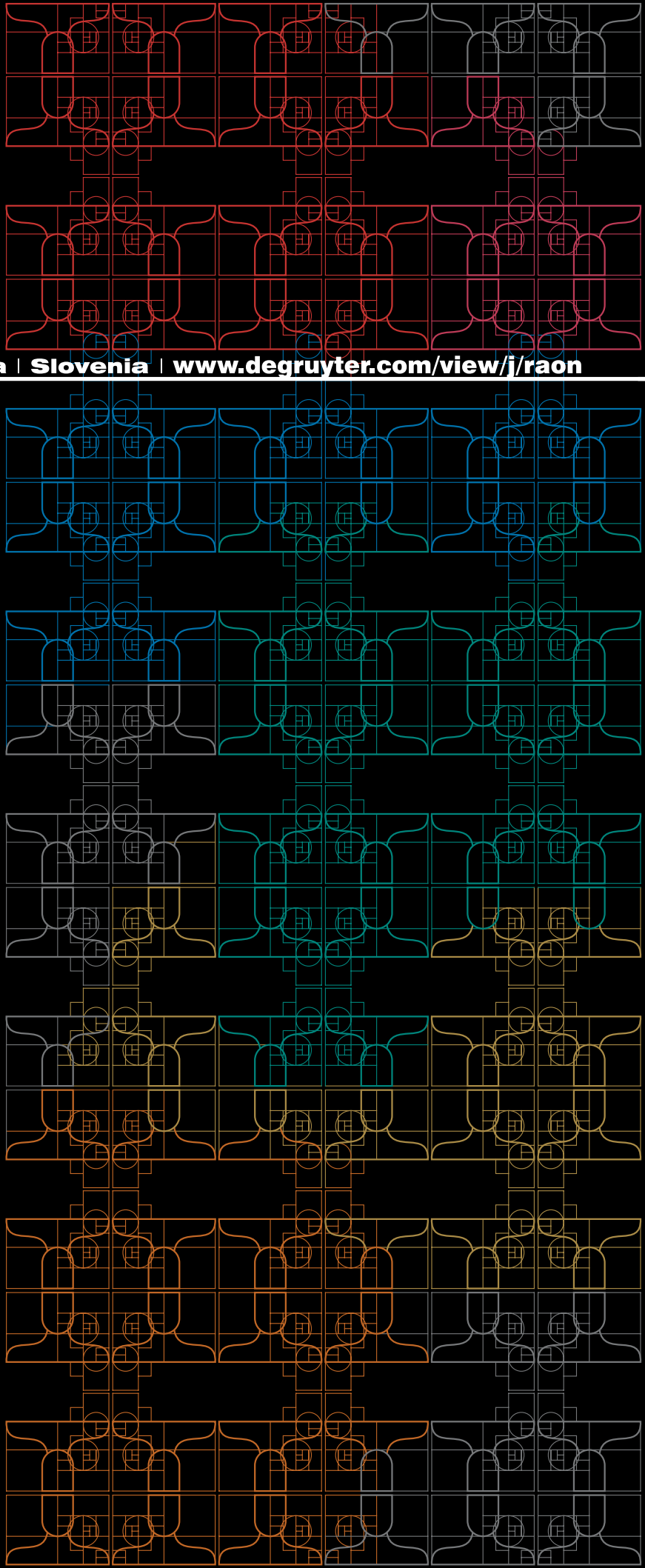

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