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NOVO

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

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

Akynzeo®
netupitant/palonosetron

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

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

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

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

review

Primary pulmonary choriocarcinoma

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Background. The aim of the study was to establish whether there are different clinical entities of primary pulmonary choriocarcinoma (PPC) that deserve different diagnostic approach and the most optimal treatment.

Patients and methods. A systematic review with PubMed search was conducted to identify studies that reported cases of PPC. The eligibility criteria were histological diagnosis of pulmonary choriocarcinoma and thorough examination of the reproductive organs to exclude potential primary choriocarcinoma in the gonads. Furthermore, to illustrate the review we additionally present a patient referred at our institution.

Results. 55 cases (17 men) were included in the review with a median age of 34 years. Women with the history of gestational event showed better survival outcome than women without the history of gestational event. Patients treated with combined modality treatment (surgery and chemotherapy) survived longer than the patients without combined modality treatment. Furthermore, multivariate analysis of prognostic factors showed that the combined modality treatment had independent prognostic significance. Size of the tumour showed significant prognostic influence in univariate and multivariate analysis.

Conclusions. PPC is an extreme rarity with variable clinical characteristics and outcome. It is important to capture and treat patients in the early stages of the disease. Women with the history of gestational event may show better survival, therefore genetic examination could help us to predict patient's prognosis. Surgery followed by adjuvant chemotherapy appears to represent the best treatment for PPC.

Key words: choriocarcinoma; pulmonary tumour; gestational event

Introduction

Choriocarcinoma is a germ cell tumour containing syncytiotrophoblastic cells and secreting human chorionic gonadotropin (hCG) hormone. Gestational choriocarcinoma originating in gonads frequently metastasizes to the lungs, but primary choriocarcinoma originating in the lung is a very rare entity. Primary extragenital choriocarcinoma most often arises in retroperitoneum, mediastinum and intracranially.

The mechanism of development of a primary pulmonary choriocarcinoma (PPC) is poorly understood. In the literature several theories have been postulated to explain the development of PPC: metastasis from primary gonadal choriocarcinoma that regressed spontaneously; origin from trophoblastic embolus related to gestational event after long period of latency; origin from retained primordial germ cells that migrate abnormally during embryogenesis; or a lung cancer that develops originally as non-trophoblastic neoplasm and later dedifferentiates.[1-5]

Lately a genetical examination helped to discriminate between gestational and nongestational origin of PPC. Maesta *et al*. proved with genetical examination that the PPC in both of their cases was of gestational origin.[6] On the other hand, Vegh *et al*. excluded gestational origin with genetical examination, despite the patient had the history of gestational event.[7]

PPC is a highly malignant intrapulmonary tumour with notoriously poor prognosis. Early diagnosis with optimal management is an impor-

tant goal, since patients captured in early stages of the disease have higher survival rate.[8] There is no standardized treatment for PPC. PPC grows rapidly and has high propensity to disseminate to other organs, such as bone, liver, brain, spleen and contralateral lung.[4] Due to undifferentiated nature of malignancy, PPC has poor response to radiation treatment.[9] The most appropriate regimen for chemotherapy seems to be BEP (bleomycin, methotrexate and cisplatin) or EMA-CO (etoposide, methotrexate, actinomycin D, cyclophospamide and vincristine).[1,6,7,8,10] Despite the absence of randomized trials to prove its superiority, the combination of EMA-CO has become the preferred regimen for initial treatment of high-risk gestational trophoblastic disease in most countries.[11]

The aim of the article was to establish whether there are different clinical entities of PPC that deserved different diagnostic and therapeutic approach. Furthermore, to illustrate the review we additionally present a patient referred to our institution. In order to elucidate clinical characteristics and to determine optimal way of management of this rare neoplasm we analysed our patient together with other 54 reported cases.

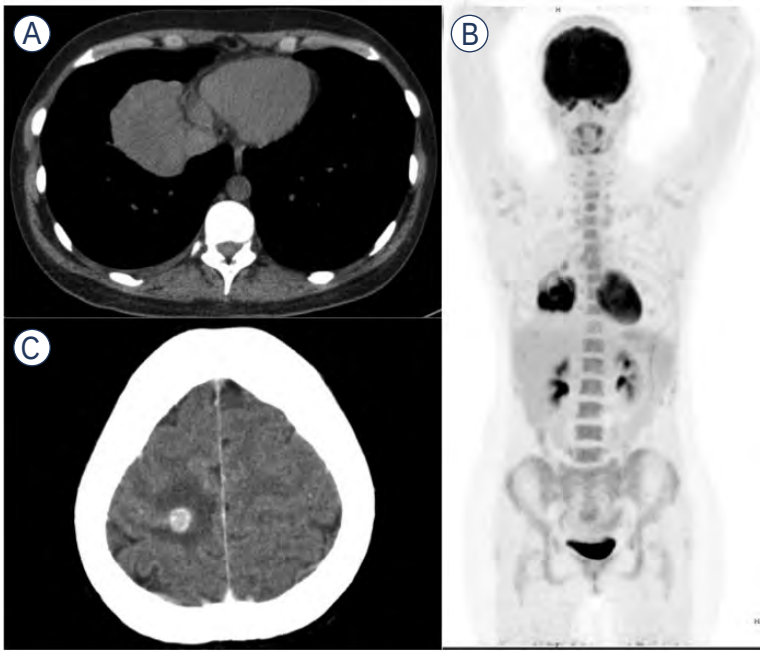

FIGURE 1. **(A)** A computed tomographic scan of the chest showing 6.4 x 5.4 x 5.9 cm pulmonary mass in right lower lobe. **(B)** FDG-PET study showing FDG accumulation in the pulmonary mass in the right lower lobe with no other abnormal accumulation found in whole body including pelvic cavity. **(C)** CT of the head showing 1 cm intracerebral mass with surrounding oedema.

Patients and methods

All published cases of PPC were collected with a PubMed search with the key word 'primary pulmonary choriocarcinoma' and the key word 'solitary pulmonary choriocarcinoma' that were published in English literature. The eligibility criteria were histological diagnosis of choriocarcinoma of the pulmonary tumour and thorough examination of reproductive organs to exclude potential primary choriocarcinoma in the gonads. Differences between patients groups according to gender were estimated using nonparametric Mann-Whitney test. Probabilities of survival were estimated using the Kaplan-Meier method and differences between patient groups were evaluated with the log-rank test. Prognostic factors were analysed using the Cox proportional hazard model. Reported values are two-sided. Statistical significance was set at $p < 0.05$.

The patient

A 35 year old woman complaining of severe right sided chest pain was admitted to our hospital. The patient had three normal term deliveries in the past 15 years and three spontaneous abortions in the past year. The chest radiograph showed a round opacity in the right lungs. A computed tomographic scan (CT) of the chest revealed the presence of 6.4 x 5.4 x 5.9 cm pulmonary mass in the right lower lobe (Figure 1A). Transthoracic needle biopsy was performed and diagnosis of poorly differentiated giant cell carcinoma (GCC) was made. To exclude additional masses FDG-PET study was performed (Figure 1B). FDG accumulation in the pulmonary mass was low, maximum standardized uptake value (SUVmax) was 2.7, and only in the periphery of the mass. No other abnormal accumulation was detected in whole body including pelvic cavity. During hospitalization the patient's condition progresively worsened with depleting levels of hematocrit, hemoglobin and platelets. Urgent right sided bilobectomy with pericard excision was performed. Histopathologic workup of excised tumour showed poorly differentiated carcinoma with trophoblast differentiation, most consistent with choriocarcinoma. Excised nodes showed no tumour infiltration.

Four weeks after surgery the patient was admitted to our hospital to start chemotherapeutical treatment. In these four weeks the patient had two episodes of epileptical seizures. CT and MRI of the head showed 1 cm intracerebral metastasis with surrounding edema (Figure 1C).

TABLE 1. Primary pulmonary choriocarcinoma: Summary of reported cases

No of patients	55
Gender, male/female	17/38
Median age all patients, years	34
Median age female, years	33
Median age male, years	60
Initial symptom	
Cough	22
Dyspnea	17
Hemoptysis	14
Chest pain	11
Asymptomatic	8
Location	
Right / Left lung	29/18
Right upper lobe	11
Right middle lobe	3
Right lower lobe	15
Left upper lobe	12
Left lower lobe	4
Bilateral	2
Tumor size, cm	
≤ 5	23
> 5, ≤ 10	13
> 10	8
Treatment	
S	10
C	8
RT	3
S + C	24
S + C + RT	3

C = chemotherapy; RT = radiotherapy; S = surgery

The patient began EMA-CO regimen chemotherapy with high dosage methotrexate. At the start of the chemotherapy plasma hCG levels were 169396 IU/L, on eighth day fell to 20883 IU/L and after three months (4 cycles of chemotherapy) the plasma hCG levels fell within normal range. Patient underwent stereotactic radiotherapy of cerebral metastasis with dose 1 x 25 Gy after the fourth cycle and afterwards received two additional cycles of EMA-CO regimen with standard dose of metotrexate. To completely rule out the origin of the tumour in reproductive tract the vaginal total hysterectomy with bilateral salpingoectomy was performed after 6 cycles of chemotherapy and histopathological examination was negative.

According to FIGO clinical and prognostic staging patient had stage IV disease with high-risk score.[12] Brain MRI was performed 7 months after stereotactic radiotherapy and showed 3 mm residual mass with no surrounding edema. Patient is on regular follow-up and is still on therapy with levetiracetam and dalteparin without evidence of relapse at 12 months following surgery.

The patient was treated according to the Helsinki Declaration. She gave a written informed consent before treatment to use her clinical data for research.

Results

Patients

We searched the literature with a PubMed search to establish characteristics of PPC using criteria described in Patients and methods. A review of the literature in English revealed 54 cases with previous report of primary choriocarcinoma originating in the lung.[1-10,13-15,17-44] The patient described in the present paper was analysed with other 54 reported cases. The profiles of the patients are summarized in Table 1; 17 men and 38 women were included with a median age of 34 years (range 0.3 to 77). At the time of discovery 47 patients (85%) produced symptoms, including persistent cough (40%), dyspnea (31%), hemoptysis (25%) and chest pain (20%). At presentation 8 tumours were asymptomatic. A few of the women have presented with hormonal problems such as amenorrhea or vaginal bleeding. In men, signs of feminisation such as gynecomastia have been observed but are uncommon.

Correlation between gender and clinicopathological factors

We analysed the relationship between gender and other clinicopathological features in patients with PPC (Table 2). Men were older than women (p = 0.000). Men had the history of smoking more often than women (p = 0.000). No statistically significant difference between genders was observed for location, size, presence of metastases, history of haemoptysis or treatment.

Survival

Only 47 cases (16 men and 31 women) reported treatment outcome. The median survival time was

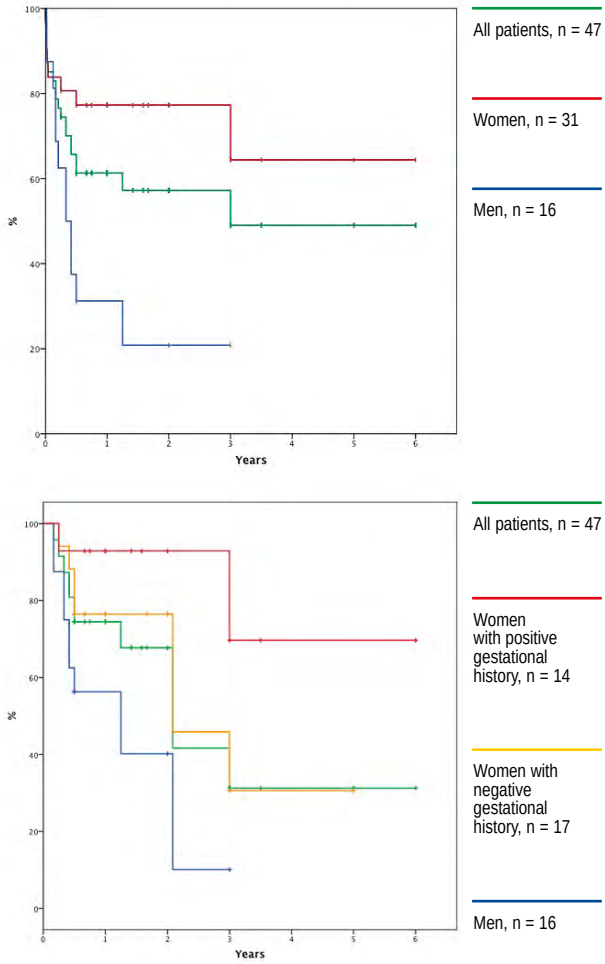

FIGURE 2. Kaplan-Meier survival curves. **(A)** Survival for all reported cases with curves showing survival for female patients was longer than for male patients (p = 0.004). **(B)** Survival of all reported cases with survival of men and curves showing survival of female patients with the history of gestational event was longer than for female patients without the history of gestational event (p = 0.040).

TABLE 2. Differences between female and male patients primary pulmonary choriocarcinoma

Characteristics		Female	Male	p-value
Age	< 40	30	4	
	≥ 40	9	13	0.000
Size, cm	< 5	17	7	
	≥ 5	12	8	0.162
Metastasis	Yes	15	10	
	No	24	7	0.161
Smoking	Yes	2	9	
	No	37	8	0.000
Hemoptysis	Yes	13	5	
	No	25	11	0.780
Treatment				
S	Yes	24	9	
	No	4	4	0.221
C	Yes	21	10	
	No	7	3	0.895
S+C	Yes	17	7	
	No	11	6	0.682

C = chemotherapy; S = surgey

8 months. The review of these 47 cases showed 1-, 2-, and 5- year survival rates of 61%, 57% and 49%, respectively. Important difference (p = 0.004) in survival time between genders was observed with women showing 1-, 2-, and 5- year survival rates of 77%, 77% and 64% while men showed 1-, 2-, and 5- year survival rates of 31%, 21% and 21%, respectively (Figure 2A).

In Table 3 univariate analysis is shown. Younger patients (< 40 years) survived longer than older patients (≥ 40 years), (p = 0.009). Patients with smaller tumours (< 5cm) survived longer than patients with larger tumours (≥ 5cm), (p = 0.000). Survival of patients without metastases at presentation was longer than for patients with metastases (p = 0.000). Patients without the history of smoking survived longer than patients with the history of smoking (p = 0.001). The women were further divided into two groups according to the history of gestational events (such as abortion, pregnancy or hydatiform mole) within the time period of less than 7 years prior the admission. Women with the history of gestational event (n = 14) had better survival outcome than women without the history of gestational event (n = 17), the difference was statistically significant (p = 0.040), (Figure 2B). Patients treated with combination of surgery plus chemotherapy survived longer than those treated with optimal supportive care or either chemotherapy or surgery alone (p = 0.001). Patients treated with chemotherapy only survived less than the patients treated with surgery only or combination of chemotherapy and surgery (p = 0.016). Patients treated with combination of surgery and chemotherapy survived longer than patients without combination of surgery and chemotherapy (p = 0.010). Treatment with surgery demonstrated no significant prognostic influence.

As shown in Table 4, multivariate analysis of prognostic factors using the Cox proportional hazards model showed that the treatment combining surgery with chemotherapy had independent prognostic significance (p = 0.007). Furthermore,

TABLE 3. Possible prognostic factors

Characteristic		n	MST (months)	1-year survival (%)	2-year survival (%)	p-value
Gender	Female	31	-	77.3	77.3	
	Male	16	4	31.3	20.8	0.004
Age	< 40	29	-	75.9	58.4	
	≥ 40	18	4	33.3	33.3	0.009
Size (cm)	< 5	20	-	80.0	80.0	
	≥ 5	16	3	25.0	0.0	0.000
Metastasis	Yes	21	4	42.9	0.95	
	No	26	-	84.6	84.6	0.000
Smoking	Yes	11	4	0.0	0.0	
	No	36	-	69.4	69.4	0.001
Female – gestational history	Positive	14	-	69.6	69.6	
	Negative	16	-	56.3	56.3	0.040
Treatment						
S	Yes	9	-	55.6	55.6	
	No	32	-	71.0	64.5	0.458
C	Yes	7	3	28.6	28.6	
	No	34	-	75.8	69.9	0.016
S+C	Yes	24	-	83.3	56.3	
	No	17	4	41.2	41.2	0.010

C = chemotherapy; MST = median survival time; S = surgery

the size of the lesion showed an independent prognostic significance (p = 0.008).

Discussion

In the present work we made a systematic review of the relevant literature. We found out that there are different clinical entities of PPC based on gender and reproductive history that show different survival. Furthermore, we showed it is important to capture and treat patients in the early stages of the disease. In addition we demonstrated that surgical treatment combined with chemotherapy is the best treatment regarding survival.

Gender difference

PPC occurs in women in younger ages than in men (33 *vs.* 60 median age), an observation already described by Umemori *et al*.[8] Therefore, it can be derived that PPC in women is more likely to occur in the reproductive period. Furthermore, there are more reported cases with women than men. Statistically significant difference in survival time between genders was observed with women showing better outcome. We further divided women in two groups due to the history of the gestational event within the time period of less than 7 years prior the admission. We chose the cut off of 7 years conservatively on the basis of three published cases with genetical examination.[6,7] In the paper by Maesta *et al.* they present two patients with gestational event within 4 years prior to PPC diagnosis and in both cases they proved it to be of gestational origin.[6] In the paper by Vegh *et al.* the patient had the last gestational event 7 years prior to admission and with fluorescence *in situ* hybridization they excluded gestational origin, no paternal chromosome was found in tissue examinated.[7] Based on this assumption we considered that women with the history of gestational event less than 7 years had better survival outcome than women without such history of gestational event.

Looking at aforementioned gender differencies, PPC seems to be of different etiology in men. Furthermore, we showed that men have a history of smoking more often than women. To support

TABLE 4. Multivariate analysis of prognostic factors, Cox proportional hazards model

S+C	HR	95% CI	p-value
Yes	0.147	0.036 – 0.598	0.007
No	1		
Smoking			
Yes	1.827	0.418–7.982	0.423
No	1		
Gender			
Male	1.266	0.248–6.466	0.776
Female	1		
Tumour size, cm			
≥ 5	6.622	1.622–27.031	0.008
< 5	1		
Age, years			
≥ 40	0.879	0.164–4.703	0.880
< 40	1		

C = chemotherapy; HR = hazard ratio; S = surgery

the theory of dedifferentiation four cases of PPC have been reported with co-existent pulmonary carcinoma.[5,10,13,14] All were male patients, in three cases PPC was sinchronous and found at autopsy, in one case the PPC was metachronous arising 6 years after diagnosis of squamous carcinoma. Problematically primary pulmonary carcinomas can produce hCG. Ikura *et al.* reported more intense expression of hCG in PPC than in hCG producing GCC.[14] Although this finding seemed to reflect the level of serum hCG the cut off point seemed to be ambigous.[15] Because of the rarity and clinicopathological similarity of PPC and hCG producing GCC the criteria for distinguishing them are unclear and the diagnosis is very difficult.[14]

Survival

The survival data should be handled cautiously because of the possibility that mostly patients with good survival were reported in the literature and the rest were neglected. Nevertheless PPC is notorious for having a poor prognosis, however, the present review shows 1-, 2-, and 5- year survival rates of 61%, 57% and 49%, which is reasonably good. Furthermore, if only female patients are observed the survival rates are even higher. Patients having smaller size tumour and being without metastases at diagnosis have higher survival rate, thus early diagnosis with optimal management is important. The outcome is still worrying but in comparison with the review article from the year 2004 by Umemori *et al.* where they reported 1-, 2-, and 5-year survival rates of 41%, 34%, 34% the outcome results are more promising. We noticed that the early reports of PPC had poorer outcome than the ones nowadays and that chemotherapeutical treatment was rarely used in the cases prior to year 1994 but if chemotherapy was used it was single modality therapy. The evolvement of chemotherapeutical treatment and better supportive therapy could influence the better outcomes seen in the present review, but we could not attribute the difference in survival rates to the specific factor due to the rarity and nonexistence of therapeutic guidelines.

Treatment

In order of finding out any relationship between treatment and survival the univariate and multivariate analysis of prognostic factors was carried out. Univariate analysis showed that treatment with chemotherapy only is not good choice of treatment however significant prognostic influence for combined treatment with surgery and chemotherapy was found. Furthermore, multivariate analysis of prognostic factors revealed that combined treatment with surgery and chemotherapy had independent prognostic significance. These findings are in concordance with already known facts that PPC is very aggressive disease, not just locally, but also by early spread to other organs.[4] Therefore, combined modality treatment (surgery and chemotherapy) represents best possible way to improve survival. Of course patients have to be fit enough for such aggressive treatment. No reports of extended survival were found in patients who underwent complete resection without chemotherapy.

Conclusions

PPC is an extreme rarity with variable clinical characteristics and outcome. It is important to capture and treat patients in the early stages of the disease. Women with the history of gestational event may show better survival, therefore genetic examination could help us to predict patient's prognosis. Surgery followed by adjuvant chemotherapy appears to represent the best treatment for PPC.

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research article

PET/CT imaging in polymyalgia rheumatica: praepubic ^{18}F-FDG uptake correlates with pectineus and adductor longus muscles enthesitis and with tenosynovitis

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Background. The role of ^{18}F-fluorodeoxyglucose positron emission computed tomography (^{18}F-FDG PET/CT) is increasing in the diagnosis of polymyalgia rheumatica (PMR), one of the most common inflammatory rheumatic diseases. In addition to other locations, increased ^{18}F-FDG accumulation has been detected in the praepubic region in some patients. However, a deeper description and pathophysiological explanation of this increased praepubic accumulation has been lacking. The aim of the presented study is to confirm a decrease in praepubic ^{18}F-FDG accumulation in response to therapy and to describe potential correlations to other ^{18}F-FDG PET/CT scan characteristics during the course of disease. As a secondary objective, we describe the pathological aspects of the observed praepubic ^{18}F-FDG uptake.

Patients and methods. A retrospective review of patients with newly suspected PMR undergoing baseline and follow up ^{18}F-FDG PET/CT between February 2010 and March 2016 is given. Those with a visually detected presence of praepubic ^{18}F-FDG accumulation were further analysed. The uptake was assessed visually and also semi-quantitatively in the defined region of interest by calculation of target-to-liver ratios. Other regions typical for PMR were systematically described as well (shoulders, hips, sternoclavicular joints, ischiogluteal bursae, spinous interspaces).

Results. Twenty-three out of 89 screened patients (26%) presented with initial praepubic ^{18}F-FDG PET/CT positivity, 15 of whom also underwent follow up ^{18}F-FDG PET/CT examination. Five out of 15 patients presented with increased ^{18}F-FDG accumulation in large arteries as a sign of giant cell arteritis. During follow up examination, decrease in ^{18}F-FDG accumulation caused by therapeutic intervention was observed in all evaluated locations in all analysed patients and no new positivity was indicated, including periarticular, extraarticular tissues or target large vessels. Praepubical accumulation of ^{18}F-FDG was diminished in all patients (15/15, 100%) after treatment with steroids.

Conclusions. Increased praepubic ^{18}F-FDG uptake in patients with PMR is relatively common and this region should be systematically evaluated during differential diagnosis of rheumatic and malignant disease. Praepubic inflammation is probably related to enthesitis and tenosynovitis at the origin of pectineus and adductor longus muscles ventrally from the pubis.

Key words: positron emission tomography, polymyalgia rheumatica, enthesitis, tenosynovitis, fluorodeoxyglucose

Introduction

Polymyalgia rheumatica (PMR) is the most common inflammatory rheumatic disease in patients older than 50 years, with a higher incidence in women. PMR shares many pathogenetic and epidemiological features with giant cell arteritis (GCA)[1], and 50% of patients with GCA also develop PMR symptomatology.[2] The typical symptoms of PMR are bilateral aching of the shoulder girdle, neck and hip girdle, and morning stiffness lasting for 30 minutes or more. These symptoms are probably related to inflammation of the subacromial, subdeltoid and trochanteric bursae, and the glenohumeral or hip joints.[3,4] The diagnosis of PMR is made primarily on clinical grounds and is bolstered by laboratory evidence of an acute phase reaction. There is no single diagnostic test for PMR, but several diagnostic and classification criteria have been suggested by some groups.[5-9] Each set of criteria has advantages and disadvantages. A PMR-associated ultrasound lesion(s) in the shoulders and/or hips is currently acknowledged as diagnostic criteria for the scoring algorithm in the differential diagnosis of PMR.[10] However, additional imaging methods for assessing rheumatic diseases are warranted.

Prolonged febrile illness with concomittant non-specific symptoms can be also a sign of PMR as well as GCA. Thus, patients may be referred during differential diagnostics of inflammatory or malignant disease to whole body positron emission tomography (PET) or a combination of PET with computed tomography (PET/CT) using ^{18}F-fluorodeoxyglucose (^{18}F-FDG).[11-14] Both GCA and PMR have their own characteristic ^{18}F-FDG PET/CT features, which may occur in a non-mutually exclusive manner.

PMR presents with increased ^{18}F-FDG accumulation in periarticular areas of shoulder and hip girdle and of sternoclavicular joints.[15-17] The other location with metabolically active inflammation in PMR patients are the extraarticular synovial structures (bursae). It appears that ^{18}F-FDG detection of extraarticular bursitis using PET/CT might be routinely achievable for PMR patients, with reasonable sensitivity (85.7%) and specificity (88.2%), by considering high ^{18}F-FDG uptake in at least 2 of 3 locations (ischial tuberosity, greater trochanter, spinous processes).[18]

Contrasting at least in part with PMR, GCA imaging typically reveals increased avidity in the wall of whole aorta, including its branches (subclavian and brachial arteries, brachiocephalic trunk, common iliac arteries and femoral arteries).[19-21] Importantly, from the clinical point of view, ^{18}F-FDG uptake in pertinent locations decreases in response to effective treatment in both PMR and GCA. Thus, ^{18}F-FDG PET/CT evaluation may be used for monitoring therapy and for follow up.[15,19,22,23]

In our previous study, increased ^{18}F-FDG accumulation was detected in the praepubic region in some patients.[17] However, a deeper description and pathophysiological explanation of this increased praepubic accumulation is needed. The aim of the present study is to confirm a decrease of praepubic ^{18}F-FDG accumulation in response to treatment and to describe potential correlations to other ^{18}F-FDG PET/CT scan characteristics and to the course of disease, and thereby to support the validation of the praepubic avidity within the general ^{18}F-FDG PET/CT features of PMR. As a secondary objective, we provide a description of the pathological aspect of the observed praepubic ^{18}F-FDG uptake.

Patients and methods

Patients with suspected new or relapsed PMR who underwent ^{18}F-FDG PET/CT examination at Masaryk Memorial Cancer Institute in Brno between February 2010 and March 2016 were retrospectively screened for visually detected presence of praepubic ^{18}F-FDG accumulation. Patients for whom follow up ^{18}F-FDG PET/CT was performed during corticosteroid therapy within the clinical remission phase were eligible for further analysis. All patient had to meet ACR 2012 diagnostic criteria for PMR. Patients who were previously treated for known PMR (with at least 15 months from the termination of therapy) were eligible as well. All patients initially provided their signed informed consent with participation on further retrospective studies and this analysis was approved by the institutional review board. Treatment consisted by prednisone, methylprednisolone or methotrexate in various dosages as listed in Table 1.

^{18}F-FDG PET/CT examination was performed utilizing the hybrid scanner Biograph 64 HR+ Siemens Erlangen, Germany. CT scan was performed in low dose CT (25 mAs eff/120 kV) as well as diagnostic or contrast enhanced CT scan (160 mAs eff/12 kV) (intravenous Iomeron 400, BRACCO, Milan, Italy). All patients had standard preparation prior to examination, including restriction of physical activity for 12 h, fasting for at least 6 h, capillary glycemia below 10 mmol/L (180 mg/dL) prior to ^{18}F-FDG administration and peroral hydration with 500-

TABLE 1. Imaging and laboratory results of all patients. Reported baseline treatment was initiated several days after baseline PET/CT

No	Disease status	Date of baseline (B) and follow up (FU) examination	Time to control exam (months)	B/FU praepubic to liver uptake ratio	B/FU FW (mm/h)	B/FU CRP (mg/dL)	B/FU treatment (mg/day)	B/ FU region of positivity of other PMR areas (target-to-liver ratio)	B/FU vascular positivity
1	R	11-18-2014	4.0	1.134	44	23	P 15	S 2.23, H 1.25, Scl 1.13, Isch 1.45	no positivity
		3-20-2015		0.576	16	1.4	P 5	H 1.11	no positivity
2	N	2-23-2010	48.7	1.234	120	49	P 15	S 1.65, H 1.24, Scl 1.24, Isch 1.17, L 1.14	no positivity
		3-15-2014		0.812	26	16.9	M 8	no positivity	no positivity
3	N	9-1-2014	3.2	2.148	120	137	P 60	S 1.87, Scl 1.45, H 2.31, Isch 1.65, L 1.12, Th 1.14, C 1.24	no positivity
		12-8-2014		0.992	35	16.7	P 10	S 1.36	no positivity
4	N	7-24-2010	6.1	1.891	120	56.8	P 40	S 1.78, Scl 1.54, H 1, Isch 2.21, L 1.08	no positivity
		1-25-2011		0.345	30	5.7	P 2.5	no positivity	no positivity
5	N	3-13-2014	12.0	1.772	60	28.5	P 20	S 2.02, H 1.47, Scl 1.24, Isch 2.02, L 1.23	V3/6
		3-13-2015		0.987	7	1	P 0	no positivity	V1/6
6	N	10-14-2013	16.9	1.298	54	45	P 20	S1.78, H1.88, Scl 1.95, L 1.25, Isch 1.16	no positivity
		3-13-2015		0.537	6	5.3	P 2.5	no positivity	no positivity
7	R	1-3-2014	13.1	1.302	77	78.3	M 16	S 1.78, H 1.87, Scl 1.35, Isch 1.65, L 1.12	V4/6
		2-6-2015		0.403	5	1.9	M 2	no positivity	no positivity
8	N	6-9-2012	33.1	1.835	50	76	P 30	S 2.21, H 2.16, Scl 1.8, Isch 1.78, L 1.2	V4/6
		3-13-2015		0.54	14	3.1	P 0	H 1.23	V2/6
9	N	6-3-2015	3.1	1.502	60	41.5	P 15	S 2.36, H 2.24, Scl 2.03, Isch 2.04, C 1.11	no positivity
		9-4-2015		0.76	16	13.4	P 10	no positivity	no positivity
10	N	1-8-2014	21.7	1.209	80	78.3	P 60	S 1.84, H1.78, Scl 1.69, Isch 1.57, L 1.23	no positivity
		10-30-2015		0.395	10	2.5	P 7.5	no positivity	no positivity
11	R	3-4-2015	8.1	1.546	62	16.5	M 48 + MTX 10/ week	S 2.66, H 2.74, Scl 2.0, Isch 1.72, C 1.47, L 1.48	V5/6
		11-5-2015		0.811	30	3.3	M 4 + MTX 10/ week	H 1.14, C 1.08, S 1.11, Scl 1.07	V3/6
12	N	2-6-2015	8.3	1.789	120	98.7	M 32	S 2.27, H 2.06, Scl 2.16, Isch 3.03, L 1.45	no positivity
		10-16-2015		0.97	40	7.5	M 8	Scl 1.14, Isch 1.21	no positivity
13	N	1-23-2015	14	1.123	70	67.5	P 15	S 1.78, H 1.87, Scl 1.69, Isch 1.57, L 1.24	no positivity
		3-24-2016		0.441	6	1.4	P 5	no positivity	no positivity
14	N	9-16-2015	5.8	1.282	74	52	P 20	S 2.44 , H 3.04, Scl 2.07, L 1.21	no positivity
		3-9-2016		0.41	24	3.2	P 7.5	no positivity	no positivity
15	N	10-26-2015	3.2	1.892	80	118.8	P 30	S 2.93, H 2.97, Isch 3.22, C 1.44, L 1.24, Th 1.17, Scl 1.79	V4/6
		2-1-2016		0.678	5	1.3	P20	no positivity	no positivity

B/C = baseline and control; C, L, Th = cervical, lumbal, thoracic interspinous space; FW = Fåhræus-Westergren test; H = hip; M = methylprednisolone; Isch = ischiogluteal bursae; MTX = methotrexate; N = newly diagnosed; P = prednisone; R = relapse; S = shoulder; Scl = sternoclavicular joint; V = vascular uptake with number indicating presence in regions from 6 measured

1,000 mL of plain water. ^{18}F-FDG (UJV Rez, Czech Republic) was administered in a dose range of 327-434 (median 366 MBq) in the baseline study and in a dose range of 301-400 (median 362 MBq) in the follow up examination. After an *in vivo* accumulation time of 55 to 75 minutes, whole body scanning from the proximal third of thighs to the skull base was performed in baseline as well as follow up study. All images were iteratively reconstructed and corrected for attenuation.

^{18}F-FDG uptake was assessed visually and also semi-quantitatively in the defined region of interest (ROI) with calculation of target-to-liver ratios. Liver ^{18}F-FDG uptake (SUVmax) was used as a reference base (measured within the ROI located in the centrum of the right liver lobe). Praepubic ^{18}F-FDG accumulation was semiquantitatively assessed as SUVmax within elliptic ROI located through both preapubic regions while keeping in the safe distance from the bladder based on the investigator discretion. Praepubic-to-liver ratio (SUVmax) was subsequently calculated.

Other regions typical for PMR were systematically described with measurement of SUVmax: shoulders, hips and sternoclavicular joints and extraarticular sites – in ischial tuberosity and between spinous processes where ischiogluteal and interspinous bursae are often presented, respectively. A target-to-liver ratio higher than 1.0 was considered positive in all mentioned regions. For paired organs, the higher value (from the right or left site) was used for target-to-liver calculation. ^{18}F-FDG uptake (SUVmax) within the typical sites for GCA was also systematically evaluated, namely in the walls of following arteries: thoracic aorta, abdominal aorta, brachial and subclavian arteries, iliac and femoral arteries. An artery wall-to-liver ratio higher than 1.0 was also considered positive. The number of positive vascular regions out of six evaluated regions is reported. Again, for paired structures, the higher value (from the right or left site) was used for target-to-liver calculation. The same scan evaluation was performed for follow up scans.

Results

Patients characteristic

From 89 screened patients, 23 (26%) presented with initial praepubic ^{18}F-FDG PET/CT positivity. 15 patients, 10 women and 5 men with a median age of 70 years, range 53 to 78, underwent also follow up ^{18}F-FDG PET/CT and met inclusion criteria for the presented analysis (Table 2). Twelve out

TABLE 2. Patients' baseline characteristics

Characteristics	Numbers (%) n= 15 (100%)
Sex, n (%)	
females	10 (66.7 %)
males	5 (33.3 %)
Age at the disease onset	
median (min-max)	70 years (53–78)
Time to follow up exam median (min-max)	8 months (3-49)

TABLE 3. Changes in analysed laboratory parameters between baseline and follow up examination

	Baseline median (min-max)	Follow up median (min-max)	p-value median (min-max)
CRP	57 mg/l (17–137)	3 mg/l (1–17)	0.001
FW	74 mm/hod (44–120)	16 mm/hod (5–40)	0.001

TABLE 4. Nuclear medicine data at baseline and follow up examination

	Baseline median (min-max)	Follow up median (min-max)	p-value median (min-max)
^{18}F-FDG	366 MBq (327–434)	362 MBq (301–400)	0.271
Praepubic / liver ^{18}F-FDG uptake	1.50 (1.12–2.15)	0.58 (0.35–0.99)	0.001

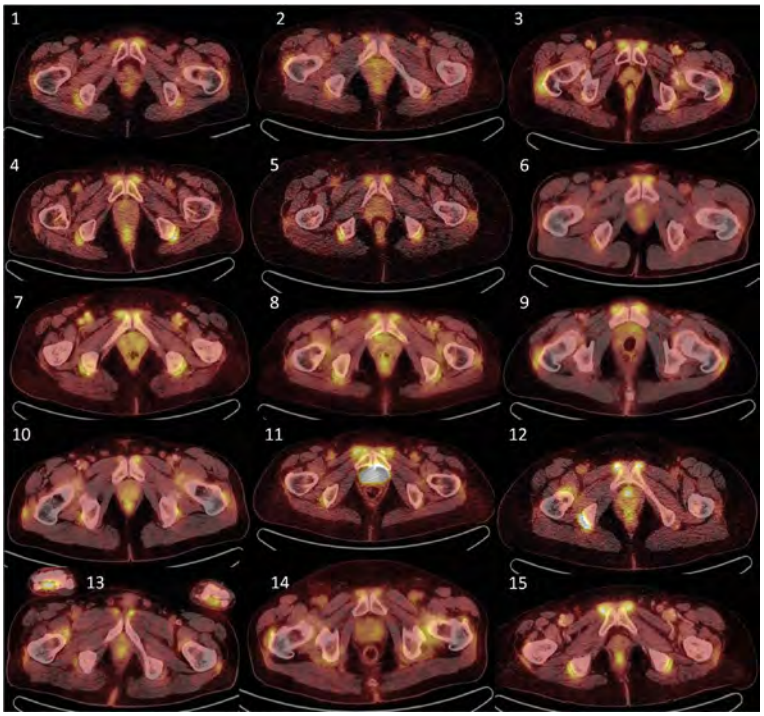

FIGURE 1. Initial ^{18}FDG-PET/CT examination of all 15 examined patients, showing transversal planes through maximal praepubic uptake. Visually detectable accumulation can be observed in all patients.

of 15 patients were classified as newly diagnosed; prior to the initiation of corticosteroid therapy, the other three patients had been previously treated for known PMR (with at least 15 months from the termination of therapy). Laboratory values for FW and CRP were obtained for all patients, ± 14 days around ^{18}F-FDG PET/CT (Table 3). Patient number 5 had accompanied giant cell arteritis confirmed histologically by temporal artery biopsy.

Baseline ^{18}F-FDG PET/CT characteristics

^{18}F-FDG PET/CT characteristic including ^{18}F-FDG dosage are summarized in Table 4. Increased ^{18}F-FDG accumulation (positivity) was observed at baseline in all patients in the praepubic region, with praepubic-to-liver ratios higher than 1.0. This accumulation was obvious by visual evaluation only, as presented in Figure 1, and this finding was always accompanied by additional positivity in ^{18}F-FDG PET/CT scans (Table 1). Other sites with increased accumulation were as follow: around the shoulder girdle in all 15 patients, around the hip girdle in 15 patients, and around sternoclavicular joints in 14 out of 15 patients. ^{18}F-FDG PET/CT positivity was observed also in extraarticular synovial structures, in ischiogluteal bursa in 14 patients and between spinous processes of the vertebrae in 14 patients, most commonly within lumbal region in 13 patients.

Five out of 15 patients presented with increased ^{18}F-FDG accumulation in large arteries as a sign of large vessel vasculitis of GCA. In those with large vessel vasculitis, ^{18}F-FDG PET/CT positivity was at least in 3 of 6 evaluated vascular regions. Increased values of FW and CRP were detected in all patients during initial examination and were correlated to newly diagnosed PMR or to its relapse (Table 3).

Follow up ^{18}F-FDG PET/CT characteristics

Given the retrospective nature of this study, it was not possible to keep a strict interval between baseline and follow up ^{18}F-FDG PET/CT scans. Follow up examinations were timed by clinical purposes rather than by experimental needs. Follow up ^{18}F-FDG PET/CT (median time 8 months, range 3-49) revealed continuing positivity around the shoulder girdle in only 2/15 patients, around hip joints in 3/15, around the sternoclavicular joint in 2/15, in extraarticular synovial structures in ischiogluteal bursae in 1/15 and in interspinous regions of cervical vertebrae in 1/15 patients. In three out of 15 patients, positivity in continuous large vessels was observed, maximally in 3 vessel regions out of 6 measured. In all evaluated locations in all analysed patients, a decrease in ^{18}F-FDG accumulation (target-to-liver ratio) was observed, and no new positivity was indicated, including periarticular, extraarticular tissues or target large vessels. Praepubic accumulation of ^{18}F-FDG was diminished in all patients after treatment with steroids. Praepubic-to-liver ratio was lower than 1.0 post therapy and this decrease in accumulation was clear by visual assessment (Figure 2).

During follow up ^{18}F-FDG PET/CT examination, all patients were undergoing steroid treatment and they reported subjective improvement in health condition, and disease remission was confirmed by attending rheumatologist. The laboratory signs of inflammation, FW, and CRP were decreased as well (Table 3).

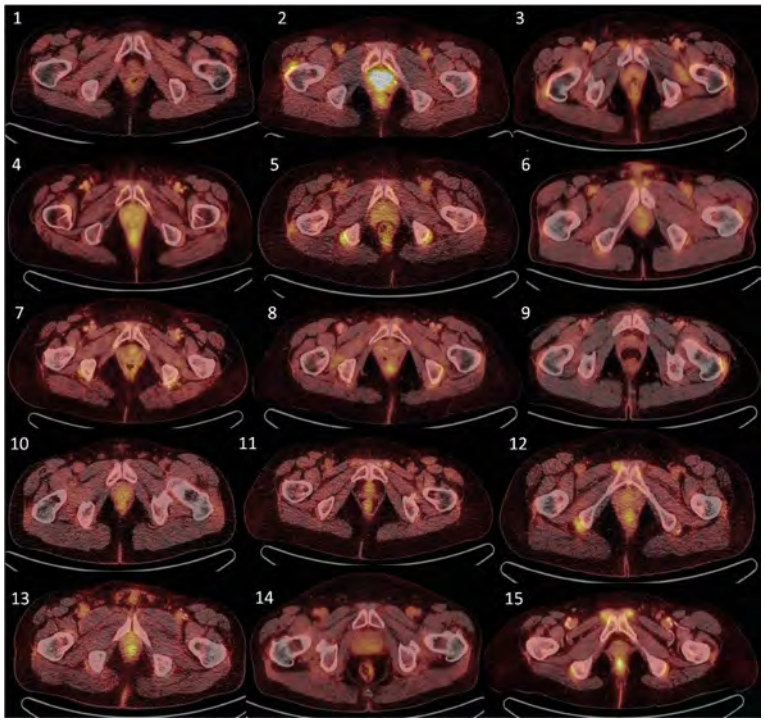

FIGURE 2. Control ^{18}FDG-PET/CT examination showing corresponding transversal slices as in Figure 1. Compared with Figure 1, decrease or complete diminishment of ^{18}FDG accumulation is observable in all patients.

Discussion

In the presented retrospective analysis of ^{18}F-FDG PET/CT findings in patients with proven PMR, 25.8% patients presented with praepubic ^{18}F-FDG uptake with fifteen patients from our cohort being able to undergo follow up ^{18}F-FDG PET/CT

examination. We were not able to perform follow up ^{18}F-FDG PET/CT scan in a prospectively defined phase of treatment; instead, the aim was to obtain ^{18}F-FDG PET/CT study during disease remission. During this follow up ^{18}F-FDG PET/CT exam, praepubic accumulation of ^{18}F-FDG was diminished in all 15 patients after treatment with steroids.

In our previous study, praepubic positivity was described only in 5/67 (8%) PMR patients representing only a relatively rare sign of the disease.[17] However, 32/67 (48%) patients were examined on an older PET scanner with the rest undergoing examination in a newer hybrid PET/CT scanner with better image resolution. Lower detectability of targed lesions may have occured on ^{18}F-FDG PET scanner alone and also underevaluation of accumulation quantification may result in false negativity (praepubic-to-liver ratio < 1.0). Even if visual accumulation was clearly positive, no patient from our previous cohort met the criterion for positivity. Given the above findings, it is reasonable to speculate that the percentage of positive preapubic accumulation in patients with active PMR would be higher when examined on a hybrid ^{18}F-FDG PET/CT rather than a PET camera. The overall number of patients with initial praepubic ^{18}F-FDG PET/CT positivity was 23/89 (25.8%). Thus, praepubic accumulation should not be considered a constant and frequent sign of ^{18}F-FDG PET/CT results as it is in periarticular ^{18}F-FDG accumulation in shoulders and hips. In cohorts from previous studies, accumulation in the shoulder groin girdle was observed in 33/35 (94%), in 12/14 (86%) and in 58/67 (87%) and in the hips in 31/35 (89%), in 12/14 (86%) and in 47/67 (70%) PMR patients, respectively.[15-17]

In a recently published report of 15 patients with PMR and of 9 patients with Elderly-Onset Rheumatoid Arthritis (EORA), praepubic ^{18}F-FDG uptake was recommended as one of the signs to enable differentiation between these two conditions.[24] The incidence of praepubic ^{18}F-FDG uptake (pectineus enthesitis) was relatively high with 9 patients from 15 evaluated which may be related to sample size bias. Patients with PMR developed significantly higher uptake compared to those with EORA.[24] Patients in the present study represent a retrospectively described cohort of patients with praepubic ^{18}F-FDG PET/CT positivity that includes post-treatment follow up examination. In light of these findings, it seems possible that increased praepubic accumulation may have been present in previously published case reports including those where axial slices indicating ischiogluteal bursitis were published.[18,25-27] Sondag *et al.* observed ^{18}F-FDG uptake in 11/50 (22%) patients; however, the cohort was a mixture of those with and without treatment with 22/50 (44%) with administration of steroids.[28] Mackie *et al.* published the first MRI findings of inflammation in the front of the symphysis in patients with PMR in 2015.[29] A similar observation was observed in our cohort (patient number 11); data not shown.

It is difficult to exactly determine the pathological background of increased ^{18}F-FDG uptake in the praepubic region in patients with PMR. The described accumulation seems to be relatively bordered and with spheric shape in some patients, while with blurry margins in others. MRI may be helpful in further evaluation of this region. Based on the MRI finding presented by Mackie *et al.* and Wakura *et al.*, we assume another type of extraarticular inflammation is responsible for the observed PET findings.[24,30] Namely, it appears to represent features of enthesitis and tenosynovitis of pectineus and adductor longus muscles. There is probably no bursa in the locations under the tendons of these muscles, which is also supported by findings on MRI, where no fluid collection with evidence of thickened wall suggestive for bursitis was observed. However, some reports have recently suggested a combination of PMR and tenosynovitis in other locations, namely in the long head of biceps brachii [31] or in extensor tenosynovitis of the hand[32] or in the vicinity of the enthesis of the rectus femoris.[24]

Our observations confirm that ^{18}F-FDG PET/CT examination seems to be an advantageous one-step diagnostic modality for detecting different variants of PMR, for assessing extent and severity, and for excluding occult malignancy. The follow up exam may be useful in monitoring disease activity including the praepubic location. It is conceivable that the praepubic region will become part of targeted examination for other imaging strategies as is US or MRI and that clinical significance and correlations will be further discovered.

Conclusions

Increased praepubic tracer accumulation is becoming an integral part of ^{18}F-FDG PET/CT evaluation of polymyalgia rheumatica, and is probably a correlate of enthesitis and tenosynovitis at the origin of pectineus and adductor longus muscles ventrally from the pubis. The findings described here were consistently presented in combination with other periarticular accumulations (shoulder and hip gir-

dle and surrounded bursae, sternoclavicular joint) and other extraarticular bursae at some distance from joints such as the ischiogluteal bursae and interspinous bursae in spine. Some patients presented with signs of large vessels vasculitis of GCA. In accordance with other ^{18}F-FDG PET/CT positive locations, praepubic accumulation was decreased in relation to PMR treatment. Our findings support the clinical value of ^{18}F-FDG PET/CT examination of patients with suspected or proved PMR.

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research article

Echocardiography and cardiac biomarkers in patients with non-small cell lung cancer treated with platinum-based chemotherapy

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Background. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer and remains an important cause of cancer death worldwide. Platinum-based chemotherapy (PBC) for NSCLC can modify outcome while the risk of cardiotoxicity remains poorly researched. We aimed to evaluate the incidence and severity of cardiac injury during PBC in patients with NSCLC and to identify patients at risk.

Methods. This was a single-centre, prospective, observational study of patients with early and advanced stage NSCLC referred for PBC. In addition to standard care, patients were examined and evaluated for cardiotoxicity before the first dose (visit 1), at the last dose (visit 2) and 6 months after the last dose of PBC (visit 3). Cardiotoxicity (at visit 2 and 3) was defined as increase in the ultrasensitive troponin T, N-terminal pro-B type natriuretic peptide or decrease in left ventricular ejection fraction (LVEF).

Results. Overall, 41 patients (mean age 61 ± 9; 54% men; 68% advanced lung cancer) were included. The median number of PBC cycles was 4. During the study period, there were no incidents of heart failure, and 3 deaths caused by tumour progression were recorded. The mean values of biomarkers and LVEF did not change significantly ($p > 0.20$). However, 10 (25%) had cardiotoxicity which was independently associated with a history of ischemic heart disease ($p = 0.026$).

Conclusions. In NSCLC, cardiac assessment and lifestyle modifications may be pursued in patients with a history of cardiac disease and in patients with longer life expectancy.

Key words: cardiotoxicity; platinum-based chemotherapy; lung cancer; cardiac biomarkers

Introduction

Currently, lung cancer is not only the leading cause of cancer-related deaths, but is also the most frequently diagnosed cancer worldwide.[1] Non-small cell lung cancer (NSCLC) accounts for 80–85% of all lung cancers. According to recent guidelines, platinum-based doublet chemotherapy is used as first line therapy in NSCLC. In addition to platinum agents (cisplatin or carboplatin), gemcitabine, pemetrexed, vinorelbine or a taxane is used.[2-4]

Cancer chemotherapeutic drugs, particularly anthracyclines, monoclonal antibodies and targeted drugs such as multiple tyrosine kinase inhibitors can induce cardiac damage.[5] In general, chemotherapy can cause cardiac damage by specifically targeting receptors on cardiomyocytes or by inducing oxidative stress, as shown in trastuzumab or anthracycline-induced cardiotoxicity, respectively.[6,7] Screening of patients before treatment and monitoring cardiac function during therapy has traditionally relied on left ventricular ejection

fraction (LVEF).[6] However, because assessment of LVEF lacks the sensitivity needed for detecting early subclinical changes, newer and more sensitive metrics of cardiotoxicity are needed. The most commonly used biomarkers to detect subclinical cardiac toxicity are cardiac troponins and atrial or B-type natriuretic peptides. Cardiac troponins reflect cardiomyocyte injury or death, whereas natriuretic peptides are released due to cardiac stretch and strain during congestive heart failure.[8,9]

In addition to the risk of cardiotoxicity related to specific anti-cancer therapy, cancer patients are at increased risk of cardiovascular (CV) disease due to older age and shared lifestyle risk factors (*e.g.*, smoking and obesity). With the introduction of new treatment modalities, the life expectancy of many cancer patients has improved substantially, and treatment-related comorbidities have become an important issue for cancer survivors. Cancer patients with longer life expectancy (childhood cancer, breast, prostate and colorectal carcinoma) are already more likely to die from CV disease than any other cause.[10] In addition, for NSCLC, new developments such as targeted therapies are showing potential for longer survival, and detection of early cardiac injury is becoming crucial for these patients.

Data regarding cardiotoxicity following platinum-based chemotherapy are scarce. There have been several studies reporting CV events and induced cardiotoxicity in animals following platinum-based chemotherapy.[11-17] Studies evaluating the long term effects of platinum-based chemotherapy in testicular cancer have shown a higher rate of CV events such as decreased LVEF and higher mortality due to CV disease in patients exposed to platinum-based regimens.[18-20] In addition, a large retrospective study showed that patients with NSCLC treated with chemotherapy and radiotherapy were more likely to develop heart failure as well as cardiac dysfunction.[21] To our knowledge, there has been only one prospective study of patients with NSCLC that evaluated cisplatin and anthracycline-based cardiotoxicity, and that study found no significant decrease in LVEF after treatment with cisplatin.[22]

Even though some degree of cardiac damage has been shown following treatment with platinum agents, it is still unclear whether platinum-based chemotherapy induces cardiotoxicity in patients with NSCLC. Hence, our aims were to evaluate cardiac function with echocardiography and biomarkers in patients with NSCLC treated with platinum-based chemotherapy and to identify those at risk for cardiotoxicity.

Patients and methods

Study design and patients

This was a single-centre, prospective, observational study. All patients with NSCLC who were referred for platinum-based chemotherapy in our clinic between June 2012 and September 2013 were screened to include those with early and advanced stage NSCLC treated with platinum-based doublets as adjuvant or metastatic therapy. Patients with adjuvant radiotherapy or concomitant chemoradiotherapy were not included in the study. Platinum doublets with vinorelbine, gemcitabine or pemetrexed were given according to guidelines and established clinical practice.[2,3] Cisplatin 80 mg/m^2 and carboplatin AUC 5 were used and modified according to the standard practice toxicity guidelines. Patients with a history, symptoms or signs of heart disease, or renal disease received carboplatin, while others received cisplatin. In addition to standard examinations, patients had evaluation of cardiac function by echocardiography and biomarkers before the first (visit 1), last cycle of chemotherapy (visit 2) and 6 months after completion of platinum-based chemotherapy (visit 3). Between visit 2 and visit 3, none of the patients was re-challenged with platinum-based chemotherapy. Physicians involved in the care of patients participating in the study were not aware of the results of biomarkers and echocardiography assessment, unless findings were pathological and would lead to a change in a patient's management. This study was conducted according to the Declaration of Helsinki and was approved by National Ethics Committee (Approval Nr. 37/07/09). All patients have received verbal and written information before written informed consent was obtained.

Biomarkers and echocardiography

Levels of biomarkers - ultrasensitive troponin T (usTnT) and N-terminal pro-B type natriuretic peptide (NT-proBNP) - were assessed at each visit. Whole blood samples were drawn by venepuncture and analysed using Roche's Elecsys® diagnostic analysis (Basel, Switzerland). A standard echocardiographic examination was performed by two experienced physicians (ML and RM). Cardiac chambers, valve abnormalities, systolic and diastolic function were evaluated. Left ventricular (LV) end-diastolic and end-systolic volumes were calculated using the Teicholtz or Simpson's biplane method.[23] The Teicholtz formula and Simpson's biplane method were used in patients with a struc-

turally normal LV and a structurally abnormal LV or diminished systolic function, respectively. Systolic function was evaluated by calculating left ventricular ejection fraction (LVEF) from LV end-diastolic and end-systolic volumes. Diastolic function was assessed using measurements of flow velocity at the opening of the mitral valve, septal tissue Doppler of the mitral annulus and volume of the left atrium. The ratio between E wave velocity at the mitral valve (E) and septal velocity of the mitral annulus (e′) was calculated (E/e′).[24]

Cut points and endpoint definitions

Elevated usTnT and NT-proBNP levels at visits 2 and 3 were defined as a > 30% increase from the visit 1 value. LVEF reduction was defined as a ≥ 10% or ≥ 5% decrease of LVEF to value ≤ 55% in asymptomatic and symptomatic patients, respectively, as suggested in trastuzumab trials.[25] Diastolic dysfunction was defined as E/e′ ≥ 15 or E/e′ < 15 and ≥ 8 with an enlarged left atrium, as recommended by the European Society of Cardiology.[24] Cardiotoxicity for an individual patient was defined as having increased usTnT or NT-proBNP or decreased LVEF at either visit 2 or 3.

Primary endpoint was cardiotoxicity, secondary endpoints were symptomatic heart failure, increased usTnT, NT-proBNP, LVEF reduction and diastolic dysfunction at visit 2 and 3, as described above.

Statistical analysis

Statistical analysis was performed using R 3.0.2 (R Development Core Team, Vienna, Austria) and the R package CVST (Krueger and Braun). Results are presented as the mean ± standard deviation for numeric variables and number (%) for nominal variables, except for the chemotherapy cycles, which are presented as median value and range. The paired t-test and Cochran's Q test were used to assess the changes at visit 2 and 3 from those at visit 1 for numeric variables and for binary variables, respectively. In univariate logistic regression models, we included cardiotoxicity as the dependent variable and sex, age, history of ischemic heart disease, history of arterial hypertension, metastatic lung cancer, number of chemotherapy cycles and usTnT, NT-proBNP and LVEF values at visit 1 as independent variables. In multivariate logistic regression models, we included cardiotoxicity as the dependent variable and history of ischemic heart disease, sex, age and LVEF at visit 1 as independent variables. For these analyses, results are presented as odds ratio (OR) with corresponding 95% confidence intervals (CI). A p value of less than 0.05 was considered to be statistically significant.

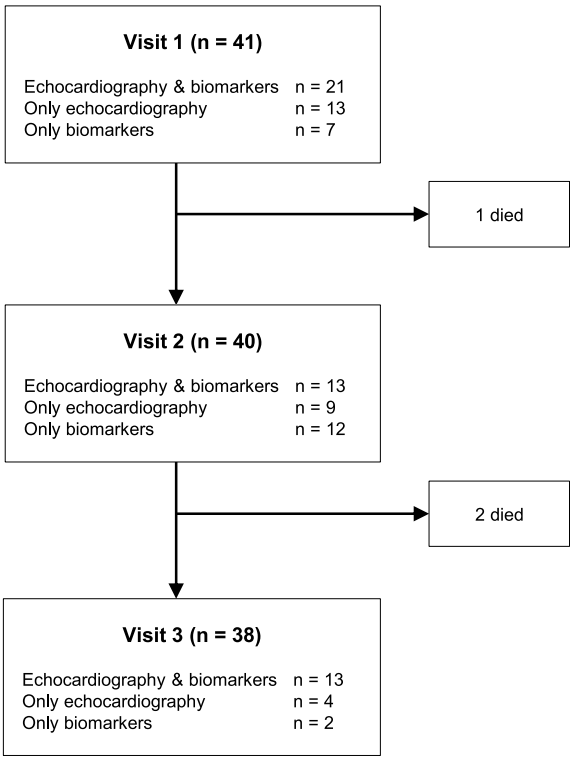

FIGURE 1. Study flowchart.

Results

Overall, 41 patients were included in the study between June 2012 and September 2013. 3 of the patients died during the study, all of them due to the cancer progression. At visit 2 and visit 3, 6 and 19 patients had an incomplete cardiac evaluation, respectively, mostly due to capacity related difficulties and patients' refusal at visit 2, and disease progression at visit 3. In all 3 visits, biomarkers were assessed in 7 patients, echocardiography was performed in 12 patients and both were assessed in 4 patients (Figure 1). All 40 patients who survived until visit 2 had information regarding primary endpoint.

Of 41 patients, 13 patients had early stage NSCLC and received adjuvant chemotherapy and 28 patients were given chemotherapy for metastatic disease (Table 2). In addition to cisplatin or carboplatin, all patients received one additional

TABLE 1. Patients' characteristics

Age – yr	61 ± 9
Male sex – no. (%)	22 (54)
Arterial hypertension – no. (%)	17 (41)
Ischemic heart disease – no. (%)	4 (10)
Cardiovascular disease – no. (%)	5 (12)
Glomerular filtration rate - ml/min/1.73 m^2	102 ± 28
Advanced disease – no. (%)	28 (68)

Where not mentioned, results are shown as mean values ± standard deviation. For calculating glomerular filtration rate, a modification of diet in renal disease study (MDRD) method was used.

TABLE 2. Chemotherapy treatment characteristics

Chemotherapy cycles – median (range)	4 (2-6)
Chemotherapy – no. (%)	
Cisplatin	28 (68)
Carboplatin	8 (20)
Both, in sequence	5 (12)
and	
Vinorelbine	11 (27)
Pemetrexed	22 (54)
Gemcitabine	8 (19)

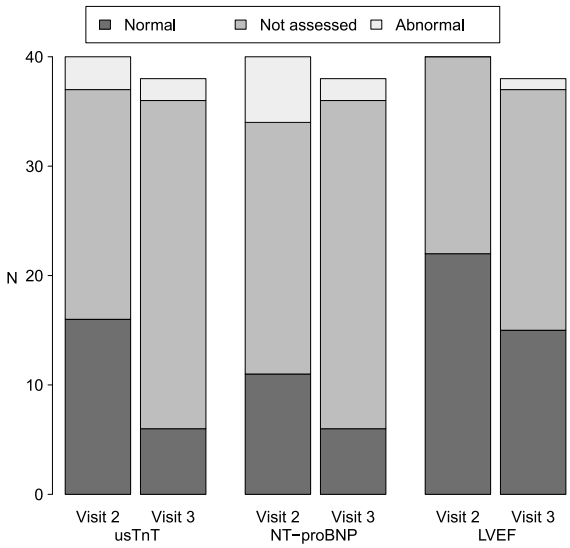

FIGURE 2. Number of patients at visit 2 or 3 with elevated usTnT, NT-proBNP and decreased LVEF form visit 1 values. Normal and abnormal was defined as > 30% increase in usTnT and NT-proBNP, and > 10% in symptomatic patients or > 5% in asymptomatic patients decrease to value lower than 50% in LVEF from visit 1 value.

LVEF = left ventricular ejection fraction; n = terminal pro BNP; NT = proBNP; usTnT = ultrasensitive troponin T

chemotherapeutic drug (vinorelbine, pemetrexed or gemcitabine).

At visit 1 or during the course of chemotherapy, none of the patients had overt symptoms or signs suggestive of heart failure or any other heart disease. CV disease was present in 5 patients (4 ischaemic heart disease and 1 peripheral artery disease). Of those with ischaemic heart disease 1 had older myocardial infarction while 3 had chronic ischaemic heart disease or angina pectoris. Differences in biomarkers and echocardiographic variables at each visit are shown in Table 3 and Figure 2. The values of usTnT and NT-proBNP gradually insignificantly decreased during the study, while the mean values of LVEF were not different between visits. ANOVA analysis included only patients with all measurements for each variable, therefore 7 and 12 patients were included in the analysis of biomarkers and LVEF, respectively. An increase of usTnT and NT-proBNP was observed in 4 and 7 patients at visit 2 or 3, respectively. None of the patients had decreased LVEF at visit 2, while 1 patient had decreased LVEF at visit 3. Patient who had decreased LVEF did not have increased values of NT-proBNP or usTnT, and one patient who had elevated NT-proBNP to more than 1800 pg/ml did not have decreased LVEF. While in some of the patients, the value of biomarkers increased, others experienced a decrease in values (Figure 3).

Primary endpoint was observed in 25% (10) out of 40 patients who survived until visit 2: 9 had increased values of biomarkers, and 1 had decreased LVEF. Univariate logistic regression models are shown in Table 4 where associations of different variables with corresponding ORs and 95% CI are given for each variable. Of all the variables tested in univariate logistic regression, history of ischemic heart disease was statistically significantly linked to cardiotoxicity (OR 12.86, 95% CI 1.16–142.88; p = 0.04). After adjusting for age, sex and LVEF value at visit 1, history of ischemic heart disease remained predictive of cardiotoxicity (OR 35.22, 95% CI 7.08–175.32, p = 0.023) (Table 5).

Discussion

Our results show that the treatment with platinum-based therapy did not induce clinically evident cardiotoxicity such as overt heart failure or other cardiac disease. Although no statistically significant or clinically important increase in the mean value for usTnT, NT-proBNP or decrease in LVEF or diastolic dysfunction was observed among the entire

TABLE 3. Biomarkers and echocardiography data

Variable	Visit 1	Visit 2	N	p-value	Visit 3	N	p-value
usTnT – pg/ml	11.4 ± 5.4	10.9 ± 5.1	19	0.93	8.3 ±3.2	8	1
>30% increase in usTnT from Visit 1 – no. (%)		3 (15.8)	19	1	2 (25)	8	1
NT-proBNP – pg/ml	266 ± 250	258 ± 378	17	0.55	226 ± 430	8	0.60
>30% increase in NT-proBNP from Visit 1 – no. (%)		6 (35.3)	17	1	2 (25)	8	1
LVEF - %	68 ± 8	67 ± 8	22	0.66	69 ± 9	16	0.63
>30% increase in LVEF from Visit 1 – no. (%)		0 (0)	22	1	1 (6)	16	1
Diastolic dysfunction – no. (%)	9 (27.3)	6 (27.3)	21	1	4 (23.5)	17	1

Results are shown as mean values ± standard deviation and differences were analysed with Paired t-test for numeric and Cochran's Q test for binomial variables.
LVEF = left ventricular ejection fraction; N = of patients with complete evaluation at visit 1 and 2 or visit 1 and 3, NT= proBNP, n-terminal pro BNP; usTnT = ultrasensitive troponin T

group, 25% of patients met the definition of cardiotoxicity. This phenomenon was largely driven by the known history of CV disease, even when adjusted to clinically relevant confounders.

Although platinum-based chemotherapy, as reported in animal models and case reports, could be associated with cardiotoxicity in patients with NSCLC, data from observational studies are lacking.[15-17] To our best knowledge, only one prospective study that investigated platinum-based cardiotoxicity in patients with NSCLC has been published.[22] The authors of that study measured LVEF with a multiple gated acquisition (MUGA) scan before and 3 months after treatment with cisplatin-gemcitabine (31 patients) or epirubicin-gemcitabine (38 patients). Study results showed a small and marginally significant decrease in the LVEF of 2% in the cisplatin-gemcitabine arm and a larger significant decrease in the LVEF of 7% in the epirubicin-gemcitabine arm. A larger decrease in the anthracycline arm was expected, which confirms that anthracycline chemotherapy is more cardiotoxic than platinum-based chemotherapy. Our results show a similar decrease in LVEF after platinum-based chemotherapy measured with echocardiography, which is less accurate for LVEF assessment but generally available, less expensive and can identify several other cardiac diseases compared to MUGA scanning. Due to similar results in both studies, echocardiography is possibly as efficient in detecting decreased LVEF as a sign of platinum-based cardiotoxicity as MUGA and represents a valid method that is more feasible for use in clinical practice.

Cisplatin and other platinum-based chemotherapy regimens are also used in other types of cancers, such as ovarian, testicular, prostatic and bladder. Some studies have measured cardiotoxicity of cisplatin in patients treated for testicular cancer.[18-20,26] Based on longer survival rates observed in patients with testicular cancer, there were several cohort studies that assessed long-term cardiotoxicity of more than 6 years.[19,20,26] The main findings of these studies were increased diastolic dysfunction and slightly decreased LVEF. Although the median age of patients diagnosed and treated for testicular cancer was approximately 35 years, several patients treated with cisplatin chemotherapy had cardiac events in the following years. Although the mean LVEF did not change after treatment, patients treated with cisplatin had a significantly higher risk for cardiovascular mortality and an increased rate of CV events compared to the general population. Similarly, in our study with a much shorter follow-up period, several patients experienced an important increase in biomarkers or

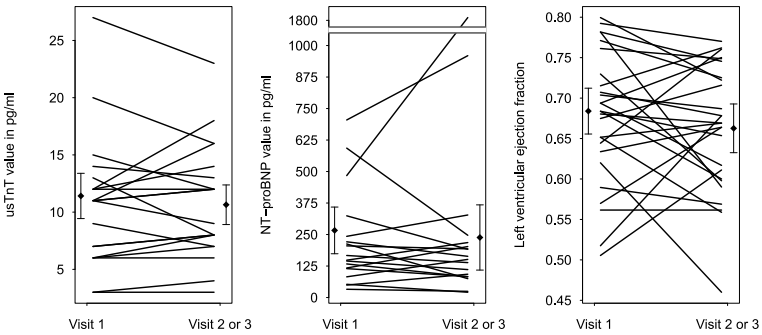

FIGURE 3. The mean with 95% CI and for each individual patient values of usTnT, NT-proBNP and LVEF at visit 1 and visit 2 or 3, whichever value was higher for usTnT and NT-proBNP and lower for LVEF.

CI = confidence interval; LVEF = left ventricular ejection fraction; n = terminal pro BNP; NT = proBNP; usTnT = ultrasensitive troponin T

TABLE 4. P values for univariate logistic regression models for cardiotoxicity

Independent variable	OR (95% CI)	p-value
Age in years	1.044 (0.955–1.142)	0.34
Male sex	0.824 (0.198–3.432)	0.79
Metastatic disease	0.765 (0.182–3.210)	0.71
History of ischemic heart disease	12.857 (1.157–142.880)	0.04
History of arterial hypertension	1.583 (0.377–6.649)	0.53
Glomerular filtration rate at Visit 1	1.000 (0.972–1.029)	1.00
No. of chemotherapy cycles	0.931 (0.474–1.827)	0.83
usTnT in pg/ml at Visit 1	0.961 (0.823–1.122)	0.62
NT-proBNP in pg/ml at Visit 1	1.000 (0.997–1.003)	0.93
LVEF in % at Visit 1	1.356 (0.000–37889.6)	0.95

LVEF = left ventricular ejection fraction; n = terminal pro BNP; NT = proBNP; usTnT = ultrasensitive troponin T

TABLE 5. Multivariate regression model for cardiotoxicity

Independent variable	OR (95% CI)	p-value
Age in years	1.044 (0.955 – 1.142)	0.52
Male sex	0.824 (0.198 – 3.432)	0.21
History of ischemic heart disease	12.857 (1.157 – 142.880)	0.026
LVEF in % at Visit 1	1.356 (0.000 – 37889.6)	0.92

OR = odds ratio; LVEF = left ventricular ejection fraction

decrease in LVEF. As none of these patients developed any symptoms or signs suggestive of heart failure or cardiac disease, the importance of this finding is uncertain. While elevations in troponin and BNP can indicate early preclinical cardiotoxicity, a decrease in LV systolic function measured by LVEF shows more extensive cardiac damage.[27] We can assume that patients who had elevations in biomarkers or lowered LVEF would have an increased risk for developing cardiac disease in the future.[28] For a minority of our patients with early lung cancer, a continuous cardiac surveillance is already planned. As we have evidence-based therapy for heart failure, screening (at least in high risk patients) should be part of routine clinical practice to detect or even prevent cardiac damage and possible cardiac events.

The findings of logistic regression model analysis suggest that patients who had a positive history of ischemic heart disease were more likely to have increased values of biomarkers or a decrease in LVEF after treatment and could be considered as those at risk and eligible for screening. Another group that would likely benefit from screening is the patients with a history of heart failure or left ventricular systolic dysfunction. With no such patients at visit 1, we were not able to test this hypothesis; given the risks and the availability of specific cardiac treatment[29,30], clinicians would hardly exclude these patients from a closer follow-up during chemotherapy.

We are aware of several limitations to this study. First, the sample size and several missing values for biomarkers and echocardiography could induce bias and limit the interpretation of the results. Patients were frequently assessed only using biomarkers or echocardiography, often only in visit 2 or visit 3, therefore complete assessment of both biomarkers and echocardiography is missing in several patients. Combining the elevated biomarker and decreased LVEF at either visit 2 or 3 allowed complete assessment of cardiotoxicity. Even though usTnT, NT-proBNP and LVEF are effected by different mechanisms, in asymptomatic patients, they are related to the development of preclinical cardiotoxicity. To further evaluate the extent of incomplete data a *post hoc* study power was calculated. For the same standard deviation and number of paired data as in our sample, we estimate that we had > 80% power to detect a > 3 pg/ml, > 250 pg/ml and > 7% increase/decrease in usTnT, NT-proBNP and LVEF, respectively. Second, blood for biomarkers was not taken immediately after the chemotherapy cycle but on day 1 of the following cycle. Therefore, any changes in biomarker values could not reflect the acute effect of the platinum-based chemotherapy cycles, as elevations in NT-proBNP and usTnT persist for 1-2 weeks, but the interval between platinum doublets used in our patients was 3 weeks.[31] Last, in our 6-month follow-up, we were unable to evaluate long term cardiotoxicity. Although patients with advanced stage NSCLC most likely would not develop long-term cardiotoxicity, patients with early stage NSCLC with longer survival could. To answer this question, future studies exploring LVEF and biomarker changes and possible development of overt heart failure in those with preclinical cardiotoxicity in early stage NSCLC patients treated with platinum-based chemotherapy are necessary.

Despite its limitations, we think that this study is important, because it is the second study to evaluate cardiotoxicity in NSCLC patients treated with platinum-based chemotherapy. We were able to detect changes in cardiac biomarkers and to identify patients who may be at risk for devel-

oping cardiotoxicity. Long-term platinum-based cardiotoxicity has already been observed in other cancers[19,20,26], and with the invention of new anticancer therapies leading to a longer life expectancy for patients with NSCLC, screening for early cardiac injury might become an important part of care for patients with NSCLC, particularly those with early disease. With other concomitant or sequential treatments, such as radiotherapy and targeted therapies, even small cardiotoxic effects of individual type of therapy could contribute to late cardiotoxicity and higher cardiac mortalities as shown in other types of cancers.[10] Detection of early cardiac injury may facilitate early therapeutic measures and healthy lifestyle modifications in patients with NSCLC who have a good prognosis, thus leading to better survival rates in those patients.

Conclusions and clinical implications

Our study shows that platinum-based therapy in patients with NSCLC did not induce clinically overt acute or subacute cardiotoxicity. Despite the fact that mean values of biomarkers and LVEF did not change significantly after treatment, 25% of patients experienced changes defined as cardiotoxic. We have shown that patients with a history of ischemic heart disease were more likely to experience an increase in laboratory biomarkers or a decrease in LVEF. Therefore, we suggest assessing LVEF and laboratory biomarkers to screen for cardiotoxicity in patients with NSCLC treated with platinum-based chemotherapy who have a history of ischemic heart disease, heart failure or asymptomatic left ventricular dysfunction. Further research should focus on patients with a history of cardiac disease, patients with longer life expectancy - especially in early stage NSCLC - and any novel biomarkers that may emerge in the future.

Acknowledgements

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research article

Contribution of diffusion weighted MRI to diagnosis and staging in gastric tumors and comparison with multi-detector computed tomography

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Background. Diagnostic performance of Diffusion-Weighted magnetic resonance Imaging (DWI) and Multi-Detector Computed Tomography (MDCT) for TNM (Tumor, Lymph node, Metastasis) staging of gastric cancer was compared.

Patients and methods. We used axial T2-weighted images and DWI (b-0,400 and b-800 s/mm^2) protocol on 51 pre-operative patients who had been diagnosed with gastric cancer. We also conducted MDCT examinations on them. We looked for a signal increase in the series of DWI images. The depth of tumor invasion in the stomach wall (tumor (T) staging), the involvement of lymph nodes (nodal (N) staging), and the presence or absence of metastases (metastatic staging) in DWI and CT images according to the TNM staging system were evaluated. In each diagnosis of the tumors, sensitivity, specificity, positive and negative accuracy rates of DWI and MDCT examinations were found through a comparison with the results of the surgical pathology, which is the gold standard method. In addition to the compatibilities of each examination with surgical pathology, kappa statistics were used.

Results. Sensitivity and specificity of DWI and MDCT in lymph node staging were as follows: N1: DWI: 75.0%, 84.6%; MDCT: 66.7%, 82%;N2: DWI: 79.3%, 77.3%; MDCT: 69.0%, 68.2%; N3: DWI: 60.0%, 97.6%; MDCT: 50.0%, 90.2%. The diagnostic tool DWI seemed more compatible with the gold standard method (surgical pathology), especially in the staging of lymph node, when compared to MDCT. On the other hand, in T staging, the results of DWI and MDCT were better than the gold standard when the T stage increased. However, DWI did not demonstrate superiority to MDCT. The sensitivity and specificity of both imaging techniques for detecting distant metastasis were 100%.

Conclusions. The diagnostic accuracy of DWI for TNM staging in gastric cancer before surgery is at a comparable level with MDCT and adding DWI to routine protocol of evaluating lymph nodes metastasis might increase diagnostic accuracy.

Key words: gastric cancer; diffusion weighted imaging; MDCT; staging

Introduction

Although there are important geographical differences worldwide, death due to gastric cancer is the third leading cause of death from cancer. When clinically detected, it has already been in the advanced stage and made metastasis.[1] Indistinct clinical presentation of gastric cancer is the main reason

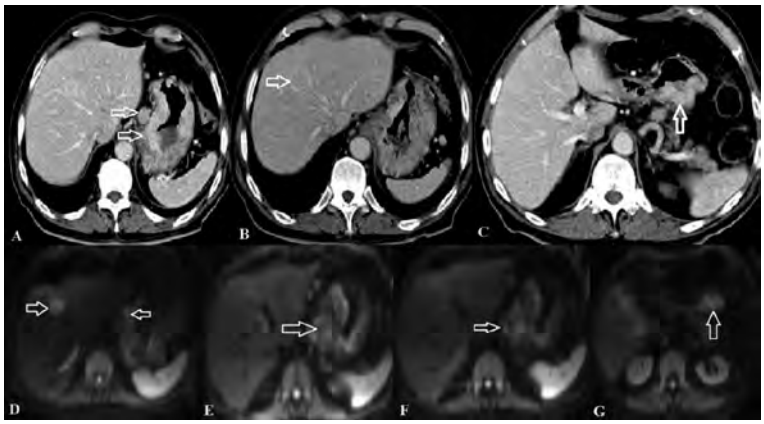

FIGURE 1. T4N3M1 C Axial MDCT (A,B,C) and DWI (D,E,D,F,G). **(A)** Axial contrasted-enhanced MDCT shows gastric corpus tumor and adjacent lymphadenopathies, **(B)** peripherally contrast enhanced lesions in the liver (metastasis), and **(C)** an invasive mass extending from the posterior of the gastric corpus to the fatty tissue (arrows). On DWI **(D-G):** on b800 DWI **(D, E)** there are diffusion restrictions in the gastric corpus and liver (Gastric tumor + liver metastasis), **(F)** restrictions that are compatible with lymphadenopathies are seen in the gastro-hepatic ligament, hepatic hilum, and the celiac axis, and **(G)** invasion of the fatty tissue in the posterior of the gastric corpus (arrow).

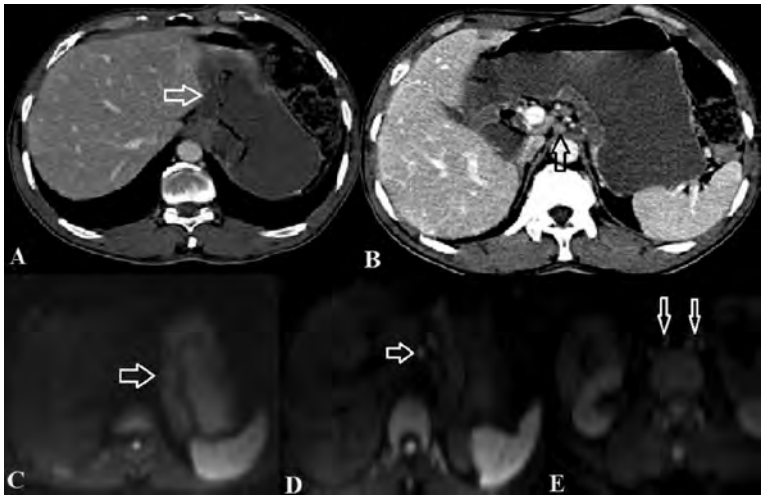

FIGURE 2. T2N3M0 C Axial MDCT **(A, B)** and DWI **(C,D,E)**. **(A,B)** axialcontrast-enhanced MDCT: Wall thickness in the gastric cardia-corpus and gastro-hepatic ligament and para-aorticlymphadenopathies, **(C, D, E)** b800 DWI images: **(C)** Diffusion restriction in the gastric cardia and corpus, **(D)** mm sized lymphadenopathiesin the gastro-hepatic ligament, and(e)para-aorticandceliac lymphadenopathies.

for late diagnosis. Symptoms are generally non-specific or all symptoms do not appear together.[2] Recently, methods such as gastroscopy, endoscopic ultrasonography (EUS), computed tomography (CT), and positron emission tomography PET/CT are generally used for the staging of gastric cancer.[3] With multi-dimensional reformatted (MPR) images and technical progress in multi-detector CT (MDCT) technology, which allows 3D imaging of the endoluminal surface of the stomach with thin-section axial images, MDCT is also generally used for the pre-operative evaluation of gastric cancer staging.[4] However, in the clinical approach, some patients are not appropriate for contrast-enhanced CT due to renal dysfunction, hypersensitivity reaction to iodine-containing contrast agents, or they are avoiding radiation exposure because of the possibility of pregnancy.[4,5] In patients with suspected gastric cancer and to whom contrasted CT cannot be applied, MRI has been mostly used for the diagnosis and staging of gastric cancer as an alternative imaging method to MDCT in the evaluation of gastric cancer.[6,7] Especially, diffusion-weighted magnetic resonance imaging (DWI), whose main feature is to reflect movement of water molecules in tissue and gather information about tissue integration, was investigated in various tumors and the value of DWI was confirmed with various studies in detecting and characterizing gastric cancers. Furthermore, it can provide information for the differentiation of benign lesions from malignant ones, detecting malignant lymphadenopathy or very small peritoneal seeding and tissue cellularity, which might be used for monitoring therapeutic efficacy. DWI, draws attention as an oncologic imaging tool.[8-10]

In our study, we tested our hypothesis that DWI might be useful in staging based on pre-operative diagnostic efficacy of DWI and MDCT in gastric cancer patients and correlation analysis of surgical pathology.

Patients and methods

Between the periods of April 2013 and May 2015, fifty-one patients with malignant gastric adenocarcinoma diagnosis were included in the current study and their diagnosis was made with endoscopic biopsy in our hospital. In 51 gastric cancer patients, who were included in research and whose preoperative clinical TNM (Tumor-Node-Metastasis) staging were made, 37 were male and 14 were female. Their average age was 61 (range: 35 – 82) years old. Approval was taken from a local ethics committee and written consent was obtained from all patients.

First, diffusion-weighted magnetic resonance imaging (DWI) and upper and lower abdomen CT were taken pre-operatively from all patients. All examinations were made with multi-slice CT device (Siemens SOMATOM Sensation 16, Germany). In the CT, kV was 120 and mAs was 150. Each pa-

tient was examined after eight hours of fasting. Stomach fullness was achieved by giving the patient one liter of water during the examination. By entering the right antecubital vein with an 18G needle, 100 ml of contrast agent was administered (Omnipaque 350 mg, GE) at a rate of 2 ml/s by an automatic injector (Medrad, vision CT injection system). Approximately 70 seconds after the start of contrast medium administration, images were obtained at 2.5 and 5 mm thick axial slices starting from the superior of diaphragm to the level of the symphysis pubis while holding their breath. In order to create multi-planar reconstruction images, CT images were reconstituted with 1 mm thick pieces, at a reconstruction interval of 1 mm. For reconstruction, thin-piece CT data were transferred to the 3D working station and coronal and sagittal MPR images were constituted with 3 mm intervals and 3 mm slice thickness.

MRI

Investigations were made with phased-array body coil as routine upper-abdomen MRI in Siemens Magnetom Symphony (Siemens, Erlangen, Germany) device. The field strength of the device is 1.5 Tesla (T), which is considered to have a high field strength. Gradient force of superconductive (Niobium-Titanium) magnetic is 30 mT/m and FOV width is 400 cm.

Before the diffusion weighted procedure, T2-weighted True-FISP at axial plane (TR, 4.4s; TE, 2.2s; Average, 2; FlipAngle, 80º; Matrix, 256 x 256; number of sections, 25; thickness of sections, 5mm; FOV, 300; Average, 2; gap between sections, 15%) sequence and after that diffusion-weighted, single-shot, echo-planar spin sequence, and chemical shift selective fat suppression technique (TR/TE, 3700/76; Matrix, 128x128; number of sections, 30; FOV, 400; gap between sections, 15%; section thickness, 5 mm; duration, 156s; PAT Factor, 2; PAT mode, parallel imaging (GRAPPA) with modified sensitivity encoding) was applied. In DWI, b values were set as 0,400 and 800 mm^2/second.

Evaluation of images

An examination was conducted after installing diffusion-weighted images to the work station. All abdominal imaging MDCT and DWI results were assessed by two radiologists (HA, AB) who had 12 and 10 years of experience, respectively. Two radiologists were informed about the localization of the lesions, which were diagnosed with gastric cancer due to endoscopic biopsy. However, they were blinded to the pathological findings. MDCT and DWI image analysis were performed. Reviewers were told to determine T(T1,T2,T3,T4) stage and N stage (N1,N2,N3) by classifying as positive or negative and M stage by classifying as MO or M1. In abdominal images, only DWI images were evaluated for staging. Conventional MR images were not assessed in the staging.

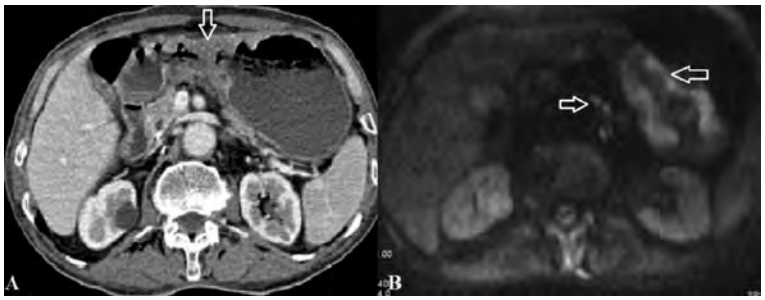

FIGURE 3. (A, B) T2N2M0 Axial MDCT and b800 DWI. **(A)** Axialcontrast-enhanced MDCT and **(B)** b800 DWI demonstrate wall thickness in the gastric corpus withdiffusion restrictions, andadjacent lymphadenopathies (mm sized) on DWI.

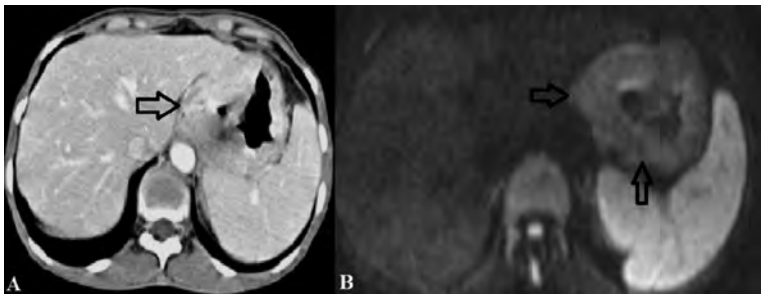

FIGURE 4. (A, B) T3N1MO Axial MDCT and DWI. **(A)** Axialcontrast-enhanced MDCT and **(B)** DWI demonstratewall thickness at gastric corpus and diffusion restriction and adjacent lymphadenopathieswere observed (arrow).

Surgical pathological analysis

Surgery was performed within the following two weeks after cross sectional imaging. Curative or palliative gastrectomy and LN dissection were applied to 48 patients out of total 51 patients who were included in this study. Open-and-closed laparotomy was applied to three patients. For each case, pathological TNM staging was reported according to the Seventh AJCC Guideline.[11]

Determination of the depth of tumor invasion (T staging)

We applied imaging criteria that were based on previous studies about T staging of gastric can-

TABLE 1. Effectiveness of DWI and MDCT in T staging according to surgical pathology result

		T2	T3	T4
DWI	Sensitivity	72.7%	71.0%	55.6%
	Specificity	77.5%	80.0%	92.9%
	PPV	47.1%	84.6%	62.5%
	NPV	91.2%	64.0%	90.7%
	Kappa (p)	0.419 (0.002)	0.488 (< 0.001)	0.506 (< 0.001)
MDCT	Sensitivity	63.6%	74.2%	66.7%
	Specificity	77.5%	80.0%	95.2%
	PPV	43.8%	85.2%	75.0%
	NPV	88.6%	66.7%	93.0%
	Kappa (p)	0.353 (0.009)	0.523 (< 0.001)	0.647 (< 0.001)

CT = Computed Tomography; DWI = Diffusion Weighted Imaging; NPV = Negative Predictive Value; PPV= Positive Predictive Value

cer and were modified according to the Seventh AJCC Guideline.[10,11] For each T stage, imaging criteria were as follows: ≤ T2 stage (≤ muscularis propria invasion), it is an abnormal growing mass or wall thickness with/without ulceration, which has trans-mural involvement and irregular external border. The thin outer layer around the tumor and clear perigastric fatty tissue is preserved. T3 stage (penetrates subserosal connective tissue without involvement of the visceral peritoneum or adjacent structures), transmural tumor involves the entire stomach wall and 1) clear perigastric fat plane around the tumor is maintained, or 2) fine perigastric oil spicules that extend throughout small and large omentum and do not spread beyond the adjacent perigastric vessels. T4 stage (invasion of serosa (visceral peritoneum) or adjacent structures) is a transmural tumor with irregular borders and perigastric oil infiltration, which involves the entire stomach wall and shows invasion beyond adjacent perigastric vessels.

Detection of regional lymph node involvement (N staging)

Positive lymph nodes can be detected according to their sizes in MDCT or MRDWI. If the shortest diameter of the largest regional lymph node is ≥ 8 mm, the patient should be considered as N positive; if it is < 8 mm, the patient should be considered as negative. If there is at least one regional lymph node that has a shortest diameter of ≥ 8 mm in DWI or there is any lymph node that shows higher signal intensity than muscle, the patient was classified as N-positive.[7]

Detection of distant metastasis (M staging)

If suspicious lesions were seen in the MDCT or DWI about distant metastasis such as the liver, adrenal gland, distant lymph nodes, and peritoneum involvement, the patient was considered to be M1.

Statistical analysis

In gastric tumors, the comparison of DWI, MDCT, and surgical pathology was made using SPSS version 20 (SPSS Inc. Chicago,IL, USA) software. TNM results of two different imaging techniques (DWI and CT) and TNM results of surgical pathology, which is accepted as the gold standard method, was compared and assessed using Pearson's chi-square (chi-square) or Fisher's exact test depending on the situation. Conformity between two different imaging techniques (DWI and CT) and surgical pathology was evaluated with the Kappa test. Additionally, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy ratio of diagnostic tests were calculated. P values < 0.05 were considered statistically significant.

Results

Pathologic TNM staging of gastric cancer

A total of 51 cases were included in the analysis. In the results of both surgical pathology and other two diagnostic tools (DWI and MD CT); there were no cases in the T1 stage and N0 stage. Because of this, T staging was made for T2 and higher and N staging was made for N1 and higher stages. T and N staging were confirmed in 51 patients to whom gastrectomy and LN dissection were applied. It was confirmed that in DWI 17 patients and in MDCT, 16 patients were stage T2. In DWI 26 patients and in MDCT 27 patients were stage T3. It was confirmed that in DWI seven patients and in MDCT seven patients were stage T4a and in MDCT one patient was stage 4b. In 51 patients, lymph node was positive. In two patients, peritoneum involvement was confirmed by radiologic evaluation and they were evaluated as M1. Other 49 patients were assessed as M0.

Diagnostic performance of T staging in gastric cancer

T staging (T2, T3, T4) diagnosis of DWI was different from surgical pathology result (p values were: 0.03, < 0.001 and 0.002 respectively). Similarly, T staging of MDCT was also different from the surgical pathology results (p values: 0.014, < 0.001, < 0.001, respectively). When we looked for Kappa compatibility test for both diagnostic tools their compatibility with surgical pathology was generally weak (Table 1).

Diagnostic performance of N staging in gastric cancer

When we compared results of the DWI and surgical pathology in the staging of the lymph node; staging of DWI (N1, N2, N3) was different from the surgical pathology (for the three stages, p < 0.001). Similarly, a significant difference was found between MDCT results and surgical pathology results in N staging (p values; 0.003, 0.009, and 0.009, respectively). When we looked for Kappa compatibility test for N staging (Table 2), we detected that DWI is compatible with surgical pathology at a moderate-fine level. However, the compatibility of MDCT was weak.

According to surgical pathology result, distant metastasis (M1) was detected in total two cases. For M1, DWI and MDCT had full compliance with the gold standard (Kappa = 1.00 p < 0.001). Sensitivity and specificity of both imaging techniques for detecting distant metastasis was 100%.

TABLE 2. Efficiency of DWI and MDCT in N staging according to surgical pathology result

		N1	N2	N3
DWI	Sensitivity	75.0%	79.3%	60.0%
	Specificity	84.6%	77.3%	97.6%
	PPV	60.0%	82.1%	85.7%
	NPV	91.7%	73.9%	90.9%
	Kappa (p)	0.549 (< 0.001)	0.563 (< 0.001)	0.649 (< 0.001)
MDCT	Sensitivity	66.7%	69.0%	50.0%
	Specificity	82.1%	68.2%	90.2%
	PPV	53.3%	74.1%	55.6%
	NPV	88.9%	62.5%	88.1%
	Kappa (p)	0.448 (0.001)	0.367 (0.008)	0.418 (0.003)

CT = Computed Tomography; DWI = Diffusion Weighted Imaging; NPV = Negative Predictive Value; PPV= Positive Predictive Value

Discussion

MRI and CT play an important role in the diagnosis and preoperative staging of gastric cancer.[7,8] Gastric cancer generally does not have any symptoms when it is at a stage at which a complete cure can be achieved. On the other hand, when clinically detected, it is generally at an advanced stage (local advanced or metastatic stage).[12] Most of our cases were detected at an advanced stage. An accurate assessment of the depth of the tumor invasion into the stomach wall, local lymph node involvement, and metastasis presence or absence is very important for the selection of therapeutic strategy. In spite of the development of methods such as computed tomography (CT), positron emission tomography (PET), magnetic resonance (MR), ultrasound (US), gastroscopy, double-contrast barium X-ray, and endoscopic ultrasound (EUS), there are still difficulties in the diagnosis and staging.[13] Preoperative staging of gastric cancers has gained more importance with the recent developments in endoscopic treatment and also minimally invasive treatments of gastric cancers such as laparoscopic surgery.[14] Recently, other MRI methods have been put into routine use, as well as conventional MR imaging. One of these methods is diffusion weighted MR imaging (DWI). Most of the studies about DWI were conducted with the Single Shot EPI (SSEPI) technique. An image is taken in a time unit of less than a second with the SSEPI technique and thus physiological movement is frozen and image artifacts are reduced.[15] We used the SSEPI technique in the current study. Diffusion weighted MR imaging makes it possible to obtain information about the perfusion and diffusion of tissue at the same time, so normal and abnormal tissues can be distinguished from each other. Various abnormalities can be detected because of this technique.[15] Today, the only imaging method that provides information about tumor cellularity is DWI.[16] This cellular density difference reflects in the DWI. Various studies have reported that diffusion weighted MRI provides an important contribution to the diagnosis in the characterization of the tumor at different sites such as the liver, pancreas, ovary, colon, cervix, bladder, prostate, and breast. In association with cellularity of tumors, it was specified that malignant tumors have more diffusion limitations compared to benign lesions.[17]

So far, very few studies compared diagnostic performance of MDCT and 1.5-3 T MRDWI for

the local staging of gastric cancer and showed that DWI is comparable with MDCT in T staging.[7,8,18] Many studies have recently shown that using MDCT with MPR imaging in T staging is accurate with a ratio of 83% and 91%, respectively.[8,19]

The current study demonstrated that DWI did not increase diagnostic performance significantly in T staging. In other studies that compared DWI and MDCT, DWI generally used for staging with conventional MRI and their accuracy values were high.[8] In our study, using only DWI images were compared with MDCT in staging. We related low accuracy ratios in T staging with this condition. DWI and MDCT results in T staging can be better as the T stage increases compared to the gold standard. As T stage progresses, the effectiveness of DWI is related to an increase of size and density of tumor cells and limitation of intracellular diffusion during the impairment of regulation of tumor cells. As a result of these features, this study revealed that DWI could define advanced gastric cancer but it was not an effective method to detect early gastric cancers (T0-T1). We detected that it was possible to have similar results with almost the same accuracy with DWI for detecting wall invasion (T2, T3, T4) in 51 patients with gastric cancer when compared to MDCT.

Although the results of our work, in terms of N staging, did not have any significant difference for diagnostic accuracy between treatment methods, it was shown that DWI had significantly higher sensitivity for evaluating metastatic lymph nodes when compared with MDCT. Lymph node involvement is one of the most important prognostic factors, especially in gastric cancer. Lymph node metastasis is frequent in gastric cancer patients.[20] However, as surgery related mortality and complications increase in the scope of analysis of the lymph nodes, it has been an issue that has been debated for a long time. In this context, for appropriate treatment strategies, local metastatic lymph nodes should be correctly considered before surgery.[21,22] To this day, the diagnostic accuracy of MDCT in the N staging of gastric cancer varies between 46% and 83%.[8,22] The weak and variable diagnostic performance might be a result of current lymph node metastasis criteria, which is generally based on lymph node size and shape; however, there can be microscopic metastasis in small sized lymph nodes and benign nodes usually can expand in gastric cancer patients when compared with a healthy population. It should be considered that lymph node size might not be adequate to distinguish metastatic lymph nodes from benign lymph nodes, especially in gastric cancer patients.[22,12] DWI also shows more diagnostic accuracy for detecting metastatic lymph nodes when compared to measurements in MRI and CT imaging. In addition to lymph node findings, there is potential to predict lymph node metastases with computerized analysis of tumor characteristics, such as primary tumor localization, diameter, Borrman types, histological types, infiltration depth, serosa invasion, molecular, and genetic markers.[7] In the current study, apparent diffusion restriction is observed in lymph nodes with millimeter dimension due to diffusion weighted MRI and these were accepted as metastatic lymph nodes were confirmed after surgical pathology results (Figure 2). Malignant lesions are generally more cellular than benign lesions and this feature can provide information about the perfusion and diffusion of a tissue. Because of this high cellularity, malignant lesions have higher signal density in DWI and this demonstrates that DWI is more beneficial than MDCT to differentiate malignant lesions from benign ones. Moreover, the accuracy of N staging with DWI was statistically higher than the accuracy of N staging with MDCT. Furthermore, when combined with other morphological characteristics, DWI reached a higher predictive power than MDCT. In our study, more lymph nodes, especially under 1 cm, were detected when evaluated with DWI. DWI signal changes could have higher predictive power than other morphological factors, including contrast uptake pattern and border irregularity for distinguishing metastatic and benign lymph nodes.

Distant metastases of gastric cancers include liver metastasis and peritoneum involvement. They can be more clearly detected by using DWI without any contrast agent when compared with MDCT. In our study, distant metastasis in two gastric cancer patients included peritoneum involvement and liver metastasis and these were detected with both DWI and MDCT. There was no statistically significant difference between these two methods. This might be due to the low number of metastatic patients in the study.

There were some limitations in this study. First of all, a relatively high proportion of patients in stage T3 might cause a statistical bias. Secondly, we did not detect any T1 stage cancer in the patient population. For this reason, further studies are required with more patients that include T1 stage cancer. Thirdly, as each lymph node was not compared separately for N staging, we could not match imaging and pathology diagnosis for each lymph node. Fourthly, as we used only qualitative

assessment in DWI without using any quantitative measurement or size criteria, further studies are required to determine optimum diagnostic criteria with DWI in TNM staging of gastric cancer. Finally, two radiologists were informed about the localization of lesions but they were blinded to other endoscopic and surgical pathological findings.

In conclusion, DWI has a potential clinical use field, especially for the assessment of node involvement in gastric cancer. In particular, the conventional MRI and DWI combination might produce accurate performance with high sensitivity and specificity in lymph nodes smaller than one centimetre. When analyzed from a clinical viewpoint, an accurate lymph node metastasis evaluation before operation facilitates lymphadenectomy surgery and may reduce the risk of complications.

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research article

Electrotransfer of plasmid DNA radiosensitizes B16F10 tumors through activation of immune response

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Background. Tumor irradiation combined with adjuvant treatments, either vascular targeted or immunomodulatory, is under intense investigation. Gene electrotransfer of therapeutic genes is one of these approaches. The aim of this study was to determine, whether gene electrotransfer of plasmid encoding shRNA for silencing endoglin, with vascular targeted effectiveness, can radiosensitize melanoma B16F10 tumors.

Materials and methods. The murine melanoma B16F10 tumors, growing on the back of C57Bl/6 mice, were treated by triple gene electrotransfer and irradiation. The antitumor effect was evaluated by determination of tumor growth delay and proportion of tumor free mice. Furthermore, histological analysis of tumors (necrosis, apoptosis, proliferation, vascularization, presence of hypoxia and infiltration of immune cells,) was used to evaluate the therapeutic mechanisms.

Results. Gene electrotransfer of plasmid silencing endoglin predominantly indicated vascular targeted effects of the therapy, since significant tumor growth delay and 44% of tumor free mice were obtained. In addition, irradiation had minor effects on radioresistant melanoma, with 11% of mice tumor free. The combined treatment resulted in excellent effectiveness with 88% of mice tumor free, with more than half resistant to secondary tumor challenge, which was observed also with the plasmid devoid of the therapeutic gene. Histological analysis of tumors in the combined treatment group, demonstrated similar mode of action of the gene electrotransfer of plasmid encoding shRNA for silencing endoglin and devoid of it, both through the induction of an immune response.

Conclusions. The results of this study indicate that irradiation can in radioresistant melanoma tumors, by release of tumor associated antigens, serve as activator of the immune response, besides directly affecting tumor cells and vasculature. The primed antitumor immune response can be further boosted by gene electrotransfer of plasmid, regardless of presence of the therapeutic gene, which was confirmed by the high radiosensitization, resulting in prolonged tumor growth delay and 89% of tumor free mice that were up to 63% resistant to secondary challenge of tumor. In addition, gene electrotransfer of therapeutic plasmid for silencing endoglin has also a direct effect on tumor vasculature and tumors cells; however in combination with radiotherapy this effect was masked by pronounced immune response.

Key words: gene therapy; electrotransfer; plasmid, irradiation, immune response, melanoma

Introduction

Electroporation is used as drug delivery system for molecules with hampered transmembrane transport.[1] It is effective for delivery of smaller molecules, as chemotherapeutics in electrochemotherapy (ECT)[1-3] and also for larger molecules, as are plasmids in gene electrotransfer (GET). GET is

recently getting a lot of scientific consideration and it is used for enhanced DNA delivery into various tissue types (*i.e.* skin, liver, kidney etc.), as well as into tumors.[4-6] Its effectiveness was first demonstrated in a wide range of preclinical studies and has thereafter proceeded to clinical oncology, veterinary and human. The results of clinical trials of GET of plasmid encoding human IL-12[7] and anti-angiogenic plasmid AMEP[8], are promising. In addition to GET of therapeutic plasmids, some plasmids devoid of therapeutic genes have also resulted in good antitumor effectiveness.[9,10] This phenomenon was observed in different tumors models and by using different plasmids. It was attributed to immune sensing of the introduced DNA as a DAMP (Damage Associated Molecular Pattern), which switch leads to activation of an immune response.[11]

Radiotherapy is one of the principal treatment modalities for primary tumors and their metastases.[12] Nowadays, irradiation is widely investigated for its associated effects on priming antitumor immunity.[13] There is growing evidence of irradiation's effect on the antitumor immune response, inducing immunogenic cell death and generating danger signals. An important danger signal after irradiation is DNA released from the nucleus of dying cells. This DNA is recognized by the immune system as a DAMP, and can promote the activation of immune response against irradiated cells.[14,15]

Studies combining tumor irradiation with GET of different therapeutic plasmid demonstrated tumor radiosensitization.[16-18] A promising approach to target tumors and its microenvironment with a combined treatment modality is through destructing abnormal tumor blood vessels. One of the promising targets, gaining on its value due to different signaling pathways from VEGF, is endoglin. It is a TGF-β coreceptor and has already demonstrated good antitumor and antimetastatic effectiveness in different tumor models when targeted with GET of plasmid.[18–22] In particular, GET of shRNA for silencing endoglin in B16F10 melanoma mice tumors that express high levels of endoglin, resulted in up to 58% of tumor cures.[23]

Tissue specific eukaryotic promoters are tightly regulated and mainly drive expression of transgene in specific cell types, although minimal unspecific expression in non-targeted tissue can also occur.[24] We constructed a plasmid containing a tissue specific promoter for endothelin and encoding shRNA for silencing endoglin. This plasmid was tested in a previous study, where the effectiveness of the plasmid with tissue specific promoter was compared to a plasmid with constitutive promoter in a tumor model that does not express targeted molecule, endoglin. *In vivo*, the effectiveness of the GET of the plasmids was comparable and resulted in significant radiosensitization, which resulted in prolonged tumor growth delay with nearly 50% of tumor free mice. Thus, the aim of this current study was to determine, whether GET of plasmid, with tissue specific promoter and encoding shRNA for silencing endoglin, can radiosensitize melanoma B16F10 tumors, which express targeted molecule, endoglin, and possibly have also some immunomodulatory effectiveness.

Materials and methods

Cell lines and plasmids

Murine melanoma cell line B16F10 (American Type Culture Collection, Manassas, VA, USA) was cultured in advanced minimum essential medium (AMEM, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 5% fetal bovine serum (FBS, Gibco), 10 mM/L L-glutamine (GlutaMAX, Gibco), 100 U/mL penicillin (Grünenthal, Aachen, Germany) and 50 mg/mL gentamicin (Krka, Novo mesto, Slovenia) in a 5% CO_2 humidified incubator at 37°C.

The plasmid with tissue specific promoter for endothelial cells, encoding shRNA for silencing endoglin (pET-antiCD105; TS plasmid) was used in experiments as the therapeutic plasmid.[18] The control plasmid, encoding shRNA with no homology to any gene in the mouse genome and with constitutive CMV promoter, was used as a negative control (pControl).[25] Amplification of both plasmids was performed in a competent *E.coli* (JM107; Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

All plasmids were isolated using JETSTAR 2.0 ENDOTOXIN-FREE Plasmid MEGA Kit (Genomed, Löhne, Germany) and diluted in endotoxin free water to a concentration of 1 μg/μL (*in vitro* experiments) and 4 μg/μL (*in vivo* experiments). Concentrations of plasmids were measured with a spectrophotometer at 260 nm (Epoch Microplate Spectrophotometer, Take3 Micro-Volume Plate, BioTek, Bad Friedrichshall, Germany) and purity of plasmid was determined by agarose gel electrophoresis and measurements of the absorbance ratio at 260 and 280 nm.

Experimental animals

All animal experiments were conducted in accordance with the guidelines for animal experiments of

TABLE 1. Response of B16F10 melanoma to different treatment modalities

Therapeutic group	DT (days)				TGD (days)			TF		SC	
	n	AM		SEM	AM		SEM	n	%	n	%
CTRL	8	1.2	±	0.1	0.0	±	0.1	0	0	-	-
pControl	8	1.9	±	0.2	0.7	±	0.2	0	0	-	-
TS	8	2.2	±	0.3	1.0	±	0.3	0	0	-	-
3 × EP	8	3.1	±	0.3	2.0	±	0.3	0	0	-	-
3 × GET (pControl)	8	9.4	±	6.5	8.2	±	2.5	0	0	-	-
3 × GET (TS)	9	9.5	±	3.2	8.6	±	3.0	4	44	3	75
IR	9	1.8	±	0.3	0.7	±	0.3	0	0	-	-
pControl + IR	9	2.5	±	0.3	1.3	±	0.3	1	11	1	100
TS + IR	8	4.0	±	1.2	2.8	±	1.2	0	0	-	-
3 × EP+IR	9	4.3	±	1.3	3.2	±	1.3	2	22	0	0
3 × GET (pControl) + IR	9	36.0	±	n/a	34.9	±	n/a	8	89	5	63
3 × GET (TS) + IR	8	32.0	±	n/a	30.8	±	n/a	7	88	4	57

AM = Arithmetic mean; DT = Tumor doubling time; Groups: (CTRL = control; EP = electric pulses; GET = gene electrotransfer; IR = irradiation; TS = pET-antiCD105); n = Number of all mice in the group; n/a = Not applicable; SC = Mice resistant to secondary challenge; SEM = Standard error; TF = Tumor free mice; TGD = Tumor growth delay

the EU Directive and the permission obtained from the Ministry of Agriculture and the Environment of the Republic of Slovenia (Permission No. 34401-1/2015/16), which was given, based on the approval of the National Ethics Committee for Experiments on Laboratory Animals).

Female C57Bl/6 mice, 6-8-week old, purchased from Envigo Laboratories (Udine, Italy), were used in the study. Before the experiment, mice were subjected to an adaptation period of 2 weeks. Animals were maintained under specific pathogen-free conditions at a constant room temperature, humidity and a 12 h light/dark cycle. Food and water were provided *ad libitum*. For induction of subcutaneous tumors, a suspension of $1x10^6$ B16F10 cells in 0.1 ml of physiological saline was injected subcutaneously into the back of the mice. The animals bearing tumors of 40 mm^3 were randomly divided into experimental groups and subjected to a specific experimental protocol. The tumor measurements were completed when the tumors reached 350 mm^3, and mice were humanely sacrificed.

Experimental groups and the number of animals in each of them were as follows and as described in table Table 1: triple injection of endotoxin-free water alone (control group; CTRL) or of pControl or TS plasmids alone, or in combination with triple application of electric pulses alone (3 × EP) or combined with plasmids (3 × GET (pControl); 3 × GET (TS)). Furthermore, the remaining groups were also the ones in combination with irradiation (IR) and other therapies described above, which are three injections of plasmids (pControl + IR; TS + IR) and in combination with electric pulses (3 × EP + IR; 3 × GET (pControl) + IR; 3 × GET (TS) + IR).

In vivo GET

In vivo GET of plasmid into subcutaneous tumors was performed 3 times every second day (on days 0, 2 and 4). 12.5 μL (4 μg/μL) of plasmid (150 μg in total) in endotoxin-free H_2O was injected intratumorally 10 min before 8 square electric pulses with a voltage-to-distance ratio of 600 V/cm, a pulse duration of 5 ms, and a frequency 1 Hz were applied. Electric pulses were generated by electric pulse generator ELECTRO CELL B10 (Betatech, L'Union, France) and delivered through 2 parallel stainless steel electrodes with 2 or 4 mm distance between them, depending on the tumor volume. After 4 pulses, electrodes were turned for 90° for 4 additional pulses to assure GET to entire tumor.

Irradiation of tumors

Tumors were locally irradiated with a single dose of 15 Gy on day 1 from the beginning of the experiment, at a dose rate of 2.16 Gy/min, using a Darpac 2000 X-ray unit (Gulmay Medical Ltd., Shepperton, UK) operating at 220 kV, 10 mA, with 1.8-mm aluminum filtration. During irradiation, mice were

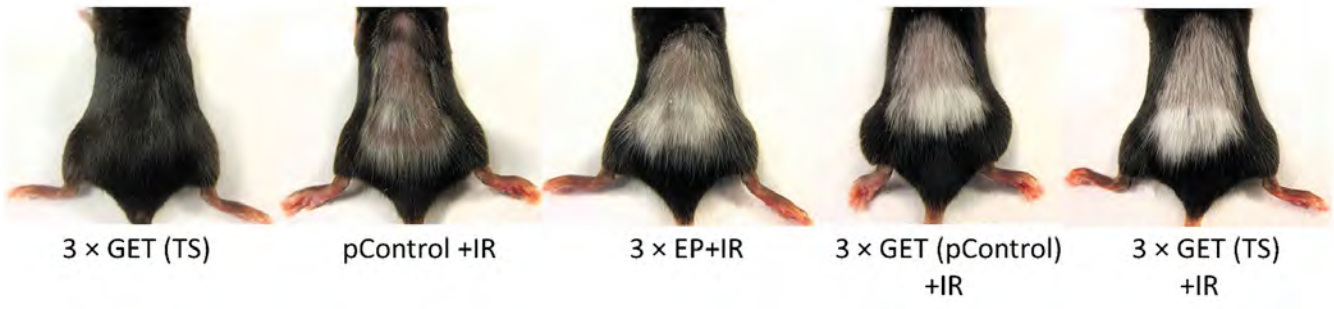

FIGURE 1. Histological sections of melanoma tumors on day 6 after the beginning of the therapy.

CTRL = control; EP = electric pulses; GET = gene electrotransfer; IR = irradiation; TS = pET-antiCD105

restrained in special lead holders with apertures for irradiation of the tumors. Due to the fixed size of the apertures, some healthy tissue (3 – 5 mm of skin surrounding the tumor) was exposed to the irradiation as well.

Tumor growth

The therapeutic potential *in vivo* was assessed by measuring the tumor size every second day and calculating tumor volume according to the formula for ellipsoid: V=axbxc π/6, where a, b and c represent perpendicular tumor diameters.[21,26] The tumor growth curves were drawn as arithmetic means (AM) with bars representing standard errors (SEM).

The tumor growth delay for each experimental group was calculated as the difference in tumor doubling times of experimental and control groups. Tumor doubling time is the number of days in which the initial tumor volume (40-50 mm^3) doubles. Mice that remained tumor free for 70 days were termed tumor free and local tumor control was deemed to have been achieved (Table 1). The weight of the mice was followed as a general index of systemic toxicity, and acute skin reaction in the whole irradiated field around the tumor was evaluated as described elsewhere.[17]

Tumor challenge of tumor free mice

After 70 days, when mice were designated as tumor free, they were challenged with a subcutaneous injection of 1×10^6 B16F10 cells in 0.1 ml of physiological saline in the right flank. Mice that at least 20 days after the challenge remained tumor free were marked as resistant to secondary challenge (Table 1, Figure 2). The growing tumors were measured twice a week and when volume of 150 mm^3 was reached mice were sacrificed and tumors were collected for further histological analysis as described below.

Histology

After therapies, at day 6, three mice from each experimental group were sacrificed. The tumors were excised, fixed in IHC zinc fixative (BD Biosciences, San Diego, CA, USA) and embedded in paraffin. Six consecutive 2-μm thick sections were cut from each paraffin block and stained as followed. To estimate the percent of the area of tumor necrosis, the first section was stained with hematoxylin and eosin. The other five sections were used for immunohistochemical (IHC) staining to evaluate percentage of hypoxic cells, cells in apoptosis, immune cells, proliferating cells and the number of blood vessels. To determine hypoxic cells, rabbit polyclonal antibodies against HIF-1-alpha (ab2185, Abcam, Cambridge, MA, USA) at dilution 1:3500, were used. In addition, apoptosis was evaluated with help of cleaved Caspase-3 (Asp175., Cell signaling Technology, Danvers, MA, USA) at dilution 1:1500, whereas immune cells (NK and CTL) were stained with help of Granzyme B (ab4059, Abcam) at dilution 1:1250. For staining proliferating cells, rabbit monoclonal antibodies against Ki-67 (clone SP6, Thermo Fisher Scientific) at dilution 1:1200 were used. The last section was stained for determination of the number of blood vessels, by using primary rabbit polyclonal antibodies against CD31 (ab28364, Abcam) at dilution 1:1000. For these sections, a peroxidase-conjugated streptavidin–biotin system (Rabbit specific HRP/DAB detection IHC kit, ab64261, Abcam) was used as the colorogenic reagent followed by hematoxylin counterstaining. From each slide and each feature (apoptosis, hypoxia, proliferation, vascularisation and immune cells), five randomly selected viable parts of each tumor were observed and captured under the light

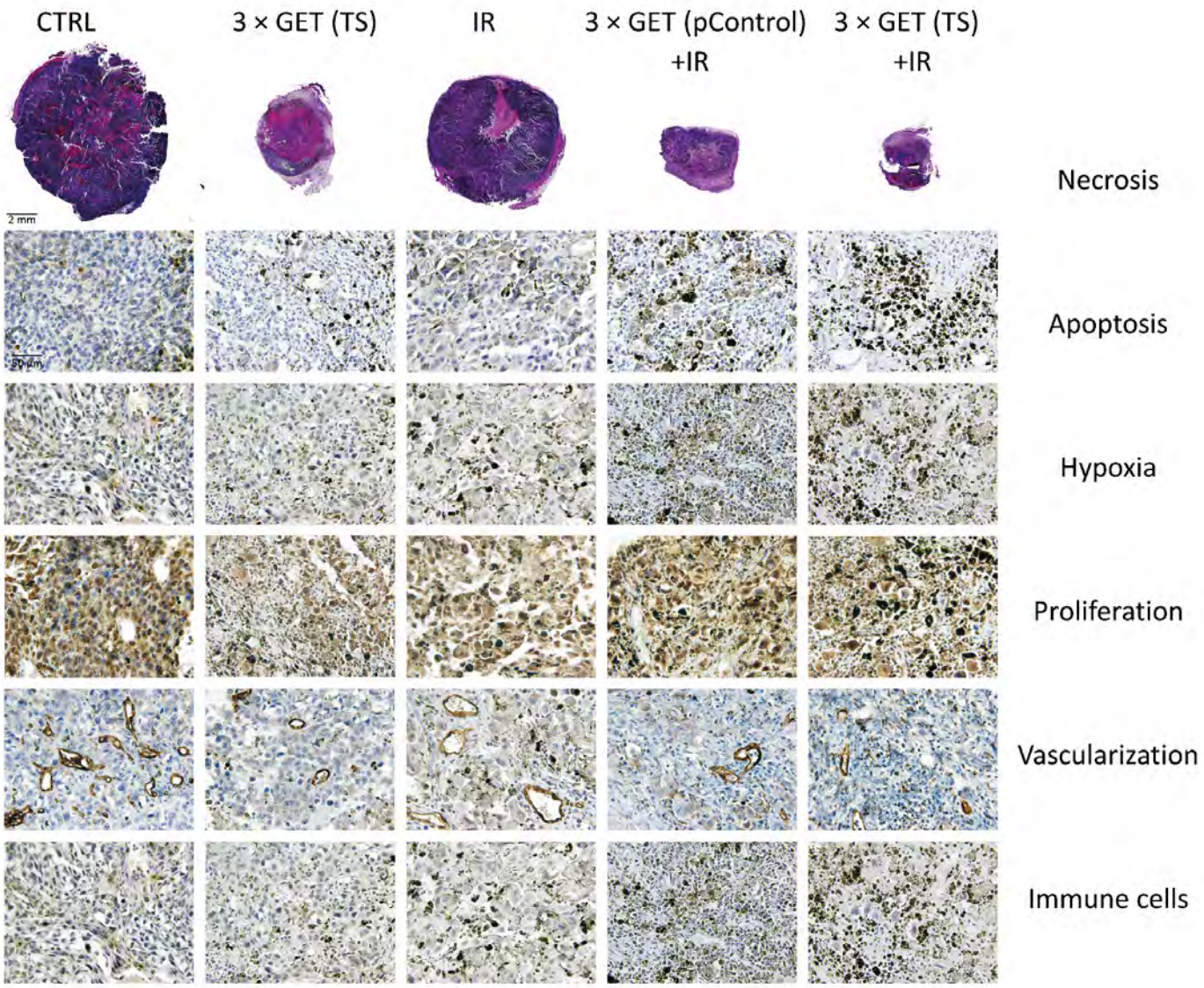

FIGURE 2. Immune response of melanoma tumors is observed by vitiligo effect.

CTRL = control; EP = electric pulses; GET = gene electrotransfer; IR = irradiation; TS = pET-antiCD105

microscopy, by DP72 CCD camera (Olympus, Hamburg, Germany) connected to a BX-51 microscope (Olympus) under 40× magnification (numerical aperture 0.85). The viable parts were analyzed in blind fashion and the results were presented as the percent (hypoxia, apoptosis, proliferation) of the cells or the number of cells (immune cells) or the structures (vascularization) positive to IHC staining. The percentage of necrosis was contributed to the tumors as whole and was also evaluated in blind fashion, as previously described.[18]

In addition, tumors that grew up to 150 mm^3 after secondary challenge of tumors, were excised, fixed and embedded in paraffin, as described above. Furthermore, from each tumor sections were cut and stained with hematoxylin and eosin to determinate morphological changes of tumor cells.

Statistical analysis

All data were tested for normality of distribution with the Shapiro-Wilk test. The differences between the experimental groups were statistically evaluated by one-way analysis of variance (one-way ANOVA) followed by a Holm-Sidak test for multiple comparison. A P-value of less than 0.05 was considered to be statistically significant. SigmaPlot Software (Systat Software, Chicago, IL, USA) was used for statistical analysis and graphical representation.

Results

Gene electrotransfer of plasmid silencing endoglin indicates a vascular targeted effects of the therapy

GET of either plasmids (pControl, TS) to melanoma tumors had statistically significant antitumor effectiveness compared to untreated tumors, which resulted in 8.2 ± 2.5 and 8.6 ± 3.0 days of tumor growth delay, respectively (Table 1). However, GET (TS) resulted in 44% of tumor free mice and 75% of them were resistant to secondary challenge, whereas in the GET (pControl) group no tumor free mice were obtained. Histological analysis (Table 2) of GET (TS) group demonstrated reduction of vascularization (14.9 ± 1.1%) and proliferating cells (49.5 ± 3.8%), whereas hypoxia (46.3 ± 3.0%) levels were increased and statistically significant to at least pertinent control groups (CTRL, pControl, TS and 3 × GET (pControl)). The levels of necrosis, apoptosis and number of infiltrating immune cells in the tumors were comparable in both GET treatment modalities (Figure 1) and statistically significant to at least CTRL, pControl and TS. These results indicated the vascular targeted effects of the GET (TS), used for silencing endoglin.

Irradiation, alone or combined either with plasmids injection or electric pulses, had minor effect on radioresistant melanoma tumors

Irradiation monotherapy with 15 Gy had minor effects on radioresistant melanoma tumor. The tumor growth delay was moderate in the groups of irradiation alone or in combination with injection of plasmids (from 0.7 ± 0.3 to 2.8 ± 1.2 days) and up to 11% of tumor free mice were obtained in the group of irradiation in combination with injection of plasmid pControl (Table 1). The results of histological analysis (Table 2, Figure 1) indicates an immunological effect of the irradiation alone or in combination with plasmids injection, since the number of infiltrating immune cells in the tumors was statistically significantly higher in comparison to control groups (CTRL, pControl, TS) and groups applying electric pulses (alone or combined with injection of plasmids (GET (pControl) and GET (TS)), and the tumor free mice were resistant to secondary challenge. Furthermore, irradiation alone caused the reduction in proliferating cell levels (53.3 ± 3.5%) and in tumor vascularization (30.1 ± 2.3%), whereas apoptosis (18.4 ± 1.8%) and hypoxia (30.2 ± 2.4%) levels were elevated. All of these results were statistically significant compared to CTRL, pControl and TS groups.

The application of electric pulses to tumors in combination with tumor irradiation resulted in better antitumor effectiveness than observed in each of these two treatment modalities alone. The tumor growth delay in combined treatment was 3.2 ± 1.3 days and 22% of tumor free mice were obtained (Table 1). The histological analysis of combined treatment demonstrated statistically significant higher levels of apoptosis (31.0 ± 2.0%) and hypoxia (51.7 ± 2.6%) in comparison to the groups of irradiation and electric pulses alone. Among these three groups no statistically significant differences were observed in the levels of necrosis. Furthermore, the analysis of levels of proliferating cells, the number of tumor blood vessels and immune cells infiltrating in the tumors, resulted in similar and moderate effectiveness of the combined treatment (3 × EP + IR) and irradiation (alone or combined with injection of plasmids), which statistically significantly differed from group of electric pulses alone. Nevertheless, in this combined treatment modality (3 × EP + IR) no tumor free mice were resistant to secondary challenge of tumors.

Combination of GET and irradiation exerts pronounced antitumor effects

The groups combining GET of plasmids (pControl, TS) and irradiation resulted in pronounced therapeutic effectiveness, with up to 89% and 88% of tumor free mice, respectively, and from those up to 63% and 57% of mice was resistant to secondary tumor challenge, respectively (Table 1). The histological analysis, of tumors excised 6 days after treatment (Table 2, Figure 1), indicated on similar mode of action of these combined treatment modalities (3 × GET (pControl or TS) + IR), since the elevation of necrosis (65.0 ± 7.6%, 79.2 ± 4.2%), number of immune cells (45.8 ± 3.0%, 46.5 ± 2.1%), reduction of tumor vascularization (18.9 ± 2.0%, 12.8 ± 1.0%) and proliferation (46.0 ± 4.8%, 46.7 ± 4.8%), did not differ between these groups. Only two statistically significant differences between the therapeutic groups combining GET (pControl, TS) and irradiation were observed which were the level of hypoxia (58.0 ± 2.8%, 66.7 ± 1.6%), and apoptosis (38.7 ± 3.1%, 50.3 ± 2.4%).

The high number of infiltrating immune cells (Table 2) in these tumors indicated on important mode of this therapeutic action; the highest and statistically significantly increased number of immune cells was observed in the groups combin-

TABLE 2. Immunohistological analysis of tumors

Therapeutic group	Necrosis (%)			Apoptosis (%)			Hypoxia (%)			Proliferation (%)			Vascularization (n of structures)			Immune cells (n of cells)		
	AM		SEM	AM		SEM	AM		SEM	AM		SEM	AM		SEM	AM		SEM
CTRL	25.0	±	2.6	11.3	±	1.6	4.7	±	1.0	92.2	±	1.3	51.6	±	3.2	7.9	±	0.9
pControl	20.0	±	2.6	11.6	±	1.0	5.4	±	1.0	92.1	±	1.2	47.1	±	4.7	8.3	±	1.1
TS	21.7	±	2.6	11.4	±	1.5	5.5	±	0.7	92.7	±	1.0	50.5	±	4.2	7.9	±	0.7
3 × EP	13.3	±	3.6	18.7	±	2.7	7.8	±	0.8	74.4	±	1.7	39.9	±	2.6	14.3	±	1.7
3 × GET (pControl)	53.3	±	7.9	19.5	±	3.0	15.3	±	2.9	75.6	±	2.4	35.3	±	4.2	15.1	±	1.6
3 × GET (TS)	65.8	±	7.6	26.7	±	2.2	46.3	±	3.0	49.5	±	3.8	14.9	±	1.1	19.3	±	1.7
IR	21.7	±	4.2	18.4	±	1.8	30.2	±	2.4	53.3	±	3.5	30.1	±	2.3	26.8	±	2.6
pControl + IR	37.5	±	8.0	18.7	±	1.9	32.3	±	2.5	54.5	±	4.7	24.5	±	1.8	26.4	±	2.1
TS + IR	43.3	±	8.0	19.9	±	1.5	31.6	±	2.3	52.0	±	2.9	23.8	±	1.6	26.4	±	2.3
3 × EP + IR	25.0	±	4.8	31.0	±	2.0	51.7	±	2.6	59.4	±	4.0	28.9	±	2.3	29.3	±	2.1
3 × GET (pControl) + IR	65.0	±	7.6	38.7	±	3.1	58.0	±	2.8	46.0	±	4.8	18.9	±	2.0	45.8	±	3.0
3 × GET (TS) + IR	79.2	±	4.2	50.3	±	2.4	66.7	±	1.6	46.7	±	4.8	12.8	±	1.0	46.5	±	2.1

AM = Arithmetic mean; Groups: (CTRL = control; EP = electric pulses; GET = gene electrotransfer; IR = irradiation; TS = pET-antiCD105); n = Number of structures or cells; SEM = Standard error

ing GET of plasmids and irradiation, followed by all the remaining groups that included irradiation (alone, TS, pControl and 3 × EP). In these groups, the immune cells infiltration was comparable and statistically significant to all of the other remaining groups. Furthermore, the immune cells infiltration was the lowest in the groups that included electric pulses (alone or with plasmids alone). Additionally, all of the mice that were tumor free after the therapies including irradiation, either alone or in combination with other modalities, had fur discoloration, known as vitiligo, indicating immune response (Figure 2). This was further confirmed with high number of mice that were resistant to secondary challenge in tumor free mice, with the exception of the group combining electric pulses and irradiation (Table 1). In these groups, in which tumors grew after secondary challenge, the growth rate and histology of the tumors were the same as after the initial induction of tumors (data not shown).

In addition, the safety of the treatment (irradiation alone or in combination with other modalities) was proven, since no body weight loss over 10%, or any other side effects were observed, except for the temporary hair loss in the irradiated area, without skin desquamation (data not shown).

Discussion

The results of this study indicate a dual effect of GET of plasmid encoding shRNA for silencing endoglin, the direct and the indirect, both having a radiosensitizing effect. The direct effect was on the tumor vasculature and also on melanoma tumor cells, whereas the indirect was observed with the use of plasmid devoid of therapeutic gene, through boosting the immune response in tumors. Furthermore, irradiation had mainly affected melanoma cells, although some effect on vasculature could also be noticed. In addition, the higher number of infiltrating immune cells in all of the groups combined with irradiation indicated an important role of the immune system. All of these effects had synergistic action and, in combined treatment modality of GET and irradiation, resulted in increased radiosensitizing effectiveness of melanoma tumors that resulted in prolonged tumor growth delay, which led to 88% of tumor free mice, of which 57% were resistant to secondary challenge of tumors.

Dual effectiveness of GET

The first direct effect of GET (TS) was on tumor vasculature, which was significantly reduced after the treatment. This vascular effect can be ascribed to the specificity of the plasmid for endothelial cells and to the endoglin silencing. This direct effect on tumor vasculature was also observed in other studies using GET of plasmid for silencing endoglin on melanoma[22,23] and other tumor models.[18,19,21] The vascular targeted effects in these studies were first confirmed in non-endoglin expressing tumors, *i.e.* murine mammary adenocarcinoma, by endotheli-

al-specific promoter[18,21] and non-specific, constitutive promoter[18–21], that resulted in pronounced antitumor effectiveness. In melanoma tumor model, B16F10-luc, the GET of plasmids silencing endoglin resulted in significant antimetastatic effectiveness.[22] Furthermore, GET of plasmid silencing endoglin, with constitutive promoter, performed on small melanoma B16F10 tumor model (4 mm^3 at the beginning of therapy) confirmed vascular targeted effects, which resulted in prolonged tumor growth delay and tumor free mice (58%), also due to nonspecific nature of constitutive plasmid that was used in this study silenced endoglin also in melanoma cells.[23] Nevertheless, in the current study, which was done on bigger tumors (40 mm^3), in addition to significant increased level of hypoxia, also decreased number of proliferating tumor cells was observed. This was attributed to the second direct effect of GET (TS), that is silencing of endoglin also in melanoma cells, since it is known that plasmid with tissue specific promoters can be leaky and can also be transcribed in non-targeted tissue.[27] Therefore, GET (TS) has dual direct effects; primarily by targeting vasculature and secondly by inhibition of proliferation of melanoma cells. These two effects together resulted in 44% of tumor free mice, from which 75% were resistant to secondary challenge of tumor cells.

Nevertheless, high level of infiltrating immune cells in the tumors, indicated also an indirect effect of this treatment, through the stimulation of immune response. This is also supported by the data obtained with GET of the plasmid devoid of therapeutic gene (pControl). In comparison to GET of therapeutic plasmid, TS, no tumor cures were obtained, however, in other measured parameters, infiltration of immune cells into the tumors, levels of necrosis and apoptosis as well as in tumor growth delay no statistically significant differences were obtained between these groups. Thus, to a certain degree, a similar mode of antitumor action can be ascribed to the GET of plasmid DNA, mainly because the values of these parameters were statistically different from pertinent control groups. Antitumor effectiveness of GET of non-therapeutic (control) plasmids was also observed in other studies[9,10], and the authors indicated that effectiveness was due to involvement of immune system. Namely, the plasmid introduced during GET can act as a DAMP that is recognized through different sensors (Pattern Recognition Receptors), leading to the activation of the signal transduction cascade that ultimately triggers the production of type 1 interferons and other cytokines.[28] These act as a link between the innate and adaptive immune response[29] and can induce the adaptive immune response against the introduced DNA and consequently the transfected cells. Furthermore, similar immune response can also be triggered by the stress[30] that is produced during the transfection procedure, like mechanical stress, heat, and ROS, that have all been previously reported after electroporation.[31,32]

Priming effect of irradiation

To target primary tumors, as was done in this study, irradiation is one of the most used treatment modalities.[33] This therapeutic approach alone or in combination with injection of plasmids, in radioresistant melanoma tumor model, resulted in moderate tumor growth delay, which was attributed to decreased proliferation of melanoma cells, increased levels of apoptosis and necrosis. Further histological analysis indicated on the radiation damage of the tumor vasculature, as a second effect of the irradiation, as seen in other studies.[34,35] This vascular damage was less pronounced than in group of GET (TS), although in comparison to GET (TS), irradiation monotherapy resulted in higher infiltration of the immune cells in the tumors. Furthermore, in the group combining irradiation and injection of therapeutic plasmid, one mouse was tumor free, which was also resistant to secondary challenge of tumor. This indicates a priming effect of irradiation combined with introduction of foreign DNA, through boosting the immune response. When irradiated cells die, they release their antigens in the context of the danger signal (DAMPS), which result in the priming of a tumor specific immune response against the released antigens. Therefore, irradiated tumors can sometimes act as a powerful individualized *in situ* vaccine[13], which is manifested as the abscopal effect of the irradiation. This was confirmed in *in vivo* studies indicating that irradiation can induce *in vivo* priming of T cells to exogenous model antigens engineered to be expressed by tumors.[13] In melanoma tumor model, priming of antitumor T cells to the model antigen ovalbumin, was more effective when single, 15 Gy dose was used, rather than 3 Gy given in 5 consecutive days.[36] Further on, another group also showed induction of antitumor T-cell responses with other antigen expression, when single 20 Gy dose was applied, but not by 5 Gy given 4 times.[37]

The combination of electric pulses and irradiation exerted moderate antitumor effectiveness that resulted in 22% of tumor free mice. Similar results

were obtained in our previous studies on murine mammary adenocarcinoma[18], sarcoma[17] and Ehrlich-Lettre ascites[38], where also 20%, 27% and 54% of tumor free mice were observed, respectively. Therefore we can assume that electroporation of tumors contributes to radioresponsiveness of melanoma, most probably due to generation of ROS[32,39] after application of the electric pules, which also resulted in significantly elevated levels of hypoxia in our histological analysis.

Immune boosting and radiosensitization by plasmid DNA

The combined treatment modalities (combination of GET (TS or pControl) with irradiation) exerted excellent, highly statistically significant antitumor effect in comparison to control groups, which resulted in 88% and 89% of tumor free mice, respectively, of which 57% and 63% of mice were resistant to secondary challenge of tumors, respectively. The results of histological analysis were similar between groups of combined treatment modality (GET + IR), regardless of the applied plasmid. The only two differences were on the levels of apoptosis and hypoxia. The analysis of the presence of the immune cells showed on the highest number of the immune cells in the groups of GET of plasmids and irradiation, followed by irradiation groups (alone or combined with injections of plasmids or combined with electric pulses). Therefore, it can be presumed that the priming effect of irradiation can be boosted by GET of the plasmid DNA to fully exert vaccinating effect. Additionally, fur discoloration, vitiligo, an immune-mediated destruction of normal melanocytes that has also been recognized as a positive prognostic indicator for treatment response[40,41], was also observed in this study in all the groups where tumor free mice were observed (except 3 × GET (TS)). In addition, majority of the tumor free mice were resistant to secondary challenge of tumors, further indicating on the development of the immune memory. The tumors in experimental groups, which were resistant to secondary challenged of tumor, were therefore radiosensibilized through GET of plasmid, since in the group combining electric pulses and irradiation, no mice resulted resistant to secondary challenge of tumor. The GET of plasmids in combination with irradiation presumably generated many danger signals that collectively define immunogenic cell death.[13]

Furthermore, the immunogenicity of tumor might also play an important role in combined treatment modality.[42] In the current study we obtained significant antitumor effectiveness and up to 89% of tumors free mice, that were up to 63% resistant to secondary challenge of tumors, when combining GET and irradiation in melanoma tumor model. Furthermore, in our previous study combining the same treatment modality on a different tumor model, murine mammary adenocarcinoma TS/A, we achieved up to 44% of tumors free mice with therapeutic plasmid silencing endoglin and 20% tumor free mice with the control plasmid.[18] We can presume that the boosting of immune response depends on the tumor type, melanoma being more immunogenic that mammary adenocarcinoma TS/A. Nevertheless, the mechanisms of these therapies are not fully elucidated. Currently, we presume that there is involvement of DNA sensors, ROS and specific immune response after irradiation, but further studies are needed.

Conclusions

The results of this study indicate that irradiation can in radioresistant mice tumors, such as melanoma, by release of tumor associated antigens serve as the target of the immune response. This can be further boosted by GET of plasmid, with or without therapeutic gene, which was confirmed by the equal radiosensitization resulting in prolonged tumor growth delay and up to 89% of tumor free mice, which kept immune memory to melanoma cells. In addition, GET of therapeutic plasmid silencing endoglin has also direct effect on vasculature and tumors cells; however in combination with radiotherapy this effect was masked by pronounced immune response.

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research article

Comparison between cryoablation and irreversible electroporation of rabbit livers at a location close to the gallbladder

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Background. The ablation of liver tumors located close to the gallbladder is likely to lead to complications. The aim of this article is to compare the safety and efficacy of irreversible electroporation (IRE) and cryoablation in rabbit livers at a location close to the gallbladder.

Materials and methods. We performed cryoablation (n = 12) and IRE (n = 12) of the area of the liver close to the gallbladder in 24 New Zealand white rabbits in order to ensure gallbladder damage. Serum aminotransferase and serum bilirubin levels were measured before and after the ablation. Histopathological examination of the ablation zones in the liver and gallbladder was performed on the 7th day after the ablation.

Results. Seven days after the ablation, all 24 animals were alive. Gallbladder perforation did not occur in the IRE group; only mucosal epithelial necrosis and serous layer edema were found in this group. Gallbladder perforation occurred in four rabbits in the cryoablation group. Serum aminotransferase and serum bilirubin levels obviously increased in both groups by Day 3 and decreased gradually thereafter. The elevation in aminotransferase and bilirubin levels was greater in the cryoablation group than the IRE group. Pathological examination revealed complete necrosis of the liver parenchyma from the ablation center to the gallbladder in both groups, but bile duct and granulation tissue hyperplasia were observed in only the IRE group. Full-thickness gallbladder-wall necrosis was seen in the cryoablation group.

Conclusions. For ablation of the liver area near the gallbladder, IRE is superior to cryoablation, both in terms of safety (no gallbladder perforation in the IRE group) and efficacy (complete necrosis and rapid recovery in the IRE group).

Key words: cryoablation; irreversible electroporation; liver; gallbladder; ablation

Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy worldwide[1], and a large proportion of patients with HCC are ineligible for tumor resection due to several factors, such as poor hepatic reserve (cirrhosis), multicentric tumors and extrahepatic disease.[2-3] In HCC patients who are not candidates for surgery or liver transplantation, the National Comprehensive Cancer Network (NCCN) guidelines recommend the use of locoregional ablative methods, such as radiofrequency ablation (RFA), microwave ablation (MWA) and cryoablation. However, when the HCC lesion is located in close proximity to structures such as the major bile duct, gallbladder and diaphragm, ablation should be performed with caution in order to avoid damaging these structures. According to Lee *et al.*, radiofrequency ablation of the area of the liver abutting the gallbladder can cause substantial

complications, including gallbladder perforation, especially when the ablation is performed without maintaining a safe distance.[4] Furthermore, it has been reported that ultrasound-guided percutaneous microwave ablation can be safely used for hepatic malignancies adjacent to the gallbladder, only when the ablation is performed under strict temperature monitoring.[5] Cryoablation is achieved using the high-voltage-dependent release of argon and helium to induce a cycle of low temperature followed by thawing in order to cause physical damage and treat tumors. Because major vessels in close proximity to the tumor can absorb large amounts of heat during ablation (known as the "heat sink effect"), cryoablation can minimize vascular injury during the ablation of liver tumors. In addition, cryoablation is associated with good control, which allows the accurate targeting of the necrotic area and results in few side effects.[8] This method might therefore be suitable for the ablation of liver lesions located near the gallbladder.[6,7] Irreversible electroporation (IRE) is a novel ablation technology that utilizes short pulses of high-voltage electrical energy to induce tissue necrosis. IRE has many special advantages, including short ablation time, preservation of the internal structure of vital organs and lack of the heat/cold-sink effect.[8] Although not yet included in the NCCN guidelines, IRE, owing to its intrinsic characteristics, might be a superior alternative to other ablation techniques for the ablation of tumors situated near the gallbladder. Therefore, IRE was selected for comparison with conventional cryoablation techniques. In this animal experimental study, we performed cryoablation and IRE of the liver area located 0.5 cm from the gallbladder in rabbits, and compared and evaluated the safety and efficacy of these two ablation techniques in order to identify the optimal ablation method for liver tumors located near the gallbladder.

Materials and methods

Experimental animals

In total, 24 healthy female New Zealand white rabbits, weighing (2.5 ± 0.2) kg each, were provided by the Animal Experimental Center of Jinan University. They were maintained in a clean, and mechanically-ventilated environment at a constant temperature. The rabbits were randomly assigned to the cryoablation group (n = 12) and IRE group (n = 12). All animals were fasted for 24 h before surgery, and intravenous access was established for intraoperative drug administration. The study was approved by the Research Animal Care and Use Committee of Guangzhou Fuda Cancer Hospital (approval number, LL201402).

Cryoablation

A cryoablation system (Cryocare™, Endocare, Irvine, CA), composed of the main body, argon/helium gas container and cryoprobes (CRYO-42; Endocare), was used for cryoablation. Rabbits were anesthetized by the intramuscular injection of 44 mg/kg·bw ketamine, intubated and connected to a respirator for mechanically controlled respiration. Intraoperative anesthesia was maintained with 1.5% – 2% isoflurane.

General anesthesia was induced and maintained by the intramuscular injection of 44 mg/kg·bw ketamine. The surgeon had 17 years of experience in cryoablation. The rabbits were fixed in a supine position on the operating table and shaved. The skin was disinfected with iodine, and an abdominal incision was made to expose the liver and gallbladder under sterile conditions. The gallbladder was pressed on the liver, and a cryoprobe with a diameter of 1.4 mm was inserted into the liver until its tip was approximately 0.5 cm away from the gallbladder. The location of the tip was monitored using an ultrasound system (DP-50Vet, Mindray, Shenzhen, China; Figure 1A). When it was confirmed that the cryoprobe was connected to the main equipment, the argon gas was released to freeze the tissue. The cryoablation protocol was a double freeze-thaw cycle consisting of a 2-min freeze and 1-min thaw; this protocol ensured that part of the gallbladder was included within the ablation zone of the ice ball (Figure 1B). No animal died during the cryoablation process. After the cryoablation, the cryoprobe was retrieved. The liver was put back into the abdominal cavity, and conventional abdominal wall sutures were placed to complete the cryoablation. The cryoablation tract was filled in with a thrombin-soaked gelatin sponge and closed with a short suture.

IRE

General anesthesia was induced with an intramuscular injection of 44 mg/kg·bw ketamine and maintained with 1.5% – 2% isoflurane. Respiration was controlled using a respirator during the operation (tidal volume: 30 ml/time, respiratory rate: 30/min, oxygen concentration: 100%). Before the IRE operation, 0.12 mg/kg·bw pancuronium was

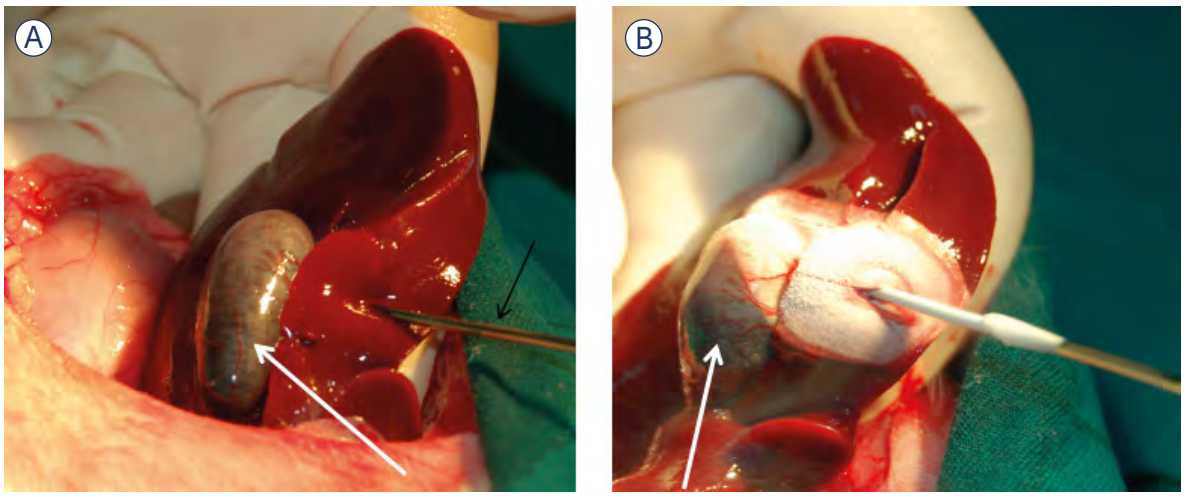

FIGURE 1. Photos of the liver and gallbladder before cryoablation. **(A)** Cryoprobe insertion. **(B)** Ice ball formation. The white arrows in **(A and B)** indicate the gallbladder. The black arrow in **(A)** indicates the cryoprobe.

intramuscularly injected to block muscle contraction. IRE was performed using the Angiodynamics Nanoknife system (AngioDynamics, Latham, NY). The Nanoknife system consists of a main body and electrode probes (catalog no. 20400104, AngioDynamics), which were used for electroporation ablation. Monopolar probes were used, which could be changed to achieve 1 – 4 cm of electrode exposure. The size of the ablation zone created depended on the exposed length of the applicator as well as the distance between the probes. When the gallbladder was exposed, two monopolar probes were carefully inserted into the liver (Figure 2A). The probes were inserted to a depth of 0.5 cm into the liver parenchyma under ultrasound guidance (DP-50Vet, Mindray, Shenzhen, China), and were kept 1.5 cm apart and 0.5 cm away from the gallbladder (Figure 3). The monopolar probes were fastened with a spacer device. The ablation parameters consisted of nine groups of ten electrical

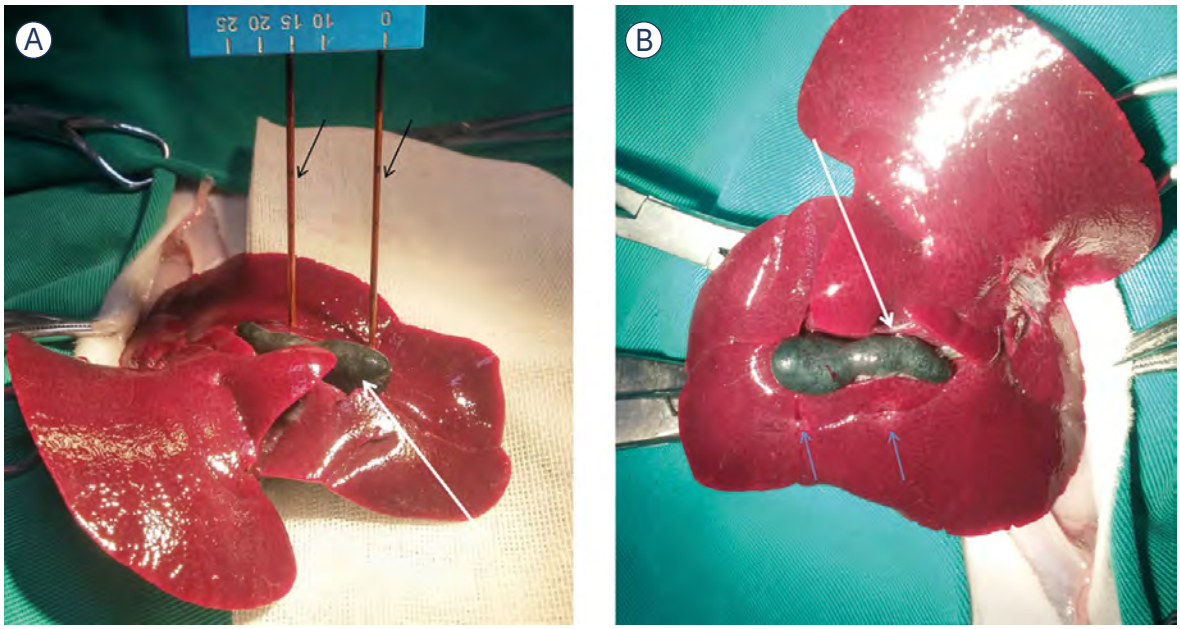

FIGURE 2. Photos of the liver and gallbladder before IRE ablation. **(A)** Electrode placement. **(B)** Immediately after the probes are pulled out, the gallbladder and liver do not show any significant changes. The white arrows in **(A and B)** indicate the gallbladder. The black arrows in **(A)** indicate the IRE probes. The blue arrows in **(B)** indicate the insertion sites of the two monopolar IRE probes.

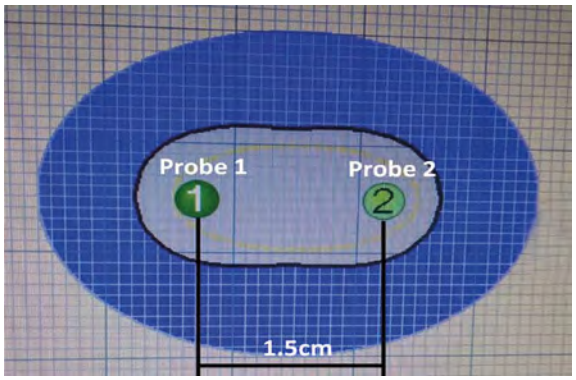

FIGURE 3. Distance between two monopolar IRE probes. The two probes are 1.5 cm apart.

pulses (total: 90 pulses), and each electrode pulse was 70-μs long; the output voltage was set at 1500 V/cm. This protocol ensured that the electric field covered part of the gallbladder (Figure 2B). Pulse delivery was synchronized with the R waves of the cardiac cycle in case of arrhythmia. One rabbit died during the IRE ablation because of tracheal intubation failure; we replaced this rabbit with another rabbit. After the IRE ablation, muscle relaxation was reversed with intramuscular injections of 5 μg/kg·bw neostigmine and 5 μg/kg·bw atropine.

Postprocedure care

The 24 experimental animals returned to consciousness under the monitoring of a senior veterinarian and were sent back to the specific pathogen-free experimental room, which was filled with fresh air and maintained at a constant temperature. All the rabbits were under meticulous care, and pain was managed with intramuscular buprenorphine (0.01 mg/kg) and oral meloxicam (0.4 mg/kg). In addition, the rabbits received daily intramuscular injections of 40 mg/kg·bw cephazolin for 3 days after the surgery in order to prevent infection. For 7 days after the surgery, the animals were observed by veterinarians, who assessed whether the rabbits had suffered any postoperative complications. Feeding was gradually restored over 3 days after the surgery in both groups of animals; however, both food and water intake were lower in the cryoablation group than in the IRE group.

Detection of serum aminotransferases and serum bilirubin

We collected blood (2 ml, from an ear marginal vein) 1 day before the operation and on days 1, 3, 5 and 7 after the ablation. The blood samples were centrifuged to obtain serum. Hepatic function was measured using an automatic biochemical analyzer (Hitachi-7100; Hitachi, Tokyo, Japan). Alanine aminotransferase/aspartate aminotransferase (ALT/AST) and serum bilirubin levels were determined with kits for in vitro diagnostic use (Biosino Biotechnology and Science Ltd, Beijing, China) by the velocity method.

Pathology

No animal died after the surgery. At 7 days after the ablation, all the animals were euthanized with an overdose of intravenous pentobarbital sodium (120 mg/kg, Sleepaway; Fort Dodge Animal Health, Fort Dodge, IA). The liver tissues from the ablation tract to the gallbladder were harvested, and the ablation lesions were cut and sectioned from the center. The maximum diameter was measured, and the lesions were photographed. Then, the tissue samples, including the abnormal and adjacent normal tissues, were fixed in 10% neutral buffered formalin, processed routinely for histology, embedded in paraffin, cut into 5-μm-thick slices and stained with hematoxylin and eosin. Pathological analyses were performed by an attending surgical pathologist.

Statistical analysis

All analyses were performed using GraphPad Prism 5 software (GraphPad Inc., San Diego, CA). The size of the ablation lesions and the serum ALT, AST and bilirubin levels were analyzed using the Student t-test. A P value of < 0.05 was considered to indicate a statistical difference, while P values of < 0.01 and < 0.001 indicated significant differences.

Results

Pathological appearance

In the cryoablation group, there were severe inflammatory adhesions between the ablation sites in the liver and gallbladder, the abdominal wall and the mesentery (12/12); the liver ablation zone was khaki-colored and oval. The lesion area had a narrow and sharp edge of light pink edematous tissue, and the ablation range obviously covered the gallbladder. There was a large gray area of necrosis adjacent to the gallbladder, and gallbladder perforation was found in four animals (Figure 4). In the IRE group, there were fewer inflammatory adhesions than in the cryoablation group (5/12 *vs.*12/12).

TABLE 1. Sizes of liver and gallbladder lesions at 7 days after the ablation ($\bar{x} \pm s$)

Ablation	Major diameter of liver lesions (cm)			Gallbladder lesions	
methods	Gallbladder side (n = 12)	Diaphragm side (n = 12)	P value	Major diameter (cm)	P value
Cryo	1.5 ± 0.4	3.8 ± 0.8	> 0.05	1.5 ± 0.4 (n = 8)	> 0.05
IRE	1.4 ± 0.4	4.1 ± 0.7	> 0.05	0.8 ± 0.2 (n = 12)	

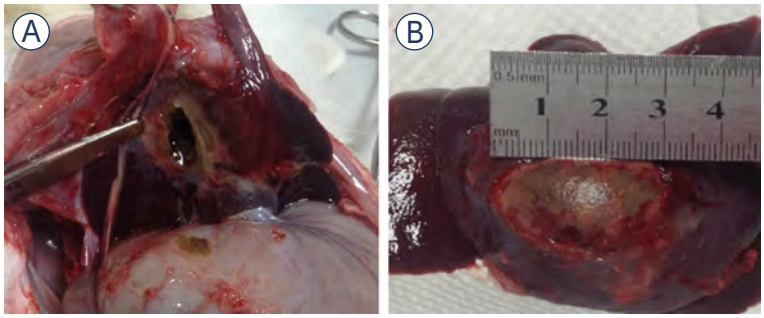

FIGURE 4. Photos of the liver and gallbladder after cryoablation. The liver as viewed from the **(A)** gallbladder and **(B)** diaphragmatic side 7 days after the ablation.

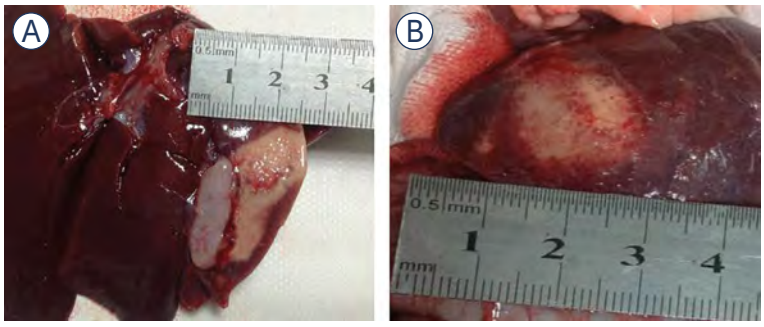

FIGURE 5. Photos of the liver and gallbladder after IRE ablation. The liver as viewed from the **(A)** gallbladder and **(B)** diaphragmatic side 7 days after the ablation.

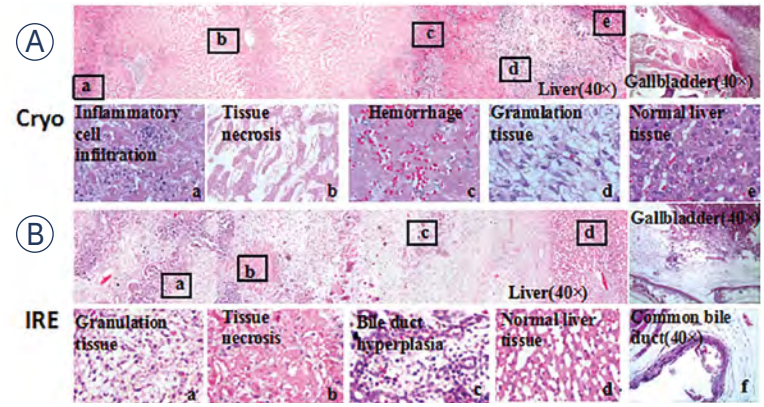

FIGURE 6. Pathological changes in the cryoablation (Cryo) and IRE ablation zones 7 days after ablation. **(A)** The top images show the ablated liver and gallbladder areas (magnification, 40×). The bottom images **(A to E)** show enlarged versions of the areas marked in the top images (magnification, 400×). **(B)** The top images and **(F)** show the ablated liver, gallbladder and common bile duct (magnification, 40×). The bottom images **(A to D)** show the enlarged versions of the areas marked in the top images (magnification, 400×).

The lesion area was yellow and oval with sharp edges. The ablated gallbladder tissue appeared grayish-white, but none of the 12 experimental animals showed gallbladder perforation (Figure 5). Details of the sizes of the ablation lesions are presented in Table 1; there were no differences in lesion size between the two groups. As gallbladder perforation was found in four animals in the cryoablation group, we recorded the major diameter of the gallbladder lesions in only eight animals in this group.

There were no intact hepatic cells in the liver slices in both the cryoablation and IRE groups. From the cryoablation tract to the liver edge, the cryolesion appeared as a pink-stained area of disintegrating tissue that included hemorrhagic congestion and tissue necrosis, and it showed nuclear disappearance, no intact cellular structures, a band of local bleeding, and abundant inflammatory cell infiltration (Figure 6A). In the IRE group, the main pathological changes consisted of small bile duct, acute granulation tissue hyperplasia, rare congestion, hemorrhage band and a small amount of inflammatory cell infiltration (Figure 6B). The most conspicuous difference between the two groups was the appearance of the gallbladder: in the cryoablation group, full-thickness gallbladder wall necrosis, muscular collapse and mucosal loss were found, and the necrotic area was large and uniformly stained red. In the IRE group, there was mucosal epithelial necrosis but no full-thickness gallbladder wall necrosis, and serous layer edema were largely concentrated in the area of the gallbladder wall near the liver.

Changes in serum aminotransferase and serum bilirubin levels

In both groups, the serum aminotransferase and bilirubin levels obviously increased on day 3, gradually decreased thereafter and recovered to normal in a week. At each time point, the serum aminotransferase and serum bilirubin levels were higher in the cryoablation group than in the IRE group (Table 2).

TABLE 2. Changes in aminotransferase and serum bilirubin levels over time ($\bar{x} \pm s$)

Hepatic function	Ablation method	d-1	d-1	d-3	d-5	d-7
ALT (U/L)	IRE	38.35 ± 3.22	167.05 ± 19.50	214.83 ± 10.37	119.60 ± 12.99	68.25 ± 10.08
	Cryo	39.20 ± 3.90	195.03 ± 9.75**	289.58 ± 12.81**	142.35 ± 57.85*	94.90 ± 9.78*
AST(U/L)	IRE	34.10 ± 0.86	47.64 ± 0.69	60.21 ± 2.55	46.78 ± 1.71	31.05 ± 0.43
	Cryo	35.26 ± 2.45	60.11 ± 3.44**	79.12 ± 2.58**	52.80 ± 3.41*	37.41 ± 2.50*
Serum bilirubin (μmol/L)	IRE	8.08 ± 2.19	26.01 ± 3.68	35.15 ± 5.70	29.09 ± 3.27	21.41 ± 3.39
	Cryo	7.59 ± 2.01	32.72 ± 4.08**	37.34 ± 6.14*	35.88 ± 4.28**	25.86 ± 5.37*

*P < 0.05 and **P < 0.01, compared with the IRE group

Discussion

In the study by Fairchild *et al.*, the ice ball extended into the gallbladder lumen (up to a mean distance of 6 mm), but no cases of gallbladder perforation occurred, even when the iceball had extended as far as 1.8 cm into the gallbladder lumen.[9] This may be explained by the theory that the bile within the gallbladdder may dissipate the cooling effect of the cyroprobe around the gallbladder fossa. However, in our study, perforation occurred in one-third of the animals, and inflammatory adhesions occurred in all the animals in the cryoablation group. We attribute this difference to the fact that in our study, the ice ball extended to a far greater distance into the gallbladder lumen, and thus, the cryodamage to the gallbladder was more severe. Furthermore, as more of the gallbladder wall was cryoablated, the "dissipating effect" of the bile was extremely weakened, and a local self-limited inflammatory response resulted in gallbladder wall edema and reactive fluid accumulation, which in turn increased the severity of the gallbladder damage.[9] In contrast, IRE caused a color change in the gallbladder, but no perforation occurred 7 days after ablation. In addition, full-thickness wall necrosis occurred only in the cryoablation group; in the IRE group, the damage to the gallbladder was confined to the mucous layer. This is because IRE is a promising, non-thermal technology that takes advantage of electric fields instead of thermal energy to induce cell death. The most distinctive feature of IRE is that it has little influence on the adjacent large vessels and can preserve the collagen matrix of tissue structures.[10-13] In addition, it does not lead to significant ductal damage if the electrodes are not placed very close to the ducts.[14] In our study, the IRE probes were 0.5 cm away from the gallbladder, and the damage to the gallbladder caused by IRE was acceptable. Thus, our findings indicate that IRE is a potentially safe modality for the ablation of hepatic tumors adjacent to the gallbladder.

Furthermore, although the lesion sizes and changes in the serum aminotransferase and serum bilirubin levels were similar in the two groups, the damage to the liver cells was more severe in the cryoablation group. This phenomenon may be explained as follows: Cryodamage mainly leads to cell necrosis, and secondary injuries can be mediated by the resultant inflammatory reaction.[15] According to current studies, thermal injury may require time to cause gallbladder perforation[16] and perforations are usually found in the acute phase, not the immediate phase.[4] In contrast, electrical injury (IRE) mainly causes cell apoptosis and tissue damage, and repair following IRE is rapid.[8] At 7 days after cryoablation, tissue destruction was still ongoing (areas of cell disruption with hemorrhage), while at the same time point, tissue repair was obvious in the IRE group (areas of bile duct hyperplasia).

Collectively, the above findings indicate that IRE is unlikely to destroy the gallbladder wall and offers the advantage of faster repair of the damaged tissue. Thus, in this study, IRE was found to be superior to cryoablation in terms of protecting the gallbladder, and was a safer procedure than cryoablation.

Complete necrosis of tumor cells with no residual tumor is the main goal of ablation. During cryoablation, ice ball formation can be precisely monitored using ultrasonography or computed tomography. It is impossible to induce tumor cell necrosis in the periphery of the ice ball because the peripheral temperature of the ice ball is 0°C[17], and the temperature that is lethal to cells is at least –20°C.[18,19] To ensure efficacious cryoablation, the edge of the ice ball is usually kept 1 cm beyond the edge of tumor, and the double freeze-thaw cycle protocol is used. In our study, the efficacy of

cryoablation of the liver area near the gallbladder reached the expected levels, as determined based on the appearance (gray-red lesion spread to the gallbladder) as well as histopathological examination (no live cells between the cryoprobe and gallbladder) of the cryozones. IRE ablation has been reported to achieve nearly 100% necrosis in the ablation zone at 24 h in a swine liver model.[8,20] The effectiveness of IRE ablation over a longer time is one of the focuses of this study. Seven days after IRE ablation, not only were the target cells destroyed completely, but the area of bile duct hyperplasia was far greater than the area of pink, disintegrating tissue. All in all, although there was a great difference between the abilities of the two therapies to destroy the liver cells, the long-term effects of both treatments were good.

The major limitation of this article is the lack of short- and long-term studies. Analysis of the pathological appearance of the gallbladder at different time points after ablation would help provide a stronger basis for the clinical application of the two ablation methods. Future experiments should use cholangiography to observe changes in the gallbladder and determine the integrity of the main bile duct.

In conclusion, we compared the safety and efficacy of cryoablation and IRE in rabbit livers, at a location close to the gallbladder. IRE provided complete ablation of the target tissue and completely retained the entire gallbladder structure. In the case of liver tumors situated close to the gallbladder, cryoablation must be performed carefully to avoid direct trauma to the gallbladder. The comparison in this article has a certain meaning in terms of the selection of instruments for liver tumor ablation. However, the clinical value of our findings and long-term curative effect of IRE remain to be confirmed.

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research article

Cancer burden in Slovenia with the time trends analysis

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Background. The aim of our study was to describe cancer burden and time trends of all cancers combined, the most frequent as well as the rare cancers in Slovenia.

Patients and methods. The principal data source was the population-based Cancer Registry of Republic of Slovenia. The cancer burden is presented by incidence and prevalence for the period 1950–2013 and by mortality for years 1985–2013. The time trends were characterized in terms of an average annual percent change estimated by the log-linear joinpoint regression. The Dyba-Hakulinen method was used for estimation of incidence in 2016 and the projections of cancer incidence for the year 2025 were calculated applying the Globocan projection software.

Results. In recent years, near 14,000 Slovenes were diagnosed with cancer per year and just over 6,000 died; more than 94,000 people who were ever diagnosed with cancer are currently living among us. The total burden of cancer is dominated by five most common cancer sites: skin (non-melanoma), colon and rectum, lung, breast and prostate, together representing almost 60% of all new cancer cases. On average the incidence of common cancers in Slovenia is increasing for 3.0% per year in last decade, but the incidence of rare cancers is stable.

Conclusions. Because cancer occurs more among the elderly, and additionally more numerous post-war generation is entering this age group, it is expected that the burden of this disease will be growing further, even if the level of risk factors remains the same as today.

Key words: cancer burden; cancer incidence; time trend; Cancer Registry of Republic of Slovenia

Introduction

Cancer is a growing public health challenge in Europe, with substantial and persistent inequalities in the incidence, mortality and survival within and between Member States.[1] There were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide.[2] Europe comprises only one eight of the total world population but has around one quarter of the global total number of cancer cases.[1,3] Without effective interventions (comprehensive cancer prevention and control policies) the burden of cancer will increase dramatically.[4] Almost 200 cancer registries are established only in Europe with the same goal of monitoring the burden of disease and the effectiveness of cancer control measures.[5,6]

Slovenia has a long tradition in establishing cancer control and it has one of the oldest population-based cancer registries in Europe – Cancer Registry of Republic of Slovenia (CRS) that was founded in 1950 at the Institute of Oncology Ljubljana. Notification of cancer has been compulsory in Slovenia since the foundation of the CRS and is prescribed by law.[7] The main source of data are notifications of cancer, gathered from all hospitals and diagnostic centres in Slovenia, exceptionally also from primary health care centres in case the patient has not been referred for further diagnostic investigations and/or treatment. Since 2010, regular annual reports[7] of CRS are supported by web-

portal SLORA (www.slora.si/en), where on-line analyses of CRS data are available.[8]

The data on cancer, that are collected by the CRS, incidence, survival and prevalence, serve together with mortality data, collected by the National Institute of Public Health as the basis for assessing the cancer burden in the country. They are important for planning and evaluation of all levels of cancer control: primary prevention, diagnosis, treatment, palliation and rehabilitation, for planning facilities and funding needed for cancer control (personnel, equipment and hospital capacities). Furthermore, they are basis for clinical and epidemiological research in Slovenia and in international multicentre studies as well as for evaluation of effectiveness of cancer screening programs.[5-7]

In Slovenia, all patients with cancer have equal access to quality treatment, which is completely covered by obligatory health insurance. Primary care is provided by health care centres and general practitioners included in the public network. Secondary health care is provided by 11 general hospitals, two tertiary Clinical Centres, in Ljubljana and Maribor and Golnik Clinic. The Institute of Oncology Ljubljana, founded in 1938, is the only national comprehensive centre for cancer diseases, involved also in research and education. Common cancers are treated in general hospitals also, but the Institute is the referral centre for rare cancers; furthermore, radiotherapy is administered only there.[9] It was estimated that health-care costs of cancer per person in Slovenia is around 70% of the European average cancer case costs.[10] Since 2010, the National Cancer Control Programme is aimed to improve all aspects of cancer prevention and care in Slovenia.[11,12]

In this study we are presenting the incidence, mortality and prevalence data collected by the Cancer Registry of Republic of Slovenia in order to describe cancer burden together with the existing time trends and projections in Slovenia, complementing the Annual Reports and SLORA web portal data.

Patients and methods

The basic data source of our analysis is the population-based Cancer Registry of the Republic of Slovenia. The mortality data are provided by the National Institute of Public Health. All the data refer to all cancer patients, residents of Republic of Slovenia at the time of diagnosis, irrespective of where they have been treated or where they have died. The CRS' quality and completeness indices suggest that cancer registration in Slovenia adequately covers the entire population. To assure the completeness and to obtain additional information on registered cancer cases, CRS is linked with several governmental and health databases. Synchronisation of data between different sources is based on comparing unique personal identification number which is assigned to every resident in Slovenia and recorded in every state registry including CRS. Using unique personal identification number guaranties data integrity, data quality and prevents data duplication. CRS links with the Central Register of Population instantaneously through secure on-line connection (24/7 availability) and daily updates information on vital status and address for each person registered by CRS. The electronic linkage to the national Mortality Database and to the breast, colorectal and cervical screening registries is performed several times per year.[13]

The data on gender, date of diagnosis, age at diagnosis, code of primary site according to the International classification of Diseases 10th edition, morphological code according to International Classification of Disease for Oncology 3rd edition, vital status and cause of death for deceased were extracted from the CRS' database for all cancer cases. The cancer burden is presented by three basic epidemiological indicators: incidence, prevalence and mortality. Incidence and mortality are absolute numbers of all newly diagnosed cancer cases or number of deceased from cancer in individuals with permanent residence in Slovenia in one calendar year. New primary cancers of the same histology in paired organs, *e.g.* in the left and right breast, are not comprised in the incidence figures, neither are any new cancers of the same histology appearing in the same organ, *e.g.* multiple lesions of the colon. Prevalence is the number of all cancer patients that are alive on a given date, regardless of when they were diagnosed with cancer. Lifetime cancer prevalence is defined as all persons living and had ever been diagnosed with cancer, while partial prevalence counts only those patients, diagnosed with cancer within a defined period of time *e.g.* 1, 5 or 10 years before the date on which prevalence is calculated. Incidence and prevalence measures are available for the period from 1950 to 2013, the mortality measures since 1985.

Incidence rates were age standardised using the world standard population[14] and represent the hypothetical crude rate assuming that the age struc-

ture in the observed (Slovenian) population is the same as in the standard population, *i.e.* if the age structure of the population would remain the same over the time. The time trends were analysed using the log-linear joinpoint regression on age standardised incidence and mortality rates, implemented in the Joinpoint Regression program.[15] The trends were characterized in terms of an average annual percent change (APC) of the incidence and mortality rates, assuming the rates change at a constant percentage every year in the analysed time period.

The method by Dyba and Hakulinen[16] was used for estimation of incidence in 2016. This model includes patients according to the period of diagnosis and their age at diagnosis for the last ten registered years. The population age structure was fitted for the current year (2016), provided by Statistical Office of the Republic of Slovenia. Projections of cancer incidence for the year 2025 were calculated applying the Globocan projection software.[2] The expected number of new cancer cases was computed by multiplying the age-specific incidence rates estimated for 2012, by the expected population (size and age structure) for 2025. This analysis assumes that recent trends in cancer incidence will continue and that predictions of the size and age structure of the future population of Slovenia are accurate.

Results and discusion

Cancer burden

In Slovenia in the year 2013 there were 13,717 (666/100,000) patients newly diagnosed with cancer, 7,442 (730/100,000) males and 6,275 (604/100,000) females. According to the EUCAN estimates Slovenia ranked in the 8th place among 40 European countries in 2012 considering all cancer sites incidence.[3] At the end of December 2013, there were 94,073 people (41,607 males and 52,466 females) alive who were diagnosed with one or more cancers at any time since the foundation of the CRS. One-year partial prevalence which includes patients, diagnosed with cancer within one year before the date of calculation was 11,101. This reflects the number of patients which are currently in the process of their primary oncological management. Further on, there were 31,148 patients included in the regular follow-up (1-4 years prevalence). According to the official data on cancer mortality 6,075 (295/100,000) patients died from cancer in 2013 in Slovenia; 3,392 (333/100,000) males and 2,679 (258/100,000) females. According to the EUCAN estimates Slovenia ranked in the 8th place among 40 European countries in 2012 considering all cancer sites mortality.[3]

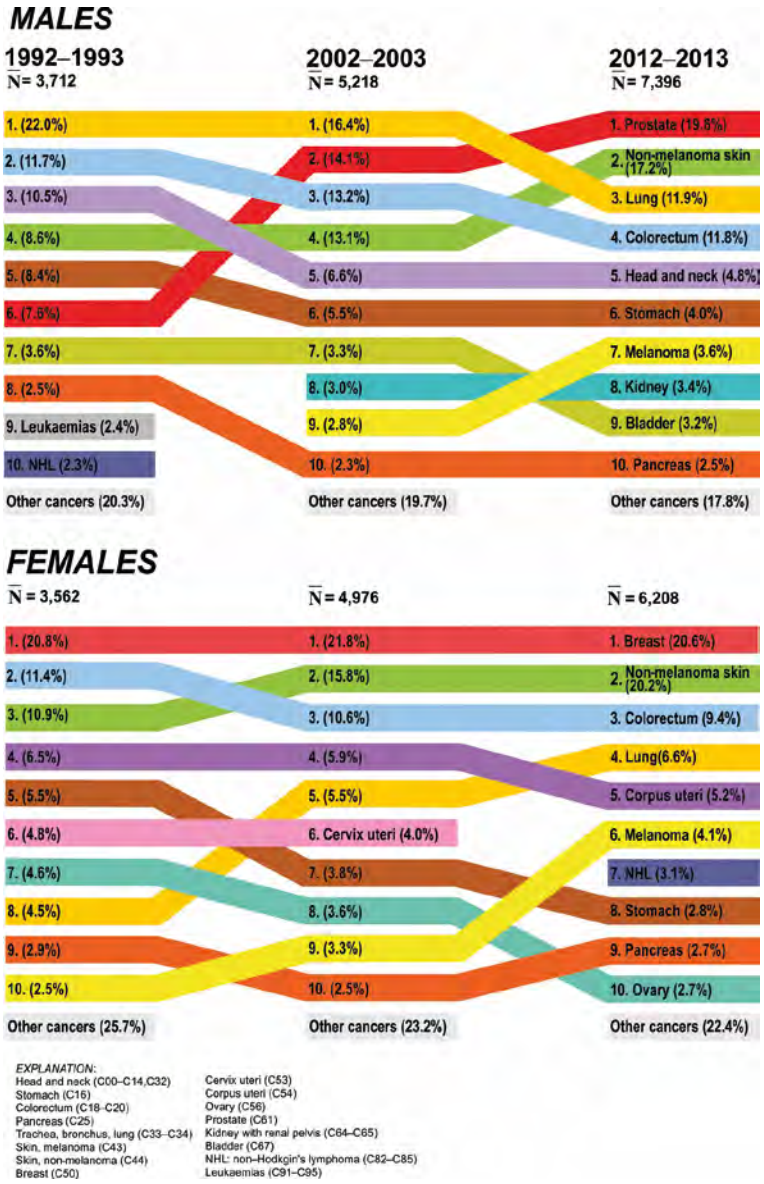

FIGURE 1. Cancer incidence - ten leading cancer sites with percentage distribution by gender, Slovenia 1992–1993, 2002–2003 and 2012–2013.

The total burden of cancer is dominated by five most common cancer sites: skin (non-melanoma), colon and rectum, lung, breast and prostate, representing almost 60% of all new cancer cases. Cancers of these sites are associated with unhealthy lifestyle, such as excessive sunbathing, unhealthy diet, smoking and excessive alcohol consumption; the aim of primary prevention is to lower the risk associated with these factors. Figure 1 shows ten

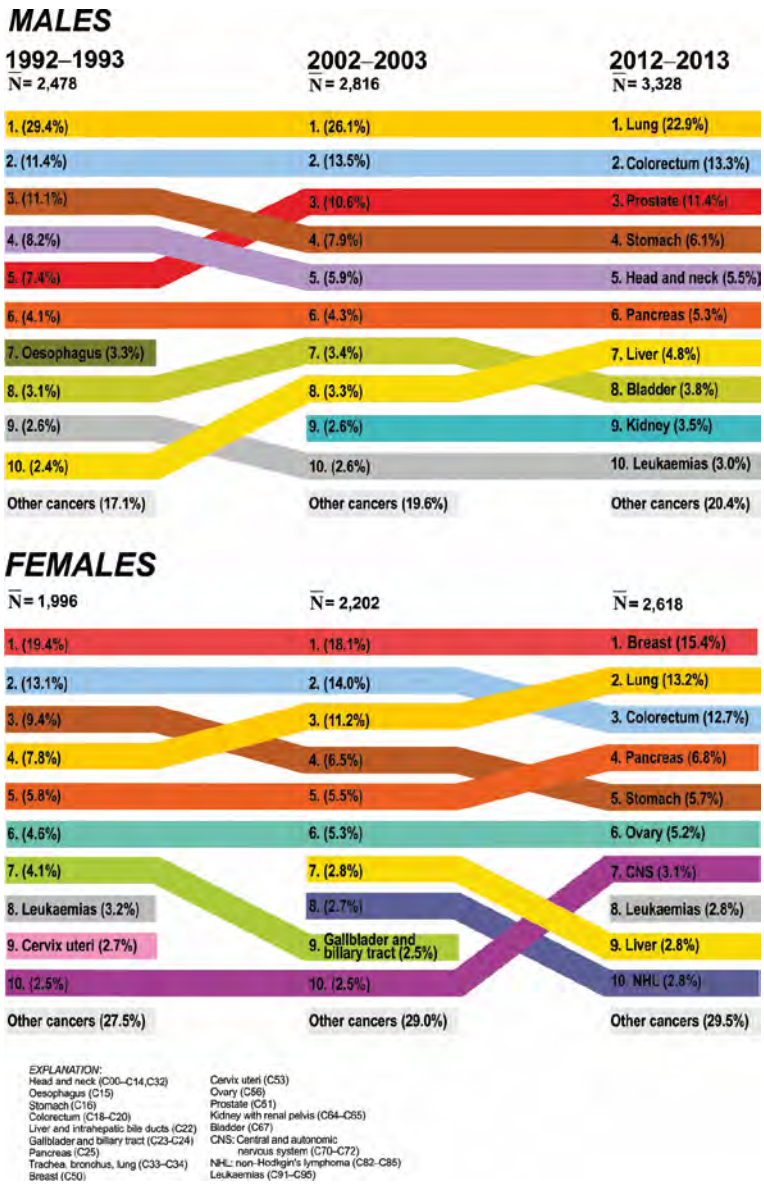

FIGURE 2. Cancer mortality - ten leading cancer sites with percentage distribution by gender, Slovenia 1992–1993, 2002–2003 and 2012–2013.

leading cancer sites with percentage distribution in three consecutive periods in Slovenia, separately for males and females. In 2013 1,485 males (20%) were diagnosed with prostate cancer in Slovenia, being the most common diagnosis in males, compared to the 6th place and less than 8% two decades ago. Lung cancer represents nowadays only 12% of all cancers in males (ranks 3rd; 869 new cases), but in previous periods it was the most common male cancer representing more than 20% of all cancers. In females in all three time periods breast is the leading cancer site with 20% of all cancer cases. In 2013 1,252 females were diagnosed with breast cancer in Slovenia. Non-melanoma skin cancer is gaining its share in female population and it is approaching breast cancer in the most recent period. Lung cancer incidence in females is disturbingly climbing – from 8th place and having a share of 4.5% two decades ago it now represents 6.6% of all newly diagnosed cases and occupying 4th place.

Ten leading cancer sites according to the mortality shares are presented in Figure 2. The non-melanoma skin cancer is rarely fatal and is not part of this list, although it is among four most common cancer diagnoses. Almost one quarter (23%) of all cancer deaths in males in Slovenia are due to lung cancer (748 deaths in 2013 in Slovenia). Another quarter of cancer death is attributed to colorectal (468 deaths in 2013) and prostate cancer (371 deaths in 2013); other cancer types cause much less cancer deaths. In females 40% of cancer deaths are attributed to breast (410 deaths in 2013 in Slovenia), lung (340 deaths in 2013) and colorectal (327 deaths in 2013) cancer. Supplementary material provides further epidemiological data for all cancers combined and for 15 most common cancer sites for the period 2009–2013 in Slovenia (SupMat 1-16).

Cancers of other sites are quite rare. In the contrast to the common definition of rare disease (prevalence less than 50/100,000), the experts from the project Surveillance of Rare Cancers in Europe (RARECARE)[17] defined the rare cancer as any cancer with crude incidence rate less than 6 per 100,000 inhabitants. According to this definition 198 groups of rare cancer were identified. They are further joined into 65 groups, which are listed in the supplementary material (SupMat 17), and further into 15 categories as defined by the RARECARE project, the later are shown on Figure 3. All rare cancers combined account for 23% of all cancers in Slovenia in period 2004–2013: annually there are around 2,880 new rare cancers (142/100,000). The rare cancer is most frequently diagnosed in digestive organs, following by head and neck, female genital organs and haematological tissue. In comparison to common cancers, rare cancers occur more often in childhood and adolescence. As in any rare disease also in rare cancers the low incidence is the main obstacle to conduct clinical trials needed to investigate and develop effective treatments. One way to overcome this obstacle would be to precisely monitor the rare cancer burden on population level and establish centres of excellence for diagnosis and treatment.[17]

The burden of cancer varies with age. Cancer is mostly the disease of the elderly since for the vast majority of cancers, incidence increases with age.

From all patients diagnosed in 2013 61% males and 58% females were aged 65 or more at diagnosis. From those born in 2013, one in two males and one in three females are expected to develop cancer by their age of 74 in Slovenian population. Cancer is a rare disease until the age of 19 years (0.6% of all cancer cases in 2013). In childhood and adolescence, the most frequent type of cancer is leukaemia. Only 1.7% of all cancers in 2013 occurred among people in the age group 20–34 years, where the most frequent malignancies are testicular cancer in males and cervical cancer in females. Among cancer patients, there is a predominance of females in the age group 35–49 years, while this ratio is reversed for the age group 50–74. Between 35–49 years, the most frequent types of malignancies in males are oral and lung cancers, while in females, breast and cervical cancers. Colorectal cancer becomes the predominant form of cancer for both sexes in the age group 50-74 years, while among older males (75+ years) prostate cancer is most frequently diagnosed. After the age of 75 years, there is a higher proportion of cancers among females, partially because of their longer life expectancy, with breast, colorectal and stomach cancers being the most common at this age.

Time trend analysis

The time trends of cancer burden in Slovenia for the last 64 years are summarized in tables and graphs available as the supplementary material (SupMat 1). Since 1950, the crude cancer incidence rate in Slovenia increased by 700% in males and by 470% in females. In the last 10 years, the average annual increase in males was 2.8% and 1.6% in females. Correspondingly, the average annual increase in age standardised incidence rate in males was 0.8% and 0.6% in females in the last 10 years. By comparing crude incidence rate to the age standardised rate where the standard population is taken to be the age structure of the Slovenian population in first year for which the graph is prepared (1961) we can assume that more than half of the crude incidence rate increase is a consequence of ageing of the population. The increase in crude mortality rate in the last 10 years was substantially slower; on average by 1.5% per year in males and by 1.7% in females. When we calculate the hypothetical rates in case, the Slovenian population would not age, the trend becomes reverse as the age standardised mortality rate is decreasing on average by 1.2% annually in males and by 0.5% in females in the last 10 years. The decreasing mortality despite increas-

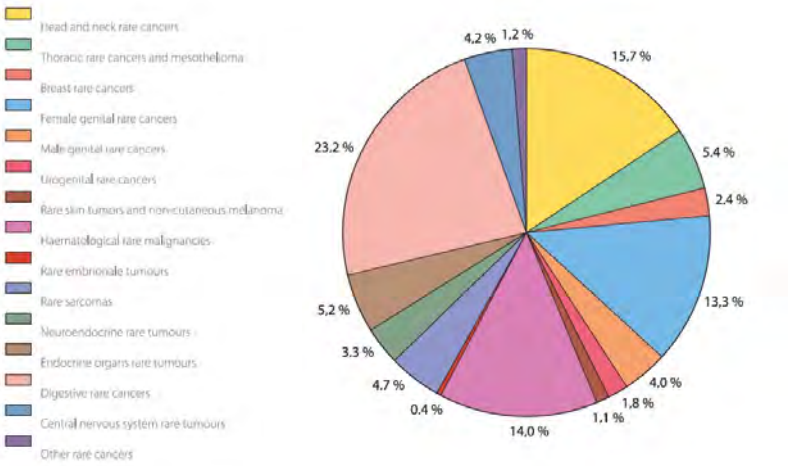

FIGURE 3. Rare cancers categorized in 15 groups, Slovenia 2004–2013.

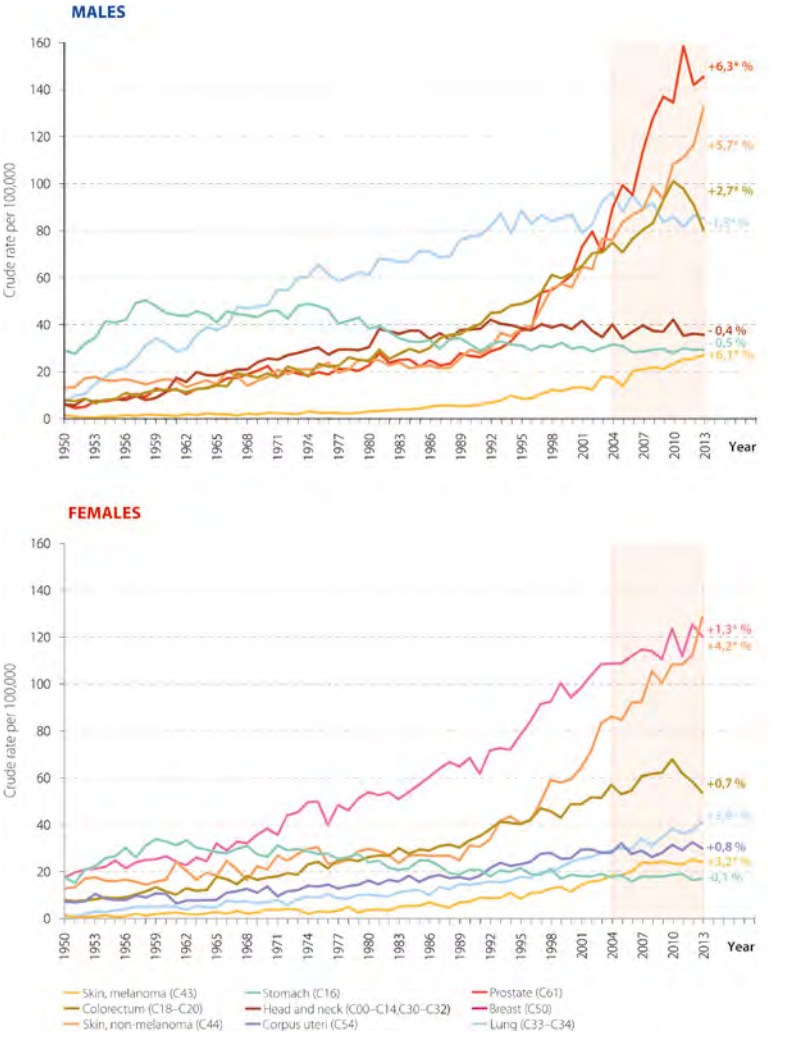

FIGURE 4. Trend of crude incidence rates of selected primary cancer sites by gender with average annual percent change in last 10 years, Slovenia 1950–2013.

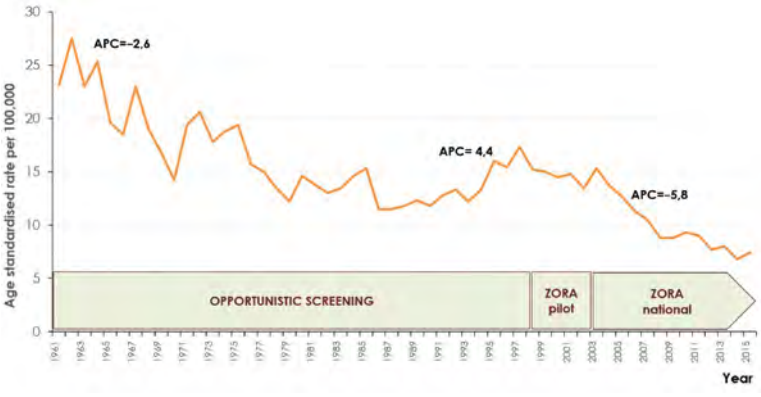

FIGURE 5. The age standardised cervical cancer incidence rates (world standard), Slovenia 1961–2015. The timeline of cervical cancer screening modalities and the average annual percent changes (APC) for the statistical significant cervical cancer incidence time trends (1961–1989, 1989–1997 and 2003–2015) are indicated.

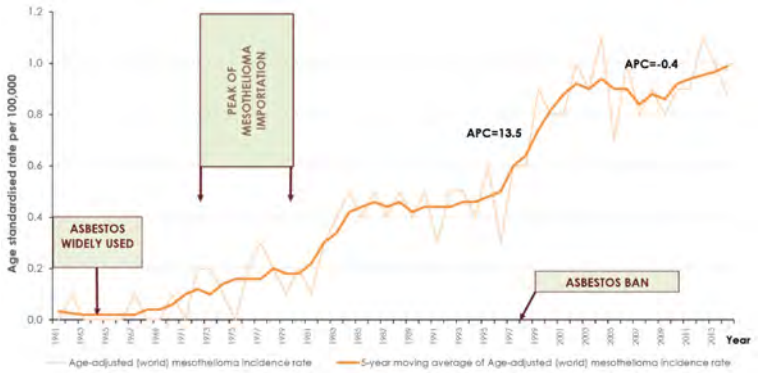

FIGURE 6. The age standardised mesothelioma incidence rates (world standard) with 5-year moving averages, Slovenia 1961–2014. The average annual percent changes (APC) are presented for the most important time segments (1998–2003 and 2003-2014) and critical years of asbestos exposure are marked.

ing incidence indicates improvement in treatment of cancer patients. It is a proof that more and more are cured of or living with and not dying from cancer.

The long-term time trends of the incidence for most common cancers by gender are plotted in Figure 4. The APCs in last 10 years are included. In males the lung cancer incidence was increasing till the beginning of the nineties, while it became stable onwards (85/100.000) and is slowly decreasing (on average by 1.3% per year) and was finally overcome by prostate cancer (average increase by 6.3% per year). The huge increase in prostate cancer incidence rate in the last years is not due to some new risk factor, but more frequent use of prostate specific antigen (PSA) test as an opportunistic screening method[9], resulting in detection of many cancers that would have never progressed or would even not have been detected during lifetime without screening, because the person would not develop any health problems.

The changes in the colorectal cancer incidence should be interpreted taking into consideration the changes in the diagnostic practice – in Slovenia the National colorectal cancer screening programme (SVIT) has been introduced in 2009.[18] The average annual increase in colorectal cancers in males in the period 2004–2011 was 5.3%, but there was a decrease of 10.5% in the following years 2011–2013, attributable to treatment of precancerous polyps that would have progressed to cancer if person would not respond to the screening invitation. In females, the incidence rate of breast, lung and non-melanoma skin cancer is increasing. Like in males also in females the colorectal cancer incidence time trends have been changed because the national screening programme SVIT has been introduced: in the period 2004–2010 the incidence increased for 3.6% annually, but in the years 2010–2013 it decreased by 6.4%.

The effect of population-based screening is even more pronounced in the case of cervical cancer. Slovenia is one of the European countries with the highest historical incidence of cervical cancer and also one of the countries with the highest decrease of cervical cancer incidence over time.[19] As presented in Figure 5, the highest peak of the cervical cancer incidence was registered in the population based CRS in year 1962 when 286 new cases were diagnosed and age standardised incidence rate was 27.5/100,000. The incidence was then decreasing till the end of the eighties by 2.6% per year on average, most likely due to the effect of the opportunistic screening. At the beginning of the nineties, the cervical cancer incidence trend has changed again with a second peak in 1997, which triggered the introduction of organised population-based cervical cancer screening programme ZORA. In 1998 and 2001 two pilot programmes of organised screening were implemented in Central and Coastal-Karst Slovenian region. The steepest decrease of incidence was observed after the implementation of programme ZORA at the national level in 2003 with APC being 5.8 in the period 2003–2015. The lowest incidence of cervical cancer to this year was registered in 2014 when 114 new cases were diagnosed and age standardised incidence rate was 6.8/100,000, which presents a difference of –20.7/100,000 in comparison with the highest recorded age standardised incidence rate in 1962.[20]

On average the incidence of common cancers in Slovenia is increasing for 3.0% each year. On

the contrary, in the group of rare cancers the incidence in stable – for all rare cancers combined the APC for the period 2004–2013 is 0.5 (SupMat 18). Nevertheless, also among rare cancers there are some specific cancer types with significant variation in incidence rate over time. One of them is malignant mesothelioma. Till the end of 2013 there were 729 mesothelioma cases registered in the population based CRS, 518 (71%) of them among men. In the period 2009–2013 there were 1.9 mesothelioma cases per 100,000 inhabitants in Slovenia; 2.8/100,000 in male population. The age standardised mesothelioma incidence rates with 5-year moving averages for the period 1961–2014 are shown in Figure 6. From 1970 on the mesothelioma incidence is irregularly increasing: the steepest increase can be observed from 1998 to 2003 (APC = 13.5). After the year 2004 the mesothelioma time trend curve is stable (APC = -0.4). In a recent age-period-cohort analysis of the presented data the highest mesothelioma risk was detected for the cohort born between 1940 and 1944.[21] The peak value of asbestos importation in 1973[22] corresponds to the peak of mesothelioma incidence curve exactly 30 years later.Considering the latency between asbestos exposure and mesothelioma development, which is around 40 years[23], these results imply that the mesothelioma peak has already been reached in Slovenia.

Projections

As CRS is using many data sources the data collection and preparation is delaying the report of cancer burden figures for 2 to 3 years. The numbers for 2013 are currently available and presented in our results. However, the estimation of the number of cancer cases was prepared for the current year, 2016. There will be 14,785 new cancer cases registered in the year 2016; 8,259 males and 6,527 females. The 2016 estimations with 95% confidence intervals for specific cancer sites are available in the supplementary material (SupMat 19). In males the number of head and neck, oesophageal, testicular and thyroid cancers is expected to be stable or even smaller. In females a smaller number is expected only for cervical cancer.

Long-term prediction of cancer incidence can help to plan cancer services and cancer control measures for the future. Due to the aging population the overall number of new cancer cases is predicted to increase further. The expected additional number of cases for some of common cancers in 2025 in Slovenia is presented in Figure 7, considering only the ageing of population. The incidence of all cancers in males will increase for 32.7% till 2025 and for 13.3% in females. In 10 years in Slovenia more than 17,000 new cancer cases are expected. It is important to note that this number does not include non-melanoma skin cancers, which is estimated to more than 2,000 additional incident cases. The increase of more than 30% is predicted for prostate, stomach, colorectal and lung cancers in males. There will be more than 600 additional prostate cancers and more than 300 additional colorectal and lung cancer cases in Slovenian male population in 2025. In females there will be only slight increase (8%) in the number of melanoma cases. More than 100 additional cases are expected in breast and colorectal cancer.

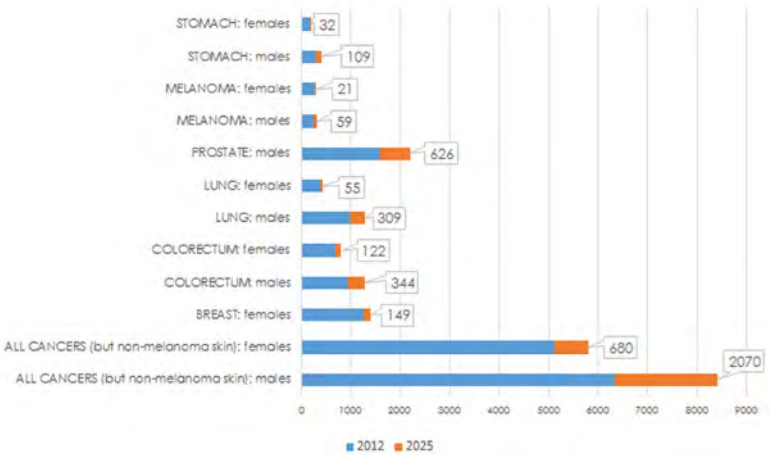

FIGURE 7. Cancer incidence of some common cancer sites in Slovenia in 2012 with the estimation of the number of expected additional cases in 2025 if only the population ageing is considered.[2]

The majority of predicted additional cases will be diagnosed in the elderly. Therefore, the median age at diagnosis will increase at population level. It is expected that in 2025 more the 70% of patients will be diagnosed at age 65 or more compared to 59% in 2013. They will on average have more concomitant diseases and less favourable health related quality of life, which render specific oncological treatment more difficult or impossible to carry out. Hence, better palliative and adjusted specific oncological treatment should be the major priority in Slovenian health care system in the next years.

However, when predicting the future cancer burden, it is not enough to consider the ageing of population only. The number of incident cancer cases depends considerably also on the risk or protective factors to which the population is exposed. In the next ten years in Slovenia we expect that the

results of the effective national smoking cessation programmes will manifest. In male population the proportion of adult male smokers has almost halved in the last thirty years[24], which in turn will lead to less smoking related cancers among men. In females the smoking cessation is not evident in Slovenian population so far and increasing number of adolescents started smoking is of concern. National policy on healthy diet and physical activity should further contribute to lower risk of common cancers.[9] In addition to success of primary preventions, the national organised cancer screening policy will have major impact on the number of new cancer patients as well as mortality from cancers for which population based screening programmes are established. Besides cervical and colorectal cancer screening, breast cancer screening will cover the whole female population in the target age group till the end of 2020, so decrease in breast cancer mortality is expected in the next decade. Considering the population ageing as well as the effects of primary and secondary prevention measures the cancer incidence is aimed to remain below 15,500 new cases in 2020 (9,000 in males and 6,500 in females) in the existing Slovenian National Cancer Control Plan.[12]

Conclusion

In 1981, Doll and Peto[25] estimated the proportion of cancer deaths attributable to known risk factors. They judged that more than a third of cancer deaths were attributable to risk factors as consequence of unhealthy lifestyle. The most important are those that are the consequence of western way of life: obesity, diet with too much energy and low in vegetables and fruit, sedentary lifestyle together with smoking and high alcohol consumption.

Changing political and economic circumstances over the past decades, resulted in very different lifestyle for most Slovenes and their exposure to carcinogens in the working and living environment, but also led to changing health care organization. Since latent period in cancer is generally longer than 20 years, it can be concluded that the effects of these changes are not fully developed yet.[26] In recent years, near 14,000 Slovenes were diagnosed with cancer per year and just over 6,000 died; more than 94,000 people who were ever diagnosed with cancer are currently living among us. Slovenian population is ageing - because cancer occurs more among older, and additionally more numerous post-war generation is coming to this ages, it is expected that the burden of this disease will be increasing further, even if the level of risk factors remained the same as today.

Control of the cancer burden can be established only by clear objectives, well-planned and financially supported interventions and constant monitoring of the quality and effectiveness of these actions. They should be summarised in national cancer control plans with well-established priorities.[27] Cancer registries, a core component of cancer health information systems, have a major role in providing data to prioritize programmes in national cancer control plans as well as to monitor the progress of their implementation. In Slovenia, we are following these recommendations by the second National Cancer Control Programme, as well as with long-standing and good-quality cancer registry.

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research article

Leiomyosarcoma of the renal vein: analysis of outcome and prognostic factors in the world case series of 67 patients

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Background. Leiomyosarcoma is a rare malignant mesenchymal tumour. Some cases of leiomyosarcoma of the renal vein (LRV) have been reported in the literature, but no analysis of data and search for prognostic factors have been done so far. The aim of this review was to describe the LRV, to analyse overall survival (OS), local recurrence free survival (LRFS) and distant metastases free survival (DMFS) in LRV world case series and to identify significant predictors of OS, LRFS and DMFS.

Methods. Cases from the literature based on PubMed search and a case from our institution were included.

Results. Sixty-seven patients with a mean age of 56.6 years were identified; 76.1% were women. Mean tumour size was 8.9 cm; in 68.7% located on the left side. Tumour thrombus extended into the inferior vena cava lumen in 13.4%. All patients but one underwent surgery (98.5%). After a median follow up of 24 months, the OS was 79.5%. LRFS was 83.5% after a median follow up of 21.5 months and DMFS was 76.1% after a median follow up of 22 months. Factors predictive of OS in univariate analysis were surgical margins, while factors predictive of LRFS were inferior vena cava luminal extension and grade. No factors predictive of DMFS were identified. In multivariate analysis none of the factors were predictive of OS, LRFS and DMFS.

Conclusions. Based on the literature review and presented case some conclusions can be made. LRV is usually located in the hilum of the kidney. It should be considered in differential diagnosis of renal and retroperitoneal masses, particularly in women over the age 40, on the left side and in the absence of haematuria. Core needle biopsy should be performed. Patients should be managed by sarcoma multidisciplinary team. LRV should be surgically removed, with negative margins.

Key words: leiomyosarcoma; renal vein; surgery, outcome

Introduction

Leiomyosarcoma (LMS) is a rare malignant mesenchymal tumour of smooth muscle origin. It represents only 5–7% of soft tissue sarcomas.[1] Approximately 2.0% of LMS originate from the smooth muscle of vessel walls, predominantly veins and 60.0% of these originate from inferior vena cava (IVC).[1] According to Gage *et al*. [1], the most common location of extracaval venous LMS is the renal vein, followed by the great saphenous, pulmonary and femoral vein. Leiomyosarcoma of the renal vein (LRV) is extremely rare. There have been some cases reported in the literature, but no

analysis of data and search for prognostic factors have been done so far. The first case was reported by Lopez Varela and Pereira Garro in 1967.[2] We present an additional case, world literature overview and the outcome of these patients.

Patients and methods

Literature overview and data collection

The search criteria in PubMed were "leiomyosarcoma" and "renal vein". In the literature 62 articles were identified describing cases of LRV. Fourteen of the articles were in Japanese, 7 in French, 4 in Spanish, 1 in Polish and 36 in English. Data from 49 articles only[2-50] were merged into a database, because out of 18 Japanese cases only 4 were reported in English articles [23,37,40,44] and the rest in Japanese articles, not accessible to us (Figure 1). The last review of Japanese cases by Kato *et al*.[42] was translated and these data included in the study. Three times the patient was discussed as different case report by two different authors.[3-4,13-14,20-26] That lowered the total number of reported cases in the last review by three.[50] In some articles more than one case was reported.[14,19,26,42,46] The authors were from the fields of urology (18/49; 36.7%), surgery (15/49; 30.6%), radiology (8/49; 16.3%), pathology (5/49; 10.2%) and internal medicine (3/49; 6.1%). A retrospective review was performed to evaluate patient demographics, tumour site, clinical presentation, operative details, tumour thrombus IVC extension, neoadjuvant and adjuvant treatment, tumour size, tumour grade, surgical margin status, time to local recurrence, time to dissemination, time to death and status at last follow up. To the authors or coauthors 18 emails were sent around the world to update the data and follow up, we received 4 replies.

Illustrative case

A 46-years old female presented in January 2014 to University Hospital Ljubljana with upper abdominal pain of 6 months duration and weight loss. Her past medical history was unremarkable. On physical examination there was a palpable mass in the left upper abdomen. Gastroscopy was not diagnostic, but computed tomography (CT) revealed a left retroperitoneal mass, 11 x 10 x 9 cm in size, interposed between the aorta and hilum of the left kidney (Figure 2). The tumour surrounded the left renal artery and the vein was not identified. Ultrasound guided fine needle aspiration biopsy (FNAB) was performed. The sample was suspicious for LMS. She was referred to the Institute of Oncology Ljubljana in February 2014 for management and treatment. A dynamic renal scintigraphy was performed for evaluation of kidney function.

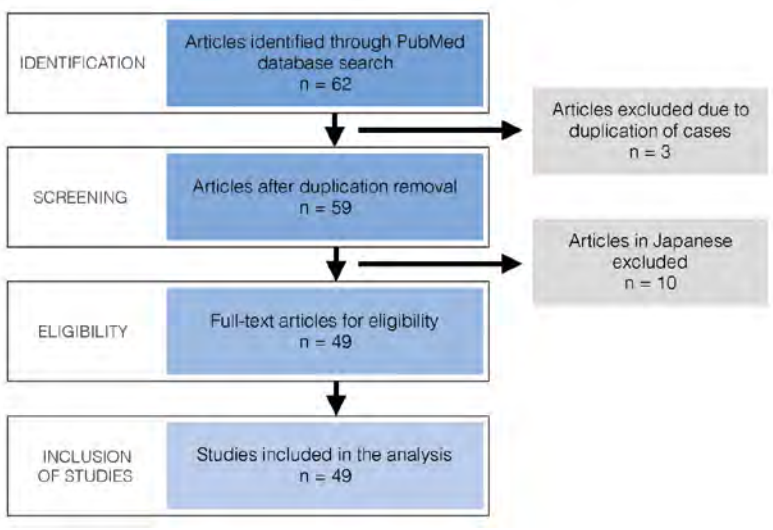

FIGURE 1. Flow chart of identification, screening, eligibility and inclusion of studies.

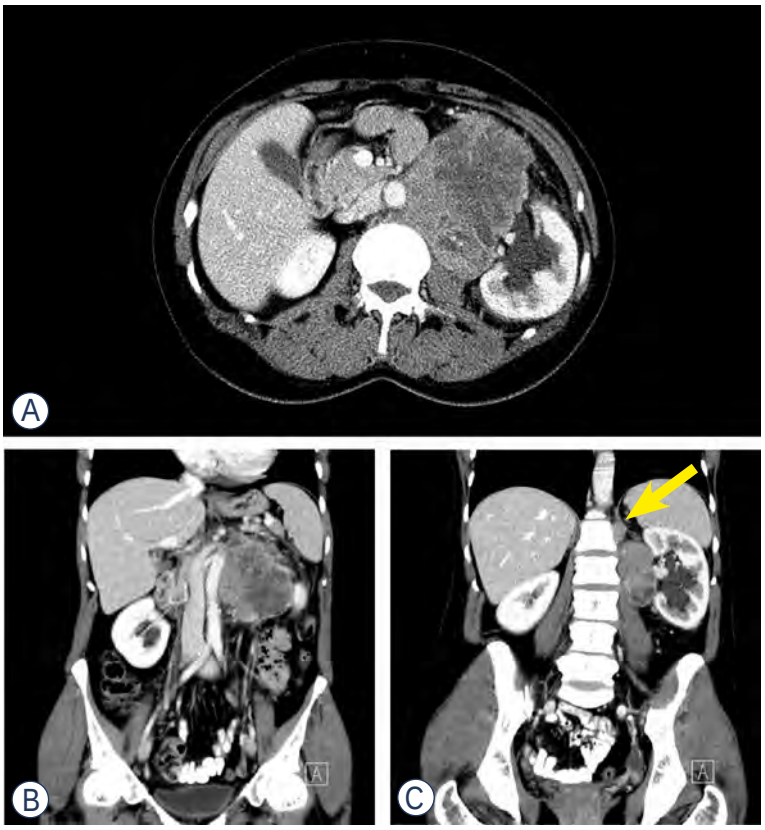

FIGURE 2. Enhanced computed tomography showing retroperitoneal tumour, interposed between the aorta and the left kidney, axial **(A)** and coronal plane **(B)**. Separately removed satellite node in coronal plane, arrow **(C)**.

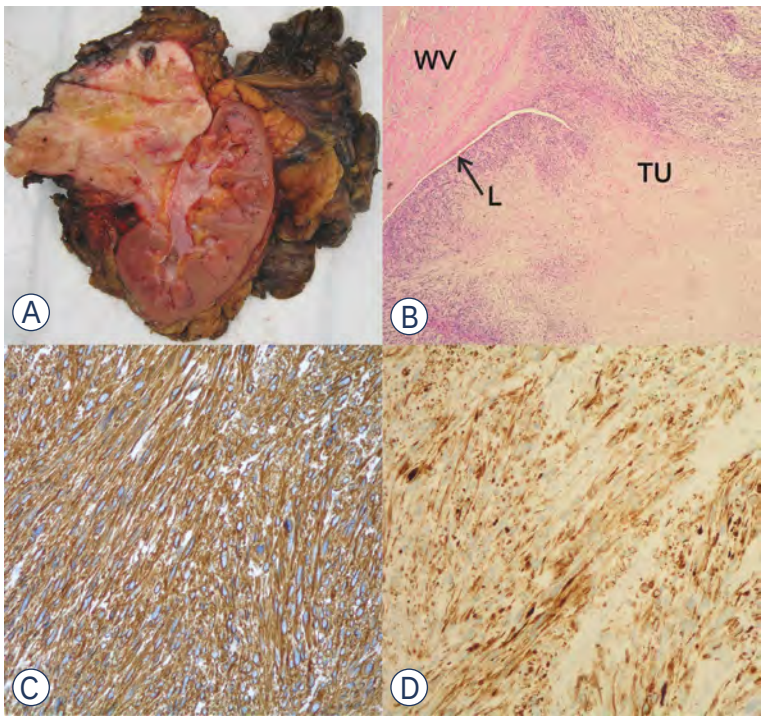

FIGURE 3. (A) A gross specimen of renal vein leiomyosarcoma. The tumour is well-circumscribed, is lying in the renal hilum, without infiltration of the renal parencyma. **(B)** Hematoxylin & Eosin stain section. Showing the vascular lumen (L) and the tumour (TU) growing from the wall of the renal vein (WV). Immunohistochemical stains for SMA **(C)** and desmin **(D)** showing strong positivity.

Excretory function of the left kidney was 47% and of the right kidney 53%. Thoracic CT revealed no metastases. After discussing the case at the multidisciplinary team (MDT) we decided to perform surgery, without preoperative core needle biopsy (CNB). Tumour was removed *en bloc* with left colon, left kidney, adrenal gland and psoas fascia. A suspicious node 3 cm in size was found intraoperative in psoas muscle close to the vertebra. It was removed separately. The main specimen weighed 1152 g. Histology confirmed a LMS, according to Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system grade 3, 12 cm in largest diameter, originating from the left renal vein, not infiltrating the surrounding organs. Surgical margins were negative. The spindle tumour cells stained positive for smooth muscle actin, desmin and focally for CD34. The separately removed node was an LMS satellite, margins were positive (Figure 3). She received adjuvant radiotherapy (RT), 59 Gy. There were no surgical or radiotherapy-related complications. In December 2014, 10 months postoperatively, liver metastases were detected on CT. After subsequent magnetic resonance imaging treatment was planned at the MDT. All 4 liver metastases, 4 to 17 mm in size were removed surgically with clear margins. She received no adjuvant treatment. In October 2015 lung metastases were detected on both sides, the largest 17 mm. She is receiving chemotherapy (ChT) with adriamycin, ifosfamide and mesna at the time of this report.

Written informed consent for all diagnostic and therapeutic procedures was obtained from the patient.

Statistical analysis

On the basis of limited data, univariate analysis was used to evaluate the following potential prognostic factors for overall survival (OS), local recurrence free survival (LRFS) and distant metastases free survival (DMFS): age, gender, tumour site, dissemination, weight loss, palpable mass, operation type, tumour thrombus IVC luminal extension, tumour size, grade, margin status, number of mitoses and neoadjuvant or adjuvant treatment. OS and LRFS were compared using log-rank test. All comparisons were two sided. P-value of 0.05 was considered statistically significant. Survival curves were calculated and plotted using Kaplan-Meier method. Cox's multivariate regression was used to identify independent prognostic variables of OS, LRFS and DMFS. The statistical program SPSS® version 22 was used for analysis.

Results

Patient and tumour characteristics

In total 67 cases were identified. The tumour predominantly occurred in women (76.1%; 51/67) and on the left side (68.7%; 46/67). The mean age at diagnosis was 56.6 years (range 27–93 years). Detailed patient and clinicopathologic characteristics are presented in Table 1, Figure 4 and 5. Histological biopsy before treatment was performed in 9 patients (13.4%; 9/67); 1 patient had biopsy during exploration[9], 4 patients had CT guided CNB[19,20,27,50], the biopsy type for 2 patients was not specified in the article[33,41] and 2 patients had biopsy through femoral approach during cavography.[36,43] FNAB before operation was performed in 1 patient[34] and in our case (3.0%; 2/67). The mean tumour size was 8.9 cm, described in 54 cases (80.6%; 54/67). System used for sarcoma grading was defined in single article.[10] Tumour grade was described in 28 cases (41.8%; 28/67), surgical margin status in 18 cases

(26.9%; 18/67) and number of mitoses in 18 cases (26.9%; 18/67). Tumour cells stained positive for smooth muscle actin in 23 cases (34.3%; 23/67), for desmin in 22 cases (32.8%; 22/67) and for vimentin in 6 cases (9.0%; 6/67). Intraluminal caval tumour thrombus was reported in 9 cases (13.4%; 9/67), IVC mural invasion in 3 cases (4.5%; 3/67), the renal parenchyma invasion in 8 cases (11.9%; 8/67) and the adrenal gland invasion in a single case (1.5%; 1/67). The data about IVC mural invasion were taken as stated in the articles.[11,15,16]

Surgery

All patients but one underwent surgery (98.5%; 66/67). Four patients had tumorectomy (6.0%; 4/67) and 60 had nephrectomy (89.6%; 60/67). One patient had attempt of laparoscopic tumorectomy, two had laparoscopic nephrectomy and one had robotic laparoscopic nephrectomy. Two patients (3.0%; 2/67) had compartment resection, tumour removed *en bloc* with (at least) adjacent segment of colon, kidney and psoas. Adrenalectomy was performed in 11 patients (16.4%; 11/67) and lymph node dissection in 6 patients (9.0%; 6/67). Tumour thrombus extended into the lumen of IVC in 9 patients (13.4%; 9/67), in 4 cases tumour was on the left side and in 5 cases on the right. In two of these patients there was also invasion of the caval wall. IVC was resected in 5 patients (7.5%; 5/67), once ligated and without reconstruction, once oversewn, once reconstructed with venous patch and once with allograft. There are no data about the type of operation on IVC for the fifth patient. Cavotomy and extraction of the tumour thrombus was performed in 3 patients (4.5%; 3/67). One patient had locally advanced tumour, with tumour extension into the right atrium and received palliative ChT only. In a patient with tumour caval wall invasion

TABLE 1. Patients data and histologic variables, treatment modalities and disease progression

Characteristic	Subgroup	Value (n = 67)	%
Age at diagnosis (year)	Mean	56.6	
	Range	27–93	
Gender	Female	51	76.1
	Male	16	23.9
Side	Left	46	68.7
	Right	21	31.3
Size (cm)	Mean	8.9	
	Range	3.5–25	
Tumour thrombus extension	IVC	9	13.4
Preoperative biopsy	Histology	9	13.4
	Fine needle aspiration	2	3.0
	No biopsy	56	83.6
Tumour grade	G1	6	9.0
	G2	8	11.9
	G3	14	20.9
	Unknown	39	58.2
Surgical margins	Negative	15	22.4
	Positive	3	4.5
	Unknown	49	73.1
Operation	Nephrectomy	60	89.6
	Tumorectomy	4	6.0
	Compartment resection	2	3.0
	No operation	1	1.5
Preoperative treatment	Embolization	3	4.5
	ChT	1	1.5
	RT + ChT	2	3.0
Intraoperative treatment	RT	1	1.5
Postoperative treatment	RT	7	10.4
	ChT	9	13.4
	ChT + RT	1	1.5
	Immunotherapy	1	1.5
Disease progression	LR	3	4.5
	M	20	29.9
	LR + M	10	14.9
	Total	33	49.3
Site of dissemination	Liver	17	25.4
	Lungs	16	23.9
	Bone	8	11.9
	Soft tissue	4	6.0

ChT = chemotherapy; G = grade; IVC = inferior vena cava; LR = local recurrence; M = metastases; RT = radiotherapy

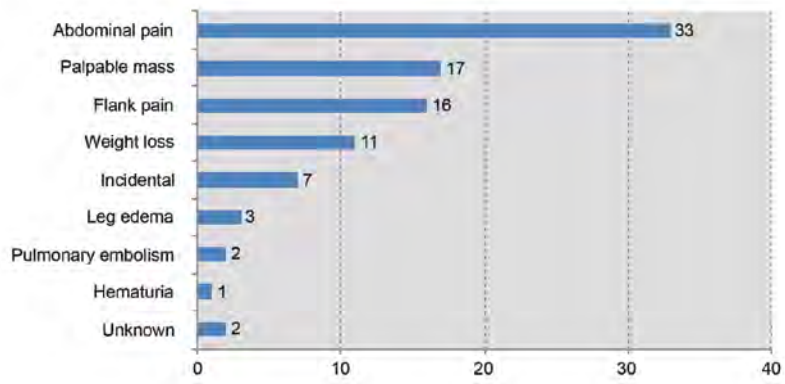

FIGURE 4. Clinical presentation of leiomyosarcoma of the renal vein cases.

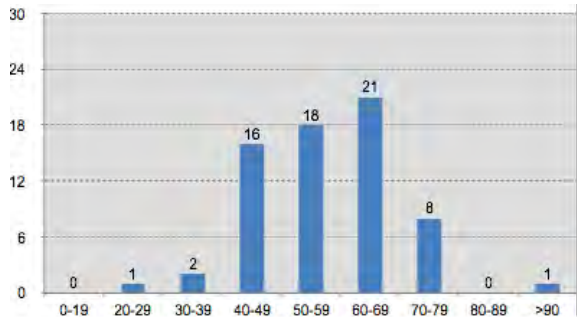

FIGURE 5. Age distribution of leiomyosarcoma of the renal vein patients.

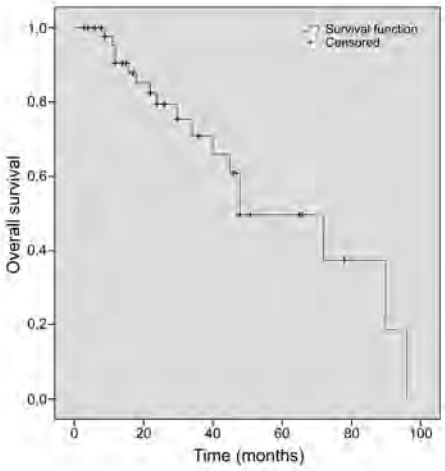

FIGURE 6. Kaplan-Meier curve of overall free survival.

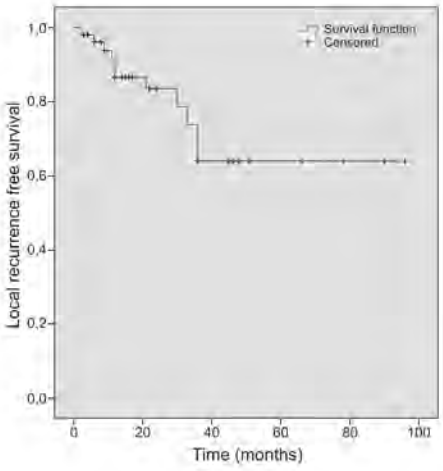

FIGURE 7. Kaplan-Meier curve of local recurrence free survival.

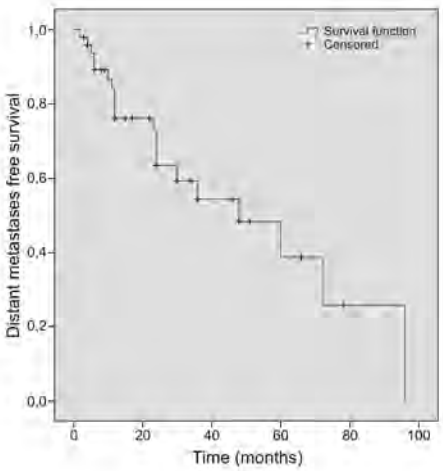

FIGURE 8. Kaplan-Meier curve of distant metastases free survival.

the IVC was reconstructed with venous patch after resection. Splenectomy was performed in 2 patients, in 1 jejunal resection and in 1 synchronous liver metastatectomy.

Treatment modalities

Three patients (4.5%; 3/67) had preoperative tumour embolization. One patient received preoperative ChT and two preoperative ChT and RT (3.0%; 2/67). Seven patients (10.4%; 7/67) received postoperative RT and 9 patients postoperative ChT (13.4%; 9/67). One patient received postoperative ChT and RT and 1 patient had immunotherapy. The information about RT and ChT as neoadjuvant and adjuvant treatment is summarized in Table 1.

Outcome

Four patients were excluded for the survival analysis, because 3 were disseminated at the time of diagnosis [19,20,26] and one was not treated surgically.[36] Two patients were alive with disease and on palliative care at the time of report.[34,46] Three patients (4.5%; 3/67) had local recurrence, 10 patients (14.9%; 10/67) had local recurrence and dissemination and 20 patients (29.9%; 20/67) had dissemination of the disease after treatment. Spread was hematogenous to different organs. In this group of 67 patients to the liver in 25.4% (17/67), lungs in 23.9% (16/67), bones in 11.9% (8/67) and soft tissue in 6.0% (4/67) (Table 1).

After the median follow up of 24 months, the OS was 79.5%. LRFS was 83.5% after median follow up of 21.5 months and DMFS was 76.1% after median follow up of 22 months. Factors predictive of OS in univariate analysis were surgical margins ($p = 0.014$), while factors predictive of LRFS in univariate analysis were IVC luminal extension ($p = 0.016$) and tumour grade ($p = 0.05$). No factors predictive of DMFS were identified in univariate analysis. Univariate analysis of OS, LRFS and DMFS are presented in Table 2. In multivariate analysis none of the factors were predictive of OS, LRFS or DMFS. Survival curves are presented in Figures 6, 7 and 8.

Discussion

Points to be discussed about the case from our institution are biopsy, surgery, adjuvant RT and treatment of liver metastases.

The patient presented to the Institute of Oncology Ljubljana because of retroperitoneal location of the tumour and cytological suspicion for LMS. Ultrasound guided FNAB was performed in another hospital. At the MDT it was not decided for CNB, because the mass was a spindle cell tumour, suspicious for LMS, with the renal vein not identified on CT, indeed suspicious for primary LRV, and because the tumour was deemed resectable and not disseminated, as such not planned for neoadjuvant treatment.

The tumour was removed with compartment resection with negative margins, in separately removed satellite margins were positive. Analysing the CT scans again after the histological report, the satellite was found on CT and it seems that the tumour was invading the psoas muscle in continuity. Surgery was planned as wide resection but was

TABLE 2. Univariate analysis of overall, local recurrence free and distant metastases free survival (log-rank)

Characteristic	Subgroup	OVERALL SURVIVAL			LOCAL RECURRENCE FREE SURVIVAL		
		Alive (n = 39)	Dead (n = 17)	p-value	No LR (n = 45)	LR (n = 11)	p-value
Age	≤ 50 50	13 26	5 12	0.285	15 30	3 8	0.519
Gender	F M	32 7	15 2	0.812	38 7	9 2	0.993
Side	L R	29 10	9 8	0.448	31 14	7 4	0.987
Weight loss	Y N	5 34	6 11	0.414	9 36	2 9	0.734
Palpable mass	Y N	9 30	6 11	0.581	12 34	4 9	0.562
Operation	Nephrectomy Tumorectomy Compartment	35 2 2	15 2 0	0.543 (nephrectomy or tumorectomy vs. compartment)	41 2 2	9 2 0	0.562
Intracaval luminal extension	Y N	5 34	3 14	0.340	4 41	4 7	0.016
Tumour size	≤ 10 cm > 10 cm Unknown	23 11 5	10 3 4	0.485 (≤ 10 cm vs. > 10 cm)	28 11 6	5 3 3	0.219 (≤ 10 vs. > 10 cm)
Grade	1 2 or 3 Unknown	4 13 22	2 6 9	0.265 (1 vs. 2 or 3)	6 12 27	0 7 4	0.05 (1 vs 2 or 3)
Margins	Negative Positive Unknown	14 1 24	1 1 15	0.014 (neg. vs. pos.)	12 0 33	3 2 6	0.096 (neg. vs. pos.)
Mitoses/10hpf	<10 ≥10 Unknown	8 2 29	2 1 14	0.782 (<10 vs. ≥10)	8 1 36	2 2 7	0.244 (<10 vs. ≥10)
Neoadjuvant/ adjuvant chemotherapy	Y N	8 31	3 14	0.987	7 38	4 7	0.337
Neoadjuvant/ adjuvant raditherapy	Y N	6 33	4 13	0.165	8 37	2 9	0.975

F = female; L = left; LR = local recurrence; M = male; N = No; R = right; Y = Yes

Characteristic	Subgroup	DISTANT METASTASES FREE SURVIVAL		
		No DM (n = 31)	DM (n = 25)	p-value
Age	≤ 50 > 50	9 22	9 16	0.805
Gender	F M	27 4	20 5	0.221
Side	L R	19 12	19 6	0.138
Weight loss	Y N	2 29	9 16	0.087
Palpable mass	Y N	5 26	10 15	0.277
Operation	Nephrectomy Tumorectomy Compartment	27 3 1	23 1 1	0.336 (nephrectomy or tumorectomy vs. compartment)
Intracaval luminal extension	Y N	4 27	4 21	0.284
Tumour size	≤ 10 cm > 10 cm Unknown	21 7 3	12 7 6	0.210 (≤ 10 cm vs. > 10 cm)
Grade	1 2 or 3 Unknown	4 9 18	2 10 13	0.131 (1 vs. 2 or 3)
Margins	Negative Positive Unknown	10 1 20	5 1 19	0.815 (neg. vs. pos.)
Mitoses/10hpf	<10 ≥10 Unknown	9 1 21	1 2 22	0.266 (<10 vs. ≥10)
Neoadjuvant/ adjuvant chemotherapy	Y N	5 26	6 19	0.683
Neoadjuvant/ adjuvant radiotherapy	Y N	5 26	5 20	0.087

DM = distant metastases; F = female; L = left; M = male; N = No; R = right; Y = Yes

marginal and R1. The operation would be optimal if both specimens would be removed *en bloc*, but the margins on the vertebra would probably be positive anyway. Because reoperation with clear margins on vertebra in case of local recurrence would probably not be possible, we decided for adjuvant RT.

According to magnetic resonance imaging liver metastases were small and resectable and that was the reason at the MDT to decide for metastasectomy.

LRV is very rare. Cases from the last literature overview in 2010 [50], cases from nonenglish literature, new reports from 2010–2015 and present case were summarised. From data gathered from these case reports, subsequent analysis and with respect to sarcoma guidelines, several observations can be made.

From the clinical point of view, LRV presents difficulties in making diagnosis, because it is uncommon, has no specific symptoms and no pathognomonic radiological features. It predominantly occurs in women (76.1%), on the left side (68.7%) and affects older population, with the peak occurring at age 60–69 years. Presenting symptoms are unspecific, abdominal pain was reported in 49.3%.

Hematuria was reported in a single case (1.5%) of LRV patients, but is present in more than one third of the cases (34.8%) of renal cell carcinoma (RCC) with venous extension.[51] Genetic predisposition may play a role in development of primary LRV, with two patients being treated for retinoblastoma and one patient having Li Fraumeni syndrome.

From the point of imaging, location of LRV is more important than the size of the tumour. It can overlap with much more common RCC with venous extension. LRV is usually located in the hilum of the kidney. The bulk of the tumour lies predominantly or entirely outside the hilar parenchyma or the tumour is limited to the renal vessels [46]. The mean tumour size in this LRV group is 8.9 cm. In a study group of 1192 patients with RCC with extension into the renal vein (23.0%) and IVC (7.0%) the mean tumour size was 8.9 cm as well.[52] It may not be possible to distinguish between these two entities by imaging. Other diagnoses considered in this location are metastatic lymph node in a patient with a history of malignancy, renal pelvis leiomyosarcoma, extremely rare as well, with around 10 cases reported in the literature[53], lymphoma, adrenal gland tumour, upper tract urothelial carcinoma, granulomatous disease and renal vein thrombus.[46]

With regard to biopsy, retroperitoneal mass is usually detected on abdominal CT scans. When imaging is not diagnostic of a retroperitoneal liposarcoma, image-guided CNB of retroperitoneal tumour is strongly recommended to obtain the sample for diagnosis. Correct diagnosis may significantly affect surgical decision and neo/adjuvant therapy.[54,55] Wilkinson *et al*.[56] from Royal Marsden, London reported, that preoperative CNB for retroperitoneal sarcoma (RPS) is safe and does not affect oncological outcome. Patients with intermediate and high-grade RPS were included. There were no intra-abdominal complications requiring early operation. The group of 90 patients with preoperative CNB was compared to a group of 60 patients, who did not have preoperative CNB. There was no significant difference in local recurrence ($p = 0.101$) or OS ($p = 0.191$). FNAB in retroperitoneal tumours rarely yields diagnostic information and should be avoided[55], but it can be performed in RCC in spite of danger of haemorrhage. In the present review preoperative histological biopsy was performed in 13.4% of cases only and FNAB in 3.0%.

With regard to treatment, the only potentially curative treatment for RPS is surgery with macroscopically complete resection.[54,55,57] The role of ChT and RT in RPS is not proven and still under investigation. It is generally recommended, that in case of RT administration, it should be delivered in the preoperative setting and possibly within a clinical trial.[54] Postoperative RT should not be administered routinely in R0 and R1 resections.[54] ChT is an option in the preoperative setting of resectable disease, is an option after surgery in case of R2 resection and is an option in case of unresectable or metastatic disease.[54]

Because of complex evaluation and treatment options patients with RPS should be managed by sarcoma MDT in a specialized reference center.[54,55]

Histologic subtype is one of the major determinants of the oncologic outcome in RPS. The most common location of LMS is the retroperitoneum[58], where it represents the second most common histological subtype after liposarcoma, accounting for 14–36% of patients in major series.[59,60] Retroperitoneal LMS has a high propensity for distant recurrence. The reported rate of distant metastases for retroperitoneal LMS at 5 years is around 40–50% and for local recurrence at 5 years around 5%.[61] Similar results are present in the present review, with the rate of local recurrence of 4.5% (3/67), distal metastases of 29.9% (20/67) and both in 14.9% (10/67), but in much shorter period of follow up.

And finally, in the present review of the literature 79.5% of the LRV patients survived at 2 years. A 5-year OS, LRFS and DMFS was not performed because of the inadequate sample size at that length of follow up. Retrospective comparisons of series of RPS patients have demonstrated 5-years OS rates of 50–70% and 5-years local control rates of 40–80%.[57] In the IVC LMS series 5-year survival has been reported between 33.0% and 53.0%.[26] Data from different large series of RPS patients have demonstrated tumour grade and surgical margin status as independent prognostic factors of OS and LRFS.[62,63] Cases from this review are dispersed world wide and through half of the century, lacking data for tumour grade (58.2%; 39/67), surgical margin status (73.1%; 49/67) and follow up (16.4%; 11/67). Because of insufficient histologic data and truncated follow up, we were not able to identify prognostic factors of OS, LRFS and DMFS in multivariate analysis.

As a retrospective analysis this study has limitations. Most of the information collected was from case reports, without significant follow up and lacking histological data. As a consequence, there was a limitation in the statistical analysis and the

conclusions that could be drawn from it, particularly in patients' outcome. However, to our knowledge, this is the largest study on this topic, and even this limited survey expands our understanding of the natural history of this rare sarcoma.

Conclusions

LRV is usually located in the hilum of the kidney. It should be considered in differential diagnosis of renal and retroperitoneal masses, particularly in women over the age of 40, on the left side and in the absence of hematuria. Core needle biopsy should be performed. Patients should be managed by sarcoma MDT. For optimal clinical outcomes, LRV should be surgically removed, with negative margins. After a median follow up of 24 months OS was 79.5%, LRFS was 83.5% after a median follow up of 21.5 months and DMFS was 76.1% after a median follow up of 22 months. Factors predictive of OS in univariate analysis were surgical margins, while factors predictive of LRFS were inferior vena cava luminal extension and grade. No factors predictive of DMFS were identified. Because of insufficient histologic data and follow up, we were not able to identify prognostic factors of OS, LRFS and DMFS in multivariate analysis.

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research article

Prognostic significance of uPA/PAI-1 level, HER2 status, and traditional histologic factors for survival in node-negative breast cancer patients

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Background. The association of HER2 status with urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) levels raises the question whether uPA/PAI-1 level carries additional clinically relevant prognostic information independently from HER2 status. The aim of our study was to compare the prognostic value of uPA/PAI-1 level, HER2 status, and traditional prognostic factors for survival in node-negative breast cancer patients.

Patients and methods. A retrospective analysis of 858 node-negative breast cancer patients treated in Maribor University Clinical Center, Slovenia, in the years 2000–2009 was performed. Data were obtained from patient medical records. The median follow-up time was 100 months. Univariate and multivariate analyses of disease-free (DFS) and overall survival (OS) were performed using the Cox regression and the Cox proportional hazards model.

Results. In univariate analysis, age, tumor size, grade, lymphovascular invasion, HER2 status and UPA/PAI-1 level were associated with DFS, and age, tumor size, grade, and uPA/PAI-1 level were associated with OS. In the multivariate model, the most important determinants of DFS were age, estrogen receptor status and uPA/PAI-1 level, and the most important factors for OS were patient age and tumor grade. The HR for death from any cause in the multivariate model was 1.98 (95% CI 0.83–4.76) for patients with high uPA and/or PAI-1 compared to patients with both values low.

Conclusions. uPA/PAI-1 level clearly carries an independent prognostic value regardless of HER2 status in node-negative breast cancer and could be used in addition to HER2 and other markers to guide clinical decisions in this setting.

Key words: node-negative breast cancer; adjuvant systemic treatment; survival; uPA/PAI-1; HER2 status

Introduction

One of the greatest challenges in the treatment of node-negative breast cancer is deciding in which patients the benefit from adjuvant cytotoxic chemotherapy would outweigh its adverse effects. Traditionally, this decision has been based on clinical and histomorphologic prognostic factors, such as patient age, tumor size, tumor grade, presence of lymphovascular invasion, and steroid hormone receptor status. Human epidermal growth factor receptor 2 (HER2) status, first used as a prognostic marker, became an important factor for predicting response to anti-HER2 therapy and is now a crucial part of this decision-making.

The serine protease urokinase-type plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1) are markers of tumor invasion and metastasis that have reached the highest level of evidence for clinical utility as prognostic factors in breast cancer.[1,2] Node-negative patients with high values of uPA and/or PAI-1 have been shown to benefit from adjuvant chemotherapy in a prospective randomized multicenter

therapy trial.[3,4] In addition, independent prognostic value of uPA/PAI-1 was confirmed in a pooled analysis of 8377 breast cancer patients that showed high levels of uPA and PAI-1 to be the strongest predictors of relapse-free survival and overall survival apart from lymph node status.[5] In spite of this evidence, these biomarkers are still not widely used in the clinic.[1]

Unfortunately, none of the large trials investigating the prognostic value of uPA and PAI-1 included HER2 status in the survival analysis. Only limited information is available on the relative prognostic impact of these factors when considered along with traditional prognostic markers in the same group of breast cancer patients. Therefore, it is still uncertain whether uPA and PAI-1 can give additional clinically relevant prognostic information after traditional prognostic factors and HER2 status have been taken into account.

To address this issue, we undertook the present study with the aim of comparing the prognostic impact of HER2 status, uPA, PAI-1, and traditional prognostic factors tumor size, grade, histological subtype, lymphovascular invasion, steroid hormone receptor status, and patient age on disease-free, overall, and breast cancer specific survival in node-negative breast cancer patients.

Patients and methods

Our retrospective analysis included all patients with lymph node-negative invasive breast cancer without distant metastases who underwent primary surgical treatment in Maribor University Clinical Center, Slovenia, in the ten-year period between January 1 2000 and December 31 2009. Exclusion criteria were neoadjuvant chemotherapy and presence of another active malignancy during breast cancer treatment. Considering the Helsinki Declaration principles the Slovenian National Medical Ethics Committee approved this study (Approval No. 55/11/13).

Clinical information on diagnosis, treatment, and follow-up was obtained from patient medical records. Survival data were completed with updated information from Slovenian Cancer Registry. Data on tumor size, histological subtype, grade, lymph node status, steroid hormone receptor status, and HER2 immunohistochemistry were obtained from original histology reports from the primary surgery. HER2 gene amplification, uPA and PAI-1 levels were obtained from our institution's Medical Genetics Laboratory. Due to economical limitations, uPA and PAI-1 could not be assessed in all patients. Some other histological data were missing in a small fraction of patients due to inconsistent hospital guidelines on histology reports in the past.

All patients underwent either modified radical mastectomy or breast conserving surgery and radiotherapy. Adjuvant systemic treatment was given according to the guidelines followed at our institution at the time and was not influenced by uPA and/or PAI-1 values. Patients who completed primary treatment were followed-up at our institution at regular intervals.

HER2 status was determined immunohistochemically and additional fluorescent in situ hybridization (FISH) with PathVysion HER-2 DNA Probe Kit (Abbott Molecular, Abbott Park, IL, USA) was performed in samples with an immunohistochemical result of 2+. Since the patients were diagnosed before the publication of new ASCO/CAP guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors[6] and human epidermal growth factor receptor 2 testing in breast cancer[7] and treated accordingly, steroid hormone receptor status and HER2 status were determined using the old guideline recommendations in order to avoid cases with diagnostic-therapeutic mismatch.

uPA and PAI-1 were analyzed prospectively from representative pieces of tumor tissue that were frozen in liquid nitrogen after histologic evaluation. The frozen samples were pulverised using a micro-dismembrator, suspended in a buffer (pH 8.5) containing 0.02 M Tris-HCl, 0.125 M NaCl and 2% Triton X-100, and shaken for three hours at 4°C. The obtained suspension was centrifuged for 30 minutes at 100,000 x g. Protein content was determined with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), and uPA and PAI-1 content was determined with commercially available ELISA assay kits (American Diagnostica, Greenwich, CT, USA). uPA and PAI-1 content was expressed as nanograms of analyte per milligram of tissue protein.

The correlations between different variables were tested with Spearman's rank correlation, Kruskal-Wallis test, Mann-Whitney U test and chi-square test, depending on the type of variables. The continuous variables uPA and PAI-1 were coded as binary variables using the previously optimized cutoffs of 3 ng uPA/mg protein and 14 ng PAI-1/mg protein to distinguish between low- and high-risk patients[8], and combined into one variable (both low versus one or both high) as previously shown

to be of greatest clinical relevance.[9] Because adjuvant trastuzumab was not available for the whole cohort, the patients were divided into three groups based on HER2 status and adjuvant trastuzumab treatment for the survival analyses: HER2 negative, HER2 positive who were not treated with adjuvant trastuzumab, and HER2 positive who were treated with adjuvant trastuzumab. Disease-free survival (DFS) was calculated from the date of primary surgery until the date of disease recurrence or death from any cause, or the date of the last follow-up visit in case of no recurrence or death. Overall survival (OS) was calculated from the date of diagnosis until the date of death from any cause, or the date of the last follow-up visit. Breast cancer specific survival (BCSS) was calculated from the date of diagnosis until the date of death from breast cancer, or the date of the last follow-up visit or death from other causes for censored patients. Kaplan-Meier method was used to calculate survival curves and univariate Cox regression was used to assess the differences between the curves in univariate analysis. Multivariate analyses were performed by applying the multivariate Cox proportional hazards model. All variables regardless of univariate analysis results were initially included in the Cox model and the method used for the model was backward stepwise likelihood ratio (LR). Model if term removed was reported separately. All tests were performed at a significance level of p=0.05 and a confidence interval (CI) of 95%. All p values were two-sided. Statistical analysis was performed using the SPSS software package v. 21 (IBM, Armonk, NY, USA).

Results

Patient characteristics

Eight hundred fifty-eight node-negative distant metastasis-free breast cancer patients who underwent primary surgery with curative intent were included in the study. The median age of the patients was 62 years (range, 24-95 years). The distribution of the traditional prognostic factors, HER2 status, uPA and PAI-1 in the study group is presented in Table 1. 787 (91.7%) patients received some kind of adjuvant systemic therapy. Of these, 132 received adjuvant chemotherapy, 522 adjuvant hormone therapy, and 133 a combination of both. Among patients who were given adjuvant chemotherapy, 79.2% received anthracycline-based therapy and the majority of the others received CMF (cyclophosphamide, methotrexate, 5-fluorouracil).

TABLE 1. Distribution of traditional prognostic factors, HER2 status, uPA and PAI-1 in the study group of node-negative breast cancer patients (N = 858)

Factors	No. of patients	%
Age	858	
≥ 40 years	821	95.7
< 40 years	37	4.3
Pathological tumor size	846	
< 2 cm	474	56.0
≥ 2 cm	372	44.0
Pathological tumor type	858	
Ductal invasive	720	83.9
Other invasive	138	16.1
Histological grade	799	
G1-2	557	69.7
G3	242	30.3
Lymphovascular invasion	795	
No	720	90.6
Yes	75	9.4
Estrogen receptor status	854	
Positive	674	78.9
Negative	180	21.1
Progesterone receptor status	803	
Positive	466	58.0
Negative	337	42.0
HER2 status	761	
Negative	610	80.2
Positive, without adjuvant trastuzumab	97	12.7
Positive, with adjuvant trastuzumab	54	7.1
uPA and PAI-1 level	332	
Both low	159	47.9
One or both high	173	52.1

Some factors could not be assessed in all tumors.

Among patients who were given hormone therapy, 28.9% received tamoxifen, 52.7% received an aromatase inhibitor, and the rest received a combination of both. Adjuvant trastuzumab was given in combination with chemotherapy in 6.4% of all patients and in 35.8% of HER2-positive patients. The median follow-up time was 100 months (range, 49–181 months).

Associations between traditional prognostic factors, HER2 status, uPA and PAI-1

Positive HER2 status was observed more frequently in younger patients ($p = 0.008$), in larger tumors ($p < 0.001$), tumor types other than ductal invasive ($p = 0.035$), tumors of a higher differentiation grade ($p < 0.001$), with lymphovascular invasion ($p = 0.018$), those with a lower expression of estrogen ($p < 0.001$) and progesterone receptors ($p = 0.002$), as well as in tumors with higher levels of uPA ($p = 0.026$) and PAI-1 ($p = 0.023$). uPA and PAI-1 levels correlated positively with each other ($r = 0.553$, $p < 0.001$). Higher uPA and PAI-1 values were seen in larger tumors ($p < 0.001$ for uPA; $p = 0.004$ for PAI-1), his-

TABLE 2. Univariate and multivariate analysis of disease–free survival in lymph node–negative breast cancer patients with a median follow–up time of 100 months. Multivariate analysis was performed in the 273 patients for whom complete data were available

	Univariate analysis		Multivariate analysis with all variables		Multivariate analysis – backward LR model		Model if term removed
	hazard ratio (95 % CI)	p	hazard ratio (95 % CI)	p	hazard ratio (95 % CI)	p	p
Age (continuous, unit = 10 years)	1.80 (1.60–2.02)	**< 0.001**	1.53 (1.20–1.96)	**0.001**	1.51 (1.19–1.93)	**0.001**	**< 0.001**
Tumor size (≥2 vs. <2 cm)	1.41 (1.08–1.83)	**0.012**	1.01 (0.56–1.80)	0.982	–	–	0.982
Tumor type (other invasive vs. ductal invasive)	1.08 (0.76–1.55)	0.658	1.28 (0.51–3.20)	0.598	–	–	0.606
Grade (G3 vs. G1–2)	1.37 (1.03–1.81)	**0.031**	1.54 (0.75–3.17)	0.240	–	–	0.240
Lymphovascular invasion (present vs. absent)	1.54 (1.03–2.29)	**0.035**	1.43 (0.65–3.15)	0.377	–	–	0.393
Estrogen receptors (negative vs. positive)	1.19 (0.87–1.61)	0.271	1.81 (0.79–4.16)	0.165	2.25 (1.24–4.09)	**0.008**	0.158
Progesterone receptors (negative vs. positive)	1.10 (0.84–1.45)	0.496	1.03 (0.48–2.23)	0.935	–	–	0.935
HER2 status		**0.005**		0.355			0.379
positive NT vs. negative	1.73 (1.21–2.47)	**0.003**	1.66 (0.83–3.31)	0.150	–	–	
positive T vs. negative	0.70 (0.35–1.43)	0.332	1.16 (0.46–2.91)	0.758	–	–	
uPA/PAI–1 (one or both high vs. both low)	2.16 (1.23–3.72)	**0.005**	1.76 (0.89–3.49)	0.106	1.99 (1.05–3.77)	**0.035**	0.098

NT = not treated with adjuvant trastuzumab; T = treated with adjuvant trastuzumab

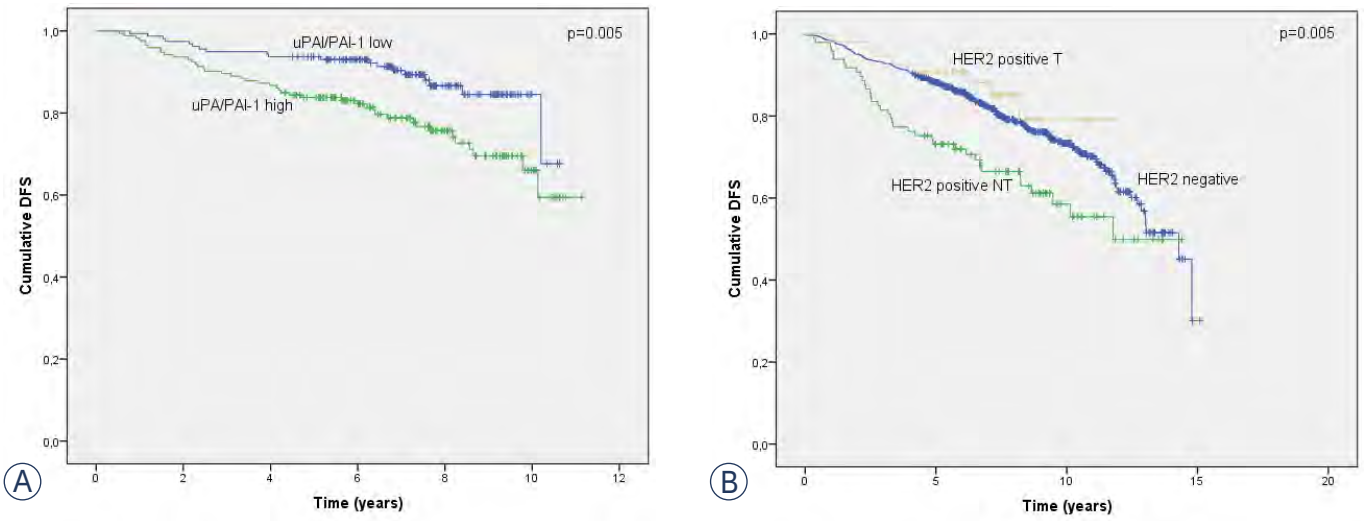

FIGURE 1. Effect of uPA/PAI-1 level and HER2 status on disease-free survival (DFS) in lymph-node negative breast cancer patients. **(A)** uPA/PAI-1 low (19 of 159 relapsed or died) versus uPA/PAI-1 high (43 of 173 relapsed or died). **(B)** HER2 negative (154 of 610 relapsed or died) versus HER2 positive not treated with adjuvant trastuzumab (NT) (37 of 97 relapsed or died) and HER2 positive treated with adjuvant trastuzumab (T) (8 of 54 relapsed or died).

NT = not treated with adjuvant trastuzumab; T = treated with adjuvant trastuzumab

tologic types other than ductal invasive ($p < 0.001$ for uPA; $p = 0.048$ for PAI-1), less differentiated tumors ($p < 0.001$ for both), and tumors with lower estrogen receptor expression ($p < 0.001$ for both). In addition, uPA but not PAI-1 was higher in tumors with lymphovascular invasion ($p = 0.047$). There were no associations between uPA or PAI-1 values and patient age or progesterone receptor status.

TABLE 3. Univariate and multivariate analysis of overall survival in lymph node-negative breast cancer patients with a median follow-up time of 100 months. Multivariate analysis was performed in the 273 patients for whom complete data were available

	Univariate analysis		Multivariate analysis with all variables		Multivariate analysis – backward LR model		Model if term removed
	hazard ratio (95 % CI)	p	hazard ratio (95 % CI)	p	hazard ratio (95 % CI)	p	p
Age (continuous, unit = 10 years)	2.18 (1.88–2.52)	**< 0.001**	1.61 (1.19–2.17)	**0.002**	1.68 (1.25–2.27)	**0.001**	**0.001**
Tumor size (≥2 vs. <2 cm)	1.38 (1.02–1.88)	**0.036**	0.94 (0.47–1.88)	0.865	–	–	0.865
Tumor type (other invasive vs. ductal invasive)	1.24 (0.83–1.85)	0.298	2.24 (0.76–6.59)	0.142	–	–	0.165
Grade (G3 vs. G1-2)	1.40 (1.01–1.94)	**0.042**	2.39 (0.95–6.02)	0.066	2.69 (1.34–5.40)	**0.005**	0.064
Lymphovascular invasion (present vs. absent)	1.34 (0.83–2.16)	0.236	1.36 (0.46–4.01)	0.582	–	–	0.594
Estrogen receptors (negative vs. positive)	1.24 (0.88–1.75)	0.224	1.80 (0.67–4.81)	0.242	–	–	0.233
Progesterone receptors (negative vs. positive)	1.01 (0.74–1.38)	0.955	0.62 (0.25–1.54)	0.299	–	–	0.279
HER2 status		0.190		0.843			0.837
positive NT vs. negative	1.37 (0.89–2.09)	0.152	0.76 (0.31–1.90)	0.562	–	–	
positive T vs. negative	0.63 (0.26–1.55)	0.318	0.90 (0.26–3.18)	0.871	–	–	
uPA/PAI-1 (one or both high vs. both low)	2.73 (1.33–5.58)	**0.006**	1.98 (0.83–4.76)	0.126	–	–	0.114

NT = not treated with adjuvant trastuzumab; T = treated with adjuvant trastuzumab

Disease-free survival

A total of 228 events occurred in the DFS analysis. Univariate and multivariate analyses of DFS are presented in Table 2. In univariate analysis, older age, tumors larger than 2 cm, grade 3, with evident lymphovascular invasion, and high uPA and/or PAI-1 (Figure 1A) were associated with worse DFS. HER2 positive patients who were not treated with adjuvant trastuzumab had significantly worse DFS than HER2 negative patients. DFS of HER2 positive patients who received adjuvant trastuzumab was not significantly different than DFS of HER2 negative patients (Figure 1B). In backward stepwise LR multivariate model, the three variables that remained significant for DFS after the final step were patient age at diagnosis, estrogen receptor status and uPA/PAI-1 level. uPA/PAI-1 level was the second most important variable after patient age in the model if term removed (Table 2).

In multivariate analysis, the HR for disease recurrence or death from any cause in estrogen receptor positive patients with high uPA and/or PAI-1 was 2.78 (95% CI, 1.28–6.03; p = 0.010) compared to those with both values low. The corresponding HR values for estrogen receptor negative, HER2 negative, HER2 positive patients who did not receive adjuvant trastuzumab, HER2 positive patients who received adjuvant trastuzumab, and triple negative patients, were 1.11 (95% CI, 0.33–3.76; p = 0.863), 1.60 (95% CI, 0.70–3.68; p = 0.268), 9.25 (95% CI, 1.06–80.82; p = 0.044), 3.31 (95% CI, 0.10–109.67; p = 0.503), and 1.08 (95% CI, 0.30–3.86; p = 0.902), respectively.

Overall survival

A total of 172 events occurred in the OS analysis. Univariate and multivariate OS analyses are shown in Table 3. Patient age at diagnosis, tumor size, grade, and uPA/PAI-1 levels (Figure 2a) were found to have a significant impact on OS in univariate analyses. HER2 (Figure 2B) and the other traditional prognostic factors were not found to influence OS in our series of node-negative breast cancer patients. In backward stepwise LR multivariate model, the remaining variables significantly associated with OS after the last step were patient age and tumor grade (Table 3). uPA/PAI-1 level was the third most important factor in the model if term removed and was removed from the model at the last step.

Breast cancer specific survival

A total of 65 events occurred in the BCSS analysis. Univariate and multivariate BCSS analyses

TABLE 4. Univariate and multivariate analysis of breast cancer specific survival in lymph node-negative breast cancer patients with a median follow-up time of 100 months. Multivariate analysis was performed in the 273 patients for whom complete data were available

	Univariate analysis		Multivariate analysis with all variables		Multivariate analysis – backward LR model		Model if term removed
	hazard ratio (95 % CI)	p	hazard ratio (95 % CI)	p	hazard ratio (95 % CI)	p	p
Age (continuous, unit = 10 years)	1.16 (0.95–1.41)	0.148	0.76 (0.51–1.12)	0.162	–	–	0.160
Tumor size (≥2 vs. <2 cm)	2.44 (1.45–4.13)	**0.001**	1.72 (0.55–5.38)	0.350	–	–	0.340
Tumor type (other invasive vs. ductal invasive)	1.20 (0.63–2.29)	0.587	3.33 (0.64–17.23)	0.152	–	–	0.196
Grade (G3 vs. G1-2)	3.58 (2.12–6.05)	**< 0.001**	7.10 (1.23–40.86)	**0.028**	10.34 (2.33–45.97)	**0.002**	**0.014**
Lymphovascular invasion (present vs. absent)	1.48 (0.70–3.12)	0.305	1.43 (0.36–5.65)	0.608	–	–	0.618
Estrogen receptors (negative vs. positive)	2.21 (1.33–3.66)	**0.002**	1.24 (0.28–5.36)	0.778	–	–	0.776
Progesterone receptors (negative vs. positive)	1.74 (1.06–2.86)	**0.028**	0.81 (0.17–3.77)	0.789	–	–	0.788
HER2 status		**0.031**		0.663			0.614
positive NT vs. negative	2.18 (1.21–3.93)	**0.009**	1.29 (0.39–4.31)	0.683	–	–	
positive T vs. negative	0.96 (0.30–3.10)	0.944	0.46 (0.06–3.68)	0.460	–	–	
uPA/PAI-1 (one or both high vs. both low)	6.46 (1.47–28.43)	**0.014**	2.79 (0.57–13.67)	0.205	–	–	0.166

NT = not treated with adjuvant trastuzumab; T = treated with adjuvant trastuzumab

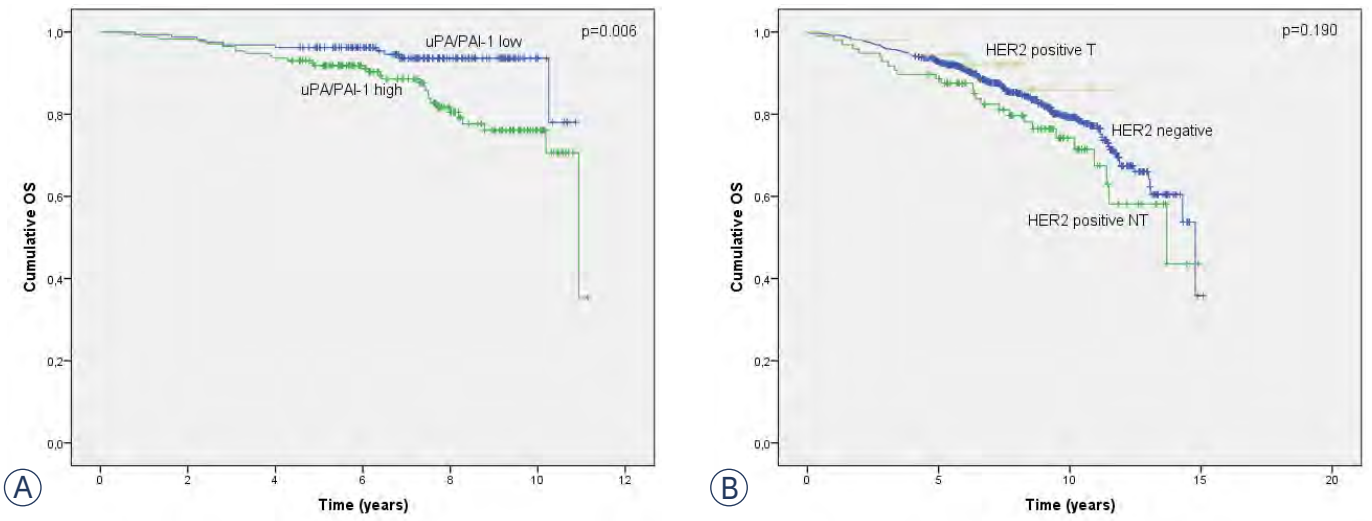

FIGURE 2. Effect of uPA/PAI-1 level and HER2 status on overall survival (OS) in lymph-node negative breast cancer patients. **(A)** uPA/PAI-1 low (10 of 159 died) versus uPA/PAI-1 high (31 of 173 died). **(B)** HER2 negative (118 of 610 died) versus HER2 positive not treated with adjuvant trastuzumab (26 of 97 died) and HER2 positive treated with adjuvant trastuzumab (5 of 54 died).

NT = not treated with adjuvant trastuzumab; T = treated with adjuvant trastuzumab

are shown in Table 4. Tumor size, grade, estrogen and progesterone receptor status, HER2 status and uPA/PAI-1 levels were associated with BCSS in univariate analyses. The only variable retained in the final multivariate model using the backward stepwise LR method was tumor grade. uPA/PAI-1 level was the third most important factor in the model if term removed.

Discussion

The results of our study indicate an important prognostic value of uPA/PAI-1 level in node-negative breast cancer patients. Even though uPA and PAI-1 values were associated with most of the prognostic factors currently in use for clinical decision-making in the adjuvant setting, multivariate analysis showed that uPA/PAI-1 level carries additional, independent prognostic information. This was particularly true for DFS analysis where uPA/PAI-1 level was the second most important variable in the survival model after age and was retained in the backward LR model after the final step along with age and estrogen receptor status. In OS analysis, the final model included only age and tumor grade, which is probably a reflection of the very strong association between higher uPA and PAI-1 values and less differentiated tumors. However, in the multivariate model that included all variables, the HR for death from any cause was 1.98 (95% CI 0.83–4.76) for patients with high uPA and/or PAI-1 compared to patients with both values low, which shows a substantial possibility of an important effect on OS that would have probably remained statistically significant in a larger sample. The same three variables were the most important in the multivariate model of BCSS as well. Here, the singular prognostic importance of tumor grade was even more evident. As opposed to DFS and OS which both included deaths from other causes as events in the analysis, younger age seemed associated with worse BCSS. It is important to emphasize that BCSS analysis must be interpreted with caution due to the small number of events. Considering the HRs and confidence intervals, it is probable that both age and uPA/PAI-1 level importantly influence BCSS and would have retained statistical significance in a larger sample. On the other hand, HER2 status only showed prognostic significance in univariate DFS and BCSS analyses when comparing HER2 negative to HER2 positive patients who were not treated with adjuvant trastuzumab. It did not remain an important determinant of DFS and BCSS in multivariate analyses and lost all prognostic value in OS analysis.

A subgroup analysis of DFS according to estrogen receptor and HER2 status showed that apart from the no longer relevant group of HER2 positive patients who did not receive adjuvant trastuzumab treatment, uPA/PAI-1 level was prognostically by far the most important in estrogen receptor positive tumors. In contrast, its prognostic value in multivariate analysis was practically null in HER2 positive patients treated with adjuvant trastuzumab and in triple negative patients. Conveniently, estrogen receptor positive patients are the ones with the highest uncertainty regarding the benefit of adjuvant chemotherapy, while the other two subgroups are generally recommended adjuvant chemotherapy ± anti-HER2 therapy regardless of other prognostic factors.

Our findings confirm those of numerous other studies that have reported uPA and PAI-1 to be statistically independent prognostic factors in lymph node-negative breast cancer patients.[10-18] However, these studies did not include HER2 status which is now an important part of the decision about adjuvant systemic therapy. Because of the observed associations between overexpression of the HER2/neu protein and tumor proteolytic factors in breast and in other cancers[19-21], HER2/neu has been suggested to up-regulate the proteolytic enzymes, including uPA and PAI-1, and thus play a direct role in tumor invasion and metastasis.[19] This possible association might be one of the additional reasons besides methodological difficulties why uPA and PAI-1 testing is still not that frequently used in the clinic in spite of the excellent evidence of its clinical utility.[1] Even so, the main reason for this inconsistency are practical issues. uPA and PAI-1 are determined using validated ELISA assay kits as described above. This measurement requires relatively large amounts of fresh or freshly frozen tumor tissue, which is not practical for clinical use, especially in needle or surgical biopsies and in small tumors. Besides, the results may not be available at the time of the histology report due to sample pooling. However, attempts to develop immunohistochemistry assays on formalin-fixed and paraffin-embedded tissues are ongoing.[1]

Among the few studies that considered the clinical relevance of both HER2 and proteolytic enzymes for survival in breast cancer patients, the results are somewhat conflicting. Harbeck *et al.*[22] showed that PAI-1 was the only independent prognostic factor for DFS and OS when considered along with tumor size, tumor grade, steroid hormone receptor status, uPA, HER2 status, MIB1, SPF, p53, and cathepsin D in 100 node-negative breast cancer patients. Similarly, Bouchet *et al.*[23] found no additional prognostic information of HER2/neu protein levels when evaluated in multivariate analysis together with uPA, PAI-1 and traditional histologic factors in the subgroup of 226 node-negative patients. They reported DFS to be independently influenced by PAI-1 and tumor size and OS by PAI-1 and uPA levels. Recently, Buta *et al.*[24] also reported

superior DFS in 73 node-negative, postmenopausal, steroid hormone receptor positive breast cancer patients with smaller tumors and low PAI-1, independent of HER2 status. On the other hand, Konecny *et al*.[19] reported that both uPA/PAI-1 level and HER2 status independently influenced DFS in addition to tumor size and nodal status in a group of 542 patients with a short follow-up not selected by nodal status. The same factors were found to be important in multivariate OS analysis as well, but this time HER2 status did not quite reach statistical significance. Interestingly, among 118 node-negative patients with long-term follow-up, Zemzoum *et al*.[25] found uPA/PAI-1 to be the only variable independently influencing DFS, and HER2 status to be the most important factor in the multivariate analysis of OS. uPA/PAI-1 and tumor grade were of borderline significance for OS. Complicating the matter even further, without reference to uPA and/or PAI-1, Schmidt *et al*.[26] reported HER2 status to be prognostically significant in node-negative breast cancer patients only when determined by FISH and not when determined immunohistochemically. In our group of 273 node-negative patients with long-term follow-up who were available for multivariate analysis, the combination of uPA and PAI-1 levels clearly carried independent prognostic value for DFS and was important although not formally statistically significant in multivariate analysis of OS as well, while HER2 status determined with the usual combination of immunohistochemistry and FISH did not prove to be an important determinant of DFS or OS in multivariate analyses regardless of adjuvant anti-HER2 treatment.

Most of the studies confirming the prognostic value of uPA and PAI-1 in breast cancer patients have focused on patients who received no adjuvant systemic treatment. In fact, the prognostic information from these biomarkers seems diminished in patients who receive adjuvant treatment, particularly adjuvant hormone therapy[27,28], indicating a possible predictive role of uPA and PAI-1 for response to therapy. However, the prognostic importance of uPA/PAI-1 level was evident in our group of patients although the vast majority received adjuvant hormone therapy and a significant fraction were given adjuvant chemotherapy. Contrastingly, based on our results, HER2 status is clearly more important in its predictive than in its prognostic role.

Our study has several limitations, the principal one being its retrospective character and the associated possibility of bias. A major problem is missing data, particularly on uPA and PAI-1 values which could only be determined in about 40% of the patients because of our institution's economical limitations and the fact that these markers still largely serve only academic purposes. Because of inconsistent histology reports in the past, some other information is missing in a fraction of patients. The total number of patients with available information was used for each univariate analysis and multivariate analyses were performed in the 273 patients in whom complete data were available. Even so, we are aware of only one study comparing the prognostic values of uPA, PAI-1, HER2, and traditional prognostic factors that included a slightly larger subgroup of 283 lymph node-negative patients.[19] Another possible limitation is the comparatively high proportion of deaths from causes other than breast cancer, which may have somewhat obscured the results of both DFS and OS analysis and is probably also the cause of such a marked association of older age and worse prognosis, an assumption confirmed by the fact that older age was associated with improved breast cancer specific survival. Furthermore, our results must be considered in the light of the unavoidable multiple analyses and the possibility of a type I statistical error. To facilitate interpretation, 95% confidence intervals and not just the p values have been stated wherever possible. On the other hand, one of the main strengths of our study in addition to the combination of analyzed prognostic factors is the long follow-up which was in the range of 49-181 months, the median of 100 months being more than three times the median follow-up of the larger study by Konecny *et al*.[19] Moreover, we believe that the scientifically sound and straightforward statistical analysis is a strength of our study as well.

Based on the results from our retrospective analysis of node-negative breast cancer patients with long-term follow-up, we conclude that the combined uPA/PAI-1 level carries important additional prognostic information, particularly for DFS, even after all traditional prognostic factors as well as HER2 status have been taken into account. We believe that routine use of uPA/PAI-1 level would further improve risk stratification and adjuvant therapy decisions in this setting, especially in estrogen receptor positive patients.

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research article

Safety and efficacy of doxorubicin-eluting superabsorbent polymer microspheres for the treatment of liver metastases from neuroendocrine tumours: preliminary results

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Background. The aim of the study was to retrospectively evaluate the symptom control, tumour response, and complication rate in patients with liver-predominant metastatic neuroendocrine tumours treated with transarterial chemoembolization using doxorubicin-eluting superabsorbent polymer (SAP) microspheres.

Patients and methods. Patients with neuroendocrine liver metastases who underwent hepatic transarterial chemoembolization using doxorubicin-eluting SAP-microspheres (50–100 µm Hepasphere/Quadrasphere Microsphere® particles, Merit Medical, South Jordan, Utah, USA) were included in this study. Pre- and post-procedure imaging studies were evaluated to assess short and intermediate-term tumour response using modified RECIST criteria. Symptom relief and procedure-related complications were evaluated.

Results. A total of 27 embolization procedures were performed on 17 patients. Twelve of 17 patients (70%) were symptomatic, including carcinoid syndrome (n = 8) and severe, uncontrollable hypoglycemia (n = 4). Eight of 12 patients (67%) had complete symptom relief, and the remaining 4 (33%) had partial relief. One patient developed ischemic cholecystitis (6%). No other hepatobiliary complications occurred. Short-term and intermediate-term imaging follow-up was available for 15/17 patients (88%) and 12/14 patients (86%) respectively. At short-term follow-up (< 3 months), 14 patients (93%) showed partial response and the remaining patient had progressive disease (7%). At intermediate-term imaging follow-up (> 3 months), partial response, stable disease and progressive disease were found respectively in 7 (58%), 3 (25%) and 2 (17%) patients.

Conclusions. Chemoembolization with doxorubicin-eluting SAP-microspheres is a safe and effective treatment option for neuroendocrine liver metastases and is associated with a low complication rate. In particular, no clinically evident liver necrosis or bile duct complications were encountered.

Key words: neuroendocrine tumour; chemoembolization; drug-eluting beads; procedure-related complications

Introduction

Among patients with neuroendocrine tumours (NET) hepatic metastases are present in 46%–93% at initial presentation.[1,2] The metastases may be responsible for hormone-related symptoms, including carcinoid syndrome and uncontrollable hypoglycemia in case of insulinoma. These clinical

symptoms can be treated effectively with somatostatin analogues in more than 70% of patients, but efficacy can decrease with time.[3] Although surgical resection of NET liver metastases can be curative and remains the treatment of choice, only 10% of patients are candidates for liver surgery.[4,5] Liver-directed transarterial therapies in nonsurgical candidates include transcatheter arterial embolization[6,7], chemo-embolization[6,8] and Yttrium-90 radioembolization.[9,10] These techniques have been used both to palliate hormone-related symptoms as well as to reduce metastatic tumour burden. Conventional transarterial chemoembolization using ethiodized oil and cytotoxic drugs has been the most popular locoregional, catheter-directed therapy to treat unresectable NET liver metastases for more than 20 years.[3,11] Drug-eluting microspheres have been used recently for both hepatocellular carcinoma[12,13] and metastases to the liver[14-16], and are associated with sustained efficacy and less systemic toxicity.[17,18] In contrast to its use in hepatocellular carcinoma[19], a high complication rate was found when using drug-eluting beads (DC/LC-beads, Biocompatibles, Farnham, UK) in neuroendocrine liver metastases, including a high risk for postprocedural formation of liver necrosis and biloma.[20-23]

We used superabsorbent polymer (SAP) microspheres (50–100 μm Hepasphere/Quadrasphere Microspheres®, Merit Medical, South Jordan, Utah, USA) loaded with doxorubicin as an alternative drug-eluting microsphere technology to treat NET liver metastases and assessed the feasibility, safety, clinical and radiological response rates in a retrospective study design.

Patients and methods

The institutional interventional radiology database was queried for patients undergoing transarterial chemoembolization using doxorubicin-eluting SAP microspheres for the treatment of liver metastases from histologically proven neuroendocrine tumours. Demographic, clinical, radiologic and laboratory data were collected. All patients were discussed at the Multidisciplinary tumour board, including medical, surgical and radiation oncologists as well as pathologists and diagnostic and interventional radiologists. All patients gave informed consent for the chemoembolization procedure and this retrospective study was approved by the local ethics committee.

Inclusion criteria for chemoembolization were symptomatic patients with liver-only or liver-predominant neuroendocrine metastases, unresponsive under chemotherapy and not a candidate for surgical resection or radiofrequency ablation or patients with progression of disease, despite optimal chemotherapeutic management. Additionally, the residual liver function allowed chemoembolization: bilirubin levels less than 2.0 mg/dl, alanine amino transferase (ALT) and aspartate aminotransferase (AST) less than five times the upper limit of normal, albumin more than 2 mg/dl.

Contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) was performed within one month before the first chemoembolization procedure. Postprocedure imaging interval was variable, depending on whether a unilobar or bilobar infusion was performed within 1 session, and ranged from 1–3 months for short-term follow-up and from 5–6 months for intermediate-term follow-up. Radiologic tumour response was assessed by size based on modified Response Evaluation Criteria in Solid Tumors (m-RECIST).

All chemoembolization procedures were performed using a standard technique as described previously for the treatment of unresectable hepatocellular carcinoma treated with doxorubicin-eluting SAP microspheres.[13] Briefly, after administration of local anaesthesia in the right groin, a 4 French (F) sheath (Boston Scientific, Natick, MA, USA) was introduced into the right common femoral artery. Selective catheterization of the celiac trunk and the superior mesenteric artery was performed with use of a 4F catheter (Impress diagnostic catheter, Merit Medical, South Jourdan, UT, USA) and followed by superselective catheterization of the right and left hepatic arteries using a microcatheter (EmboCath Plus or Maestro 2.8, Merit Medical, South Jourdan, UT, USA). Depending on number and distribution of the metastatic lesions the drug-eluting microspheres were delivered to segmental or lobar hepatic artery/arteries. If tumour burden was more than 50% of total liver volume, 2 separate lobar infusions 1 month apart were performed. No antibiotics were administered before or during the procedure. All patients had intact biliary sphincters; analgesics or antiemetics were administered if needed. All patients received somatostatin analogues (Sandostatin, Novartis Pharma, Vilvoorde, Belgium) before the start of the procedure. For each procedure, one vial of 25 mg dry SAP microspheres with a nominal dry diameter of 50-100 microns (Hepasphere/Quadrasphere Microspheres, Merit Medical, South Jordan, UT, USA), was prepared in the hospital pharmacy. The SAP microspheres were mixed with doxorubicin

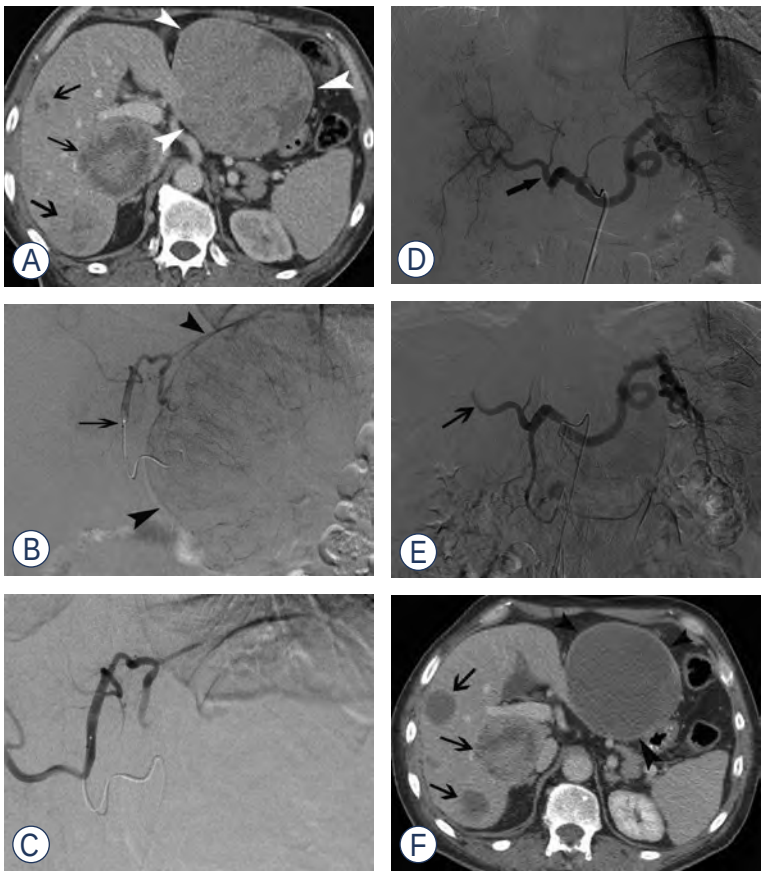

FIGURE 1. A 63-year-old male patient presented with a carcinoid of the lung and diffuse bilobar liver involvement. **(A)** Portal venous phase contrast-enhanced CT-scan confirms diffuse metastatic involvement of both liver lobes (white arrowheads at the level of the largest metastasis in the left liver lobe; black arrows at the level of multiple smaller lesions in the right liver lobe); Selective angiogram of the left hepatic artery **(B)** before and **(C)** after chemoembolization with doxorubicin-eluting SAP-microspheres (arrow at the level of the micro-catheter in the left hepatic artery); Selective angiogram of the celiac trunk **(D)** before and **(E)** after chemoembolization with doxorubicin-eluting SAP-microspheres (arrow shows stasis of contrast at the level of the right hepatic artery); **(F)** Portal venous phase contrast-enhanced CT-scan 10 weeks after initial chemoembolization shows marked decrease in volume and enhancement of most of the metastatic lesions in left and right liver lobes.

(Pharmachemie BV, Haarlem, Netherlands) and suspended in 10 ml sodium chloride 0.9% and 10 ml iodinated contrast medium (Visipaque 270, GE Healthcare, Oslo, Norway). The standard doxorubicin dose was 75 mg/m^2 (square metre of body surface area), which was reduced to 50 mg/m^2 or 25 mg/m^2 in cases of elevated bilirubin or cytopenia. The injection of the doxorubicin-eluting SAP microspheres was stopped once the feeding arteries were completely occluded as confirmed by hand-injected hepatic angiography at completion. If residual hypervascular blush was identified on confirmation angiography after chemoembolization, an additional vial of bland polyvinyl alcohol particles (nsPVA) of 250–355 micron caliber (Contour, Boston Scientific Corporation, Natick, MA, USA) was injected.

Patients were followed clinically every 2 months on an outpatient basis; early and late adverse events were noted in the patients' electronic hospital records. Chemo-embolization-related toxicity was assessed according to the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0. Assessment of symptom control after chemoembolization was performed 4–6 months after the initial chemoembolization by the referring medical oncologist. Overall survival probabilities were estimated by the Kaplan-Meier method.

Results

Seventeen patients (9 women, 8 men) with histologically proven gastrointestinal, pancreatic or pulmonary neuroendocrine tumours metastastic to the liver were included. Patients' age, site and type of the primary tumour, tumour grade based on the Ki-67 index, extrahepatic tumoural disease, and previous surgical and medical therapies are summarized in Table 1. No patient presented with portal vein thrombosis or bile duct dilatation at the time of chemoembolization procedure.

In 5 patients (29%), chemoembolization of right and left liver lobes was performed in separate sessions. Both right and left liver lobes were treated in 8 patients (47%) during the same session (Figure 1). For the remaining 4 patients (24%), isolated right hepatic lobe chemoembolization was performed.

In all 27 chemoembolization procedures the whole vial of doxorubicin-loaded SAP microspheres could be injected. The total median dose of doxorubicin injected per session was 133 mg (min 25 mg – max 150 mg). In 4 of 27 procedures (15%), one additional vial of PVA particles was injected after the SAP microspheres. Procedure-related toxicity is summarized in Table 2. Management of these complications included percutaneous gallbladder drainage (n = 1), medical management of carcinoid and insulin storm (n = 2) and analgesics and antiemetics (n = 14). No procedure-related liver necrosis or biloma was found during immediate or late follow-up.

Short-term and intermediate-term imaging follow-up was available for 15/17 patients (88%) and 12/14 patients (86%) respectively. On short term follow-up, partial response and progressive dis-

TABLE 1. Patients' demographic data

Variable	Number
Number of patients included	**17**
Age (years)	
Mean	56
SD	13.9
Range	18–82
Sex	
M	8
F	9
Primary tumour	
Intestinal NET	6
Pancreatic NET	2
Pulmonary NET	2
Pancreatic insulinoma	4
Unknown	3
Surgical resection of primary tumour	
Intestinal NET	5
Pancreatic NET	2
Pulmonary NET	2
Pancreatic insulinoma	1
Total	10
Tumour grade (Ki-67 index)	
Ki-67 < 2%	4
Ki-67 2–20%	6
Ki-67 > 20%	4
Unknown	3
Tumour burden (% of total liver volume)	
0–10%	4
10–20%	3
20–50%	5
> 50%	5
Extrahepatic metastatic disease	16
Lymphadenopathy	7
Bone	5
Lung	3
Spleen	1
Adrenal	3
Brain	1
Ovary	2
Pancreas	2
Peritoneum	1
Skin	1
Previous treatment	
Surgical resection of liver metastases	1
Radiofrequency ablation of liver metastases	1
Radiotherapy for brain metastases	1
Radiotherapy for bone metastases	1
Interferon	3
Everolimus	7
m-TOR-inhibitor	1
Sunitinib	4
Somatostatine analogue	15
Diazoxide	3

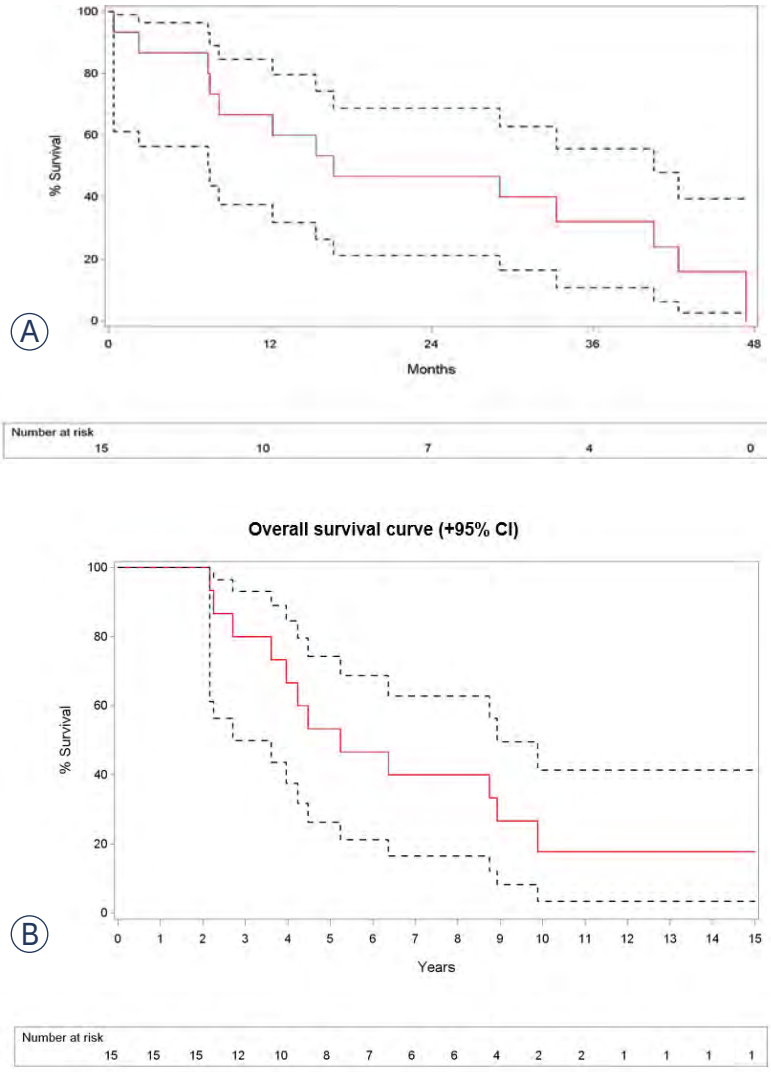

FIGURE 2. (A) Kaplan-Meier overall survival estimate shows an overall survival (with 95% confidence interval) at 12, 24 and 36 months after TACE of 67% (lower limit 38% - upper limit 85%), 47% (lower limit 21% - upper limit 69%) and 32% (lower limit 11% - upper limit 56%); **(B)** Kaplan-Meier overall survival estimate shows an overall survival (with 95% confidence interval) at 5, 10 and 15 years after diagnosis of 53% (lower limit 26% - upper limit 74%), 18% (lower limit 3% - upper limit 41%) and 18% (lower limit 3% - upper limit 41%).

ease according to m-RECIST criteria were found in 14 (93%) and 1 (7%) patients respectively. On intermediate term follow-up, m-RECIST-based partial response, stable disease and progressive disease was found respectively in 7 (58%), 3 (25%) and 2 (17%) patients.

Eight of 17 patients (47%) presented with carcinoid syndrome-related symptoms including diarrhea (n = 7; 41%) and flushing (n = 7; 41%); 5 of these 8 patients (63%) had complete symptom relief after chemoembolization. The remaining 3 pa-

TABLE 2. Procedure-related toxicity

Toxicity	grade	number of patients	percentage (%)
Ischemic cholecystits	3	1	6
Carcinoid storm	2	1	6
Insulin storm	2	1	6
Postembolization syndrome	2	14	82

Grading of procedure-related toxicity is based on National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0.

tients had moderate relief of symptoms with persisting signs of diarrhea (n = 2) and flushing (n = 3). Three of 4 patients with liver metastases related to primary insulinoma of the pancreas (pre-interventional mean glycemia values of 57 mg/dl (range 39–83 mg/dl)) had complete normalisation of their glycaemic values after the chemoembolization procedure. One patient had a partial clinical response with a residual glycemic value of 100 mg/dl. One patient with predominant symptoms of abdominal pain did not respond to the chemoembolization treatment. Overall survival of the studied patients is summarized in Figure 2 with an estimated survival at one year after TACE of 67%, and an estimated survival at five years after diagnosis of 53%.

Discussion

This retrospective observational study shows a high rate of symptomatic relief after chemoembolization for neuroendocrine liver metastases using doxorubicin-eluting SAP-microspheres: 5 of 8 patients with carcinoid syndrome-related symptoms showed complete symptom relief after the first session of chemoembolization and 3 of 4 patients with preinterventional hypoglycemia presented with complete normalisation of their glycemic values. These results are consistent with symptomatic responses after conventional chemoembolization showing symptom relief in 52%–86% of cases. These encouraging results are even higher when the treatment is performed as a first line therapy, with up to 70% complete symptomatic response and 20% partial response.[3,6,8,11]

This study also demonstrates substantial radiologic response rates, with 93% of patients showing partial response or stable disease at 3 month follow-up, based on modified RECIST criteria. These results are consistent with response rates using doxorubicin-eluting DC Beads (BTG-Biocompatibles, Farnham, UK) or using conventional ethiodized oil-based chemoembolization, which range between 57% and 80%.[8,20,21] These high response rates potentially can also be achieved in patients with high tumour burden, as suggested in the present study, with tumour burden ranging from 8% to 58%. Bland embolization without the addition of any chemotherapeutic agent also has demonstrated high response rates similar to those of chemoembolization.[3,6,11] Although most of the studies dealing with catheter-directed liver interventions for neuroendocrine metastases are based on chemo-embolization, however, no retro- or prospective comparative study has demonstrated a significant difference in response rate or other benefit for chemoembolization compared to bland embolization.

Toxicity related to SAP microsphere-based chemoembolization for neuroendocrine liver metastases is mainly limited to the post-embolization syndrome which was observed in 82% of cases. Only one case of post-embolization cholecystitis was observed, which was treated by percutaneous drainage, and one instance each of post-embolization carcinoid (n = 1) and insulin (n = 1) storm occurred despite prophylactic administration of somatostatin analogues before chemoembolization. Importantly, we did not observe any cases of post-embolization liver necrosis, liver abscess or biloma. Post-chemoembolization liver abscess formation is a risk in patients with biliodigestive anastomosis or biliary stents in situ, despite the prophylactic administration of broad spectrum antibiotics.[8,24] In this series there were no patients with previous bile duct surgery or intervention. In other studies post-embolization liver necrosis and intrahepatic biloma-formation were found in nearly half of the cases using small calibre (100–300 μm) doxorubicin-loaded DC Beads, but not after conventional, ethiodized oil-based chemoembolization.[20-22,25] It is unclear why this high number of biloma cases was found after DC/LC Bead chemoembolization for neuroendocrine liver metastases; it is hypothesized that small calibre microspheres will penetrate deeper in the (normal) residual liver parenchyma and thereby induce irreversible ischemia to the liver and biliary tree tissues.[20] This serious side effect was seen more often in patients with a smaller tumour burden compared to patients with a higher tumour load, stressing the fact that the residual normal liver parenchyma is sensible to ischemic changes when small calibre microspheres are injected.[20] SAP microspheres used in this study had

a diameter of 50–100 μm in their dry state, swelling to 200-400 μm once hydrated in saline solution. This mechanism of expansion and subsequent larger diameter, thereby resulting in a more proximal embolization when injected into the liver[26-28], might prevent any necrotic complication.

Finally, we did not observe procedure-related liver failure, even in patients with considerable tumour burden, bilobar treatment, or in patients heavily pretreated by somatostatin analogues and different types of other drugs.

Limitations of this study are multiple. First, this study deals with a very heterogeneous patient population presenting with both symptomatic and asymptomatic neuroendocrine liver metastases who were previously treated with various types of chemotherapeutics and other medical treatments. Second, the patient sample is too small to draw firm conclusions with regard to symptom and tumour responses. Third, late response rates as well as overall survival also depend on prior, concomitant and further treatment options and not on the chemoembolization procedures alone. Last, no comparative analysis with other locoregional or medical treatments was performed.

In conclusion, this study suggests that doxorubicin-eluting SAP microspheres appear to be a safe and potentially effective treatment option in the treatment of neuroendocrine liver metastases and this interventional technique might be proposed as an alternative locoregional treatment option for conventional ethiodized oil-based chemoembolization.

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research article

Long-term outcomes of high dose treatment and autologous stem cell transplantation in follicular and mantle cell lymphomas – a single centre experience

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Background. Advanced follicular lymphoma (FL) and mantle cell lymphoma (MCL) are incurable diseases with conventional treatment. The high dose treatment (HDT) with autologous stem cell transplantation (ASCT), however, offers a certain proportion of these patients the prospect of a prolonged disease-free and overall survival. The aim of this study was to investigate the event free survival (EFS) and overall survival (OS) in patients with FL and MCL treated with ASCT.

Patients and methods. Seventeen patients with FL and 29 patients with MCL were included, 15 of them were transplanted to consolidate the response to second line treatment and 24 to consolidate their first remission, respectively. All were conditioned with total body irradiation (TBI) and high dose cyclophosphamide between 2006 and 2014 and all were transplanted with peripheral blood stem cells.

Results. The estimated 5-year OS for FL was 87.8% (95% confidence interval [CI] 59.5%–96.8%) and for MCL 79.3% (95% CI 56.1%–91.1%), respectively. The estimated 5-year EFS for FL was 76.0% (95% CI 48.0%–90.3%) and for MCL 69.8% (95% CI 45.5%–84.8%), respectively. There were no secondary hematological malignancies observed in either group.

Conclusions. Based on above results, the ASCT with TBI is a good treatment option in terms of long-term survival for patients with follicular and mantle cell lymphoma demonstrating a relatively low rate of late toxicities and secondary malignancies.

Key words: follicular lymphoma; mantle cell lymphoma; autologous stem cell transplantation; overall survival; hematological malignancies

Introduction

Follicular lymphoma (FL) is nowadays still an incurable disease using standard chemotherapy.[1] Even though it is very sensitive to chemo- and radiotherapy relapses remain the main treatment failure. Significant changes were made in the past two decades, a great gain was the addition of rituximab to the standard CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone) to prolong progression-free survival (PFS).[2,3] Rituximab is now the golden standard in the first line treatment[2], it also improves the overall survival (OS) in relapsed FL patients.[4] In a Cochrane review in 2012, the authors demonstrated that high dose treatment (HDT) with autologous stem cell

transplantation (ASCT) improves the progression-free survival in comparison with chemotherapy or immuno-chemotherapy in previously untreated patients with FL, but does not prolong the OS.[5] There is also evidence that HDT with ASCT brings benefits to patients with relapsed FL.[5,6] A consensus was made in 2013 by the European Group for Blood and Marrow Transplant stating that the SCT is appropriate in patients with first chemo-sensitive relapse to consolidate remission, especially in patients with a short response after immuno-chemotherapy or with high-risk follicular lymphoma international prognostic index (FLIPI). They also pointed out that the HDT with ASCT is appropriate in second or subsequent chemo-sensitive relapses.[1]

Allogeneic transplantation and autologous transplantation are both possible. A higher relapse rate was observed in autologous SCT, whereas no significant difference in the disease-free survival or OS was found.[7,8] Allogeneic SCT showed a lower risk for disease recurrence[9,10], and that also applies for the reduced intensity conditioning SCT.[8] However, higher treatment-related mortality after allogeneic SCT than after autologous was objectified. One of the factors which contributed significantly to the higher treatment-related mortality was the total body irradiation (TBI).[9]

The mantle cell lymphoma (MCL) is also an incurable disease and successful treatment is still a challenge. In the past few years, many induction regimens were tested for their efficacy.[11-14] Since 2013, we use in our centre the alternation of rituximab-CHOP (R-CHOP) regimen with R-high dose cytarabine-based regimen for younger patients, as it was shown that it gives a better OS and a higher proportion of partial remission (PR) to complete remission (CR) conversions than other regimens.[15] Nevertheless, the HDT and ASCT remain an attractive option for those with chemo-sensitive disease regardless of the induction regimen applied.[12,14-16] In relapsed or refractory disease, long-term disease-free intervals have not been established, the reduced intensity conditioning transplantation is here an option.[16] Secondary hematological malignancies after the SCT remain an important issue with an estimated 5 year risk of 3.8%[17], although some authors report of minimum hematological malignancies or none at all.[15] The allogeneic SCT offers a lower relapse rate but a higher non-relapse mortality resulting in OS similar to ASCT.[18] However, it was also shown by Romera *et al.* that intensive chemotherapy with R-hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone) alternating with R-high dose methotrexate-cytarabine is also a suitable treatment option for treatment of MCL without ASCT.[19]

The aim of this study was to investigate the event free survival (EFS) and OS in patients with FL and MCL treated with ASCT.

Patients and methods

The study population includes 17 patients with FL and 29 patients with MCL who underwent ASCT after HDT conditioning with TBI (fractionated 6 x 200 cGy during three days' period) and high dose cyclophosphamide (2 x 60 mg/kg) between 2006 and 2014 and whose conventional treatment and the TBI were performed at the Institute of Oncology Ljubljana and the transplantation procedure at the University Clinical Centre Ljubljana, Department of Hematology. The study was conducted according to the Helsinki Declaration, all patients were aged 18 or above and patient's informed consent was obtained.

According to national guidelines, FL patients were transplanted in their second remission and only three patients with adverse prognostic factors were consolidated for their first remission. Conversely, MCL patients were transplanted already in their first remission and exceptionally only five patients (with low risk international prognostic index for MCL [MIPI]) were consolidated for their second or later remission. All patients included in the study were transplanted with stem cells collected from peripheral blood. All patients were also treated with maintenance rituximab post ASCT concordant with the latest studies[20,21] and were treated with rituximab containing regimens in their induction treatments. Twenty-six MCL patients received R-CHOP regimen as their first induction treatment and 3 patients received R-CHOP as the second line treatment. In the FL group, only 10 patients started with R-CHOP as the first line induction treatment and 7 patients received R-CHOP after failure of first line treatment without rituximab.

The disease status prior to ASCT was evaluated according to revised Cheson's criteria.[22] The OS was measured from the time of HDT/ASCT to death from any cause. The EFS was measured from the time of HDT/ASCT to progression, recurrence of the disease, or death from any cause. The non-relapse mortality was defined as death from any cause without progression or relapse of lymphoma. The EFS and OS were calculated according to the Kaplan-Meier method and differences between

TABLE 1. Patients' characteristics

	Follicular lymphoma group N = 17	Mantle cell lymphoma group N = 29
Sex (F/M)	7 (41.2%) / 10 (58.8%)	6 (20.7%) / 23 (79. 3%)
Age at diagnosis in years, median	46 (range 29–62)	54 (range 38–65)
FL grade I/II IIIa	 13 (76.5%) 4 (23.5%)	 /
Pleomorphic/blastoid MCL	/	10 (34.5%)
Distribution according to FLIPI or MIPI low risk/intermediate/high risk	4 (23.5%) / 9 (52.9%) / 4 (23.5%)	24 (82.8%) / 4 (13.8%) / 1 (3.4%)
HDT/ASCT After first line treatment* After second line treatment or later*	 3 (17.6%) 14 (82.4%)	 24 (82.7%) 5 (17.2%)
Disease status prior HDT/ASCT CR PR	 5 (29.4%) 12 (70.6%)	 13 (44.8%) 16 (55.2%)
Documented rituximab treatment before HDT/ASCT	17 (100%)	29 (100%)

* according to national guidelines; CR = complete remission; F = female; FL = follicular lymphoma; FLIPI = follicular lymphoma international prognostic index; HDT/ASCT = high dose treatment/autologous stem cell transplantation; M = male; MCL = mantle cell lymphoma; MIPI = international prognostic index for mantle cell lymphoma; N = number; PR = partial remission

subgroups were analyzed by the log-rank test and by the Cox proportional hazards model. The non-relapse mortality was estimated using the cumulative incidence function. The program used was R Statistical software (R for Windows, version R-3.1.2, University of Auckland) and GraphPad Prism programme (version 3.02, GraphPad Software, USA).

The survival outcomes of three patients with histologically confirmed transformation of FL into the diffuse large B-cell lymphoma at the time of HDT/ASCT were analyzed separately.

Results

Patients' characteristics and disease status prior to ASCT

Patients' characteristics are shown in Table 1. In the FL group, there was a slight male predominance. Median age at diagnosis of lymphoma was 46 years. Thirteen patients had FL grade I/II and 4 patients had FL grade IIIa. None of the patients had FL grade IIIb. Four of them were considered as low risk patients (0–1 points) according to FLIPI, 9 were in the intermediate group (2 points) and 4 of them in the high risk group (3–5 points). Three patients (17.6%) were transplanted to consolidate their first remission, 14 (82.4%) were transplanted as a consolidation after treatment for their first relapse. Five (29.4%) of them were in CR before transplantation, 12 (70.6%) of them had PR. In the MCL group, there were 23 males and 6 females. Median age at diagnosis was 54 years. There were 10 pleomorphic/blastoid variants of MCL in the study population. Twenty-four patients had MIPI below 5.7 (low risk group), 4 patients between 5.7 and 6.2 (intermediate risk group) and one patient was in the high risk group (MIPI above 6.2). Twenty-four patients (82.7%) were transplanted in their first remission, 3 patients (10.3%) were transplanted to consolidate their second remission and 2 patients (6.9%) for later than second remission. Thirteen (44.8%) of them had CR before transplantation and 16 (55.2%) of them had PR.

Overall and event free survival

The estimated 5-year OS for FL was 87.8% (95% confidence interval [CI] 59.5%–96.8%) and the median OS has not been reached yet with a median follow-up time of 57.5 months (range 15–102 months) (Figure 1A). The FLIPI score did not influence the OS (p = 0.365, data not shown) and also the disease status had no significant impact on the OS (p = 0.570, hazard ratio (HR) 2.188, 95% CI HR 0.120–47.29).

The estimated 5-year OS for MCL was 79.3% (95% CI 56.1%–91.1%), and the median OS has not been reached yet with a median follow-up time of 31.5 months (range 2.5 months–95.5 months) (Figure 1B). The MIPI index had no impact on OS (p = 0.776, data not shown) and the same is true for

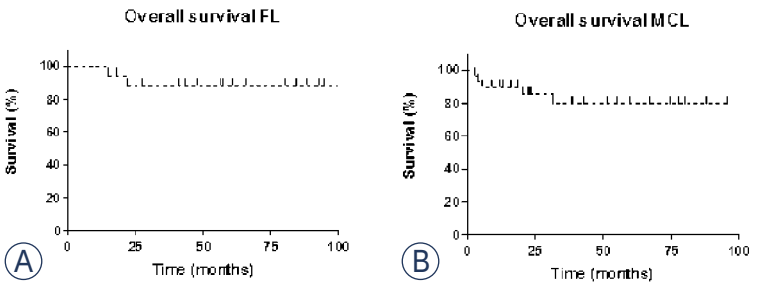

FIGURE 1. (A) Overall survival of follicular lymphoma (FL) patients (n = 17); **(B)** Overall survival of mantle cell lymphoma (MCL) patients (n = 29).

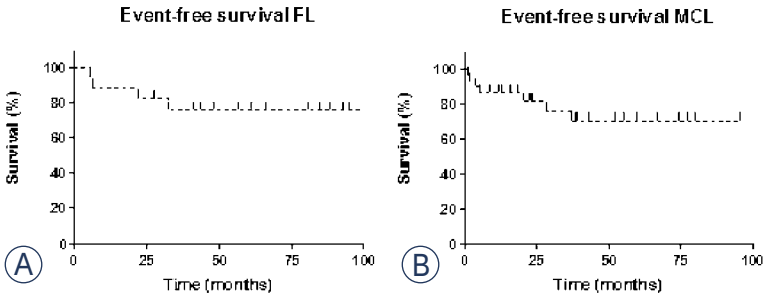

FIGURE 2. (A) Event-free survival of follicular lymphoma (FL) patients (n = 17); **(B)** event-free survival of mantle cell lymphoma (MCL) patients (n = 29).

the CR or PR status prior to transplantation (p = 0.668, HR 0.679, 95% CI HR 0.117–3.952).

The estimated 5-year EFS for FL was 76.0% (95% CI 48.0%–90.3%) and is shown in Figure 2A. The FLIPI score did not influence the EFS (p = 0.124, data not shown) and also the disease status (CR or PR) prior to ASCT did not (p = 0.851, HR 0.806, 95% CI HR 0.0936–7.056).

The estimated 5-year EFS for MCL was 69.8% (95% CI 45.5%–84.8%) and is shown in Figure 2B. The MIPI index had no impact on the EFS (p = 0.486, data not shown) and the same holds true for the CR or PR status prior to transplantation (p = 0.391, HR 0.485, 95% CI HR 0.099–2.472).

Non-relapse mortality and secondary malignancies

In FL group, 2 patients (11.8%) died. One patient transformed into the diffuse large-B cell lymphoma and died due to complications of a massive transformation and in one patient the cause of death was unrelated to lymphoma. In MCL group, 5 patients (17.2%) died. Two died of progression of MCL, one died due to histologically confirmed JC virus leukoencephalopathy and two of causes unrelated to lymphoma. The estimated probability of non-relapse mortality after 5 years equals 0.063 in FL (95% CI 0–0.186) and 0.116 in MCL (95% CI 0–0.245).

In the FL group, 1 patient (5.9%) developed ovarian cancer (treated with surgery) and 1 patient (5.9%) bladder cancer, but there were no hematological malignancies. In MCL group, there were also no hematological malignancies, 1 patient was just suspected to have myelodysplastic syndrome due to prolonged thrombocytopenia but this suspicion was later on rejected. One patient (3.4%) developed prostate cancer and is receiving hormone treatment.

Transformations

Survival analyses were done also for the 3 patients with transformation of FL into the diffuse large-B cell lymphoma at the time of HDT and ASCT. Median age of these patients was 42 years (range 39–52). There were 2 males and one female. The FLIPI scores were 0, 1 (low risk group) and 2 (intermediate risk group). The disease status prior transplantation was CR in all three cases, one patient was transplanted to consolidate the second remission and the other two to consolidate their third remission. They were all conditioned with TBI and high dose cyclophosphamide. Two of them died, one is still alive. With a median survival of 39.5 months the difference between the OS of those 3 patients and our FL group was not significant (p = 0.062, data not shown). The EFS, however, was in these patients significantly shorter (median EFS of 15 months) than in our FL group (p = 0.003, HR 6.805, 95% CI HR 3.414–495.6).

Discussion

Follicular lymphoma

The estimated 5-year OS for our FL patients is 87.8% and the median OS has not been reached. Compared to the study of El-Najjar *et al.*, the survival of our patients is superior since their 5-year OS for TBI-based HDT was 77% while for carmustine, etoposide, cytarabine, melphalan (BEAM) treated patients it was 74%. The difference between their treatment arms was statistically significant (p = 0.042).[23] Due to our relatively small sample our CI is relatively wide, but the results are encouraging.

Our projected 8-year OS was also 87.7%, since we had no further events in that period, but the 8-year estimation is not so reliable as the sample gets smaller. Thereby, our results resemble more than ASCT studies the allogeneic transplantation

studies, for example a study of Khouri *et al*, where they reported the 11-year OS of 78% achieved by the addition of ^{90}Y to the fludarabine and cyclophosphamide conditioning regimen.[24] Van Besien *et al*. conducted a study comparing allogeneic and autologous SCT. The TBI conditioning regimen was more used for allogeneic transplantation, which differs from our study. Their 5-year adjusted probabilities of survival after allogeneic transplantation were 51% and TBI was associated with higher transplant related mortality but a lower recurrence of the disease.[9] Our study confirms that the TBI including regimen is also appropriate for ASCT with a low rate of secondary malignancies and late toxicities.

The estimated 5-year EFS of our patients was 76.0% (95% CI 48.0%–90.3%) which is also better than in El-Najjar *et al.* study, where the 5-year EFS for TBI-based HDT was 58%. Like in case of OS, the EFS was also significantly worse in patients with BEAM-based HDT (49%, $p < 0.001$).[23] Eighty-two point four percent of our patients were transplanted for their first relapse, with the HDT and ASCT being an established treatment regimen for relapsed disease in the rituximab era.[25-27] Sebban *et al.* namely showed no benefit of HDT and ASCT after first line treatment regarding the EFS and OS in comparison to standard chemotherapy.[25]

The significant difference in EFS between our patients with FL and those whose lymphomas transformed to the diffuse large-B cell lymphoma ($p = 0.0034$) is interesting despite the fact that our study groups were relatively small. These results emphasize the importance of appropriate patient selection for transplantation procedure as the outcome of the patients with transformation cannot compare to our FL group (at least partially on account of a small number of transformations but also on account of a significantly worse prognosis of patients having the transformation), showing that FL patients with a transformation to the diffuse large-B cell lymphoma are not ideal candidates for TBI and high dose cyclophosphamide followed by ASCT. However, the transformation cannot be predicted and for these patients an alternative salvage option should be sought - for example a different conditioning regimen.

Mantle cell lymphoma

In our study population, the estimated 5-year OS for MCL was 79.3% (95% CI 56.1%–91.1%) and the median OS has not been reached. Also in this population, our results are comparable to the ones reported by other authors[11,28,29], for example by Dreyling *et al.* where they compared survival after TBI and ASCT versus maintenance with α-interferon after completion of induction therapy. The 3-year OS was 83% after ASCT versus 77% in the IFN group ($p = 0.18$).[11] The prospective trial of Nordic Lymphoma Group, however, reports of a 4-year OS of 81% and a 4-year EFS of 63%, but their patients were conditioned with the BEAM regimen.[30] We can also estimate the 7-year OS for our MCL as 79.3% since there were no additional events, but that estimation is not as reliable as the 5-year OS.

In a study by Oinonen *et al.*, the median EFS was set at 39 months[31], while in our group the median has not been achieved yet with a median observation period of 31.5 months. Delarue *et al.* revealed the same, comparing the BEAM treatment to TBI - the median EFS of patients receiving BEAM was 55 months, whereas the median EFS for the TBI regimen group has not been reached ($p = 0.05$).[15] This again proves that TBI conditioning regimen is safe and appropriate for ASCT in MCL.

The ASCT performed in line of first line treatment to consolidate the first remission was documented to have a significantly better EFS and OS compared to delayed ASCT.[11,32] In our study, the impact of early or late transplantation on OS and EFS was not statistically significant (OS: $p = 0.141$, HR 0.284, 95% CI HR 0.015–1.82; EFS: $p = 0.393$, HR 0.497, 95% CI HR 0.057–3.080). That could be due to our relatively small study group and a small proportion of transplantations to consolidate the second or further remissions. Most of our patients were namely transplanted after first line treatment.

TBI conditioning

The TBI regimen has been linked to secondary malignancies, mostly hematological - myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML).[17,26,33-35] The numbers vary among different studies - they range from a 5-year estimated risk of 3.8%[17] to a 7-year cumulative probability of 8.9%[34] and a 6-year incidence of 12%.[33] The therapy-related-MDS/therapy-related-AML (t-MDS/t-AML) also depended on amount of radiation used for TBI. In Metayer *et al.* multicenter case-control study, there was a nonsignificant excess risk of tMDS/tAML with use of conditioning regimens with TBI, compared with no TBI (relative risk [RR] = 2.0; 95% CI 0.95–4.18; $p = 0.07$). The dose-response analysis, however, revealed that the excess risk was limited to patients receiving TBI doses of 13.2

Gy (RR = 6.6; p = 0 .003). Those with lower TBI doses (5–12 Gy) had no evidence of elevated risk of tMDS/tAML.[34] Hypotheses on cellular level are that HDT and TBI cause similar cellular and genetic damages as the pre-transplant therapy to the hematopoietic progenitor cells and therefore provide opportunity for causing damage to the stem cells. Reinfusion of peripheral blood progenitor cells with previously damaged DNA can result in clonal abnormal hematopoiesis.[35] In this study, we had no hematological malignancies in either of our groups. Even though the number of our patients is relatively small we may assume that TBI with high doses of cyclophosphamide is a safe method of conditioning prior to transplantation in regard of the secondary hematological malignancies.

Conclusions

Follicular lymphoma and mantle cell lymphoma remain incurable diseases even in modern era. Treatment modalities are improving and transplantation of stem cells is gaining a more and more important role each year. We present good long term survivals after autologous stem cell transplantation following conditioning with TBI and high dose cyclophosphamide with minimal toxicities and low incidence of secondary malignancies. Our study also demonstrates a low relapse rate and therefore we conclude that the autologous stem cell transplantation represents a reliable option for treatment of follicular and mantle cell lymphoma with good long term results.

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research article

Incidence of positive peritoneal cytology in patients with endometrial carcinoma after hysteroscopy vs. dilatation and curettage

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Background. The aim of the study was to compare the frequency of positive peritoneal washings in endometrial cancer patients after either hysteroscopy (HSC) or dilatation and curettage (D&C).

Patients and methods. We performed a retrospective analysis of 227 patients who underwent either HSC (N = 144) or D&C (N = 83) and were diagnosed with endometrial carcinoma at the University Medical Centre Maribor between January 2008 and December 2014. The incidence of positive peritoneal cytology was evaluated in each group.

Results. There was no overall difference in the incidence of positive peritoneal washings after HSC or D&C (HSC = 13.2%; D&C = 12.0%; p = 0.803). However, a detailed analysis of stage I disease revealed significantly higher rates of positive peritoneal washings in the HSC group (HSC = 12.8%; D&C = 3.4%; p = 0.046). Among these patients, there was no difference between both groups considering histologic type (chi-square = 0.059; p = 0.807), tumour differentiation (chi-square = 3.709; p = 0.156), the time between diagnosis and operation (t = 0.930; p = 0.357), and myometrial invasion (chi-square = 5.073; p = 0.079).

Conclusions. Although the diagnostic procedure did not influence the overall incidence of positive peritoneal washings, HSC was associated with a significantly higher rate of positive peritoneal cytology in stage I endometrial carcinoma compared to D&C.

Key words: endometrial carcinoma; peritoneal cytology; FIGO staging; hysteroscopy; dilatation and curettage

Introduction

The diagnosis of endometrial cancer can be made preoperatively by obtaining a sample of endometrial tissue either with office endometrial biopsy, most commonly done with a Pipelle aspiration catheter, hysteroscopy (HSC), or dilatation and curettage (D&C).[1] The latter two procedures are most commonly used in Slovenia. HSC has been shown to be highly accurate in diagnosing endometrial cancer[2,3] and is considered a gold standard.[1] Conflicting evidence has been published in the past regarding the risk of intraperitoneal spread of malignant cells after HSC with the use of distension media.[4-7] In 2007, a retrospective study from our institution reported a significantly higher incidence of positive peritoneal washings after HSC compared to D&C.[8] However, only 24 patients in this study had undergone HSC compared to 122 who were diagnosed with D&C.[8] In recent years, HSC has become an established diagnostic tool at our institution and is now performed more frequently than D&C. The aim of our present study was to find out whether the difference in the incidence of positive peritoneal washings between HSC and D&C persists after including a higher number of patients with hysteroscopy.

Patients and methods

This retrospective study included all consecutive patients who had endometrial carcinoma diagnosed preoperatively with either D&C or HSC between January 2008 and December 2014 at the University Medical Centre Maribor, Slovenia. The study included patients who had more than one D&C or more than one HSC. Patients who had undergone both D&C and HSC were excluded from the study. The study was approved by our institution's ethics committee (Approval No. 13-03/15, November 26, 2015). All patients signed a written informed consent that their medical records can be used for research matters retrospectively.

HSC was performed in the office setting or under general anesthesia. Saline solution warmed to body temperature was used as the distension medium. In the office setting, the distension medium was installed into the pressure cuff and the intrauterine pressure was set between 80–150 mmHg. Intrauterine pressure was controlled with the Vario Flow device (Pelta, Slovenia) in operative HSC.[9] D&C was performed under general anesthesia. Curettage of the cervical canal and the uterine cavity was performed separately. Tissue samples for histologic examination were obtained during both procedures.

During the final surgery for endometrial carcinoma, samples of peritoneal washings from the pouch of Douglas were obtained for cytologic examination. Irrigation of the peritoneal cavity with saline solution was performed to obtain samples in cases with no free fluid. The samples were inspected by an expert cytopathologist. In cases of suspicious peritoneal cytology additional calretinin, MOC 31, HBME 1 and Ber-EP4 immunostaining was performed during the clarification process. In cases of small numbers of positive cells after immunostaining peritoneal cytology was described as suspicious. We therefore included suspicious results in the analysis of positive peritoneal cytology.

The primary statistical outcome was the incidence of positive peritoneal washings after HSC and after D&C. A detailed analysis of tumour histopathologic characteristics was performed including histopathologic type, tumour differentiation, depth of myometrial invasion, lymphovascular invasion and FIGO stage. Different types of endometrial carcinomas were identified in the study population with endometrioid carcinoma representing the majority of cases (N = 211; 93.0%). Other carcinomas (serous adenocarcinoma: N = 8; clear cell adenocarcinoma: N = 8) were assigned into non-endometriod group for the purpose of the study. Tumour differentiation was reported as good, moderate or poor. The depth of myometrial invasion was reported as no invasion, less than half of myometrium or more than half of myometrium. Patients treated before 2009 who had been staged according to the 1988 FIGO classification were restaged according to the new 2009 FIGO classification for statistical analysis. The time interval from diagnosis to final surgery was also analyzed.

Statistical analysis was performed with SPSS software version 22.0 (IBM, Armonk, NY, USA). Descriptive analysis, chi-square test and t-test of independent samples were performed as applicable. A p value of less than 0.05 was considered statistically significant.

TABLE 1. Histologic characteristics of the patients with endometrioid endometrial carcinoma (N = 211) and non-endometrioid endometrial carcinoma (N = 16)

Histologic parameter	Endometrioid endometrial carcinoma	Non-endometrioid endometrial carcinoma	p*
Tumour differentiation			**< 0.001**
G1	125 (59.2%)	2 (13.3%)	
G2	63 (29.9%)	4 (26.6%)	
G3	23 (10.9%)	9 (60.0%)	
Myometrial invasion			
none	8 (3.8%)	1 (6.3%)	**0.888**
less than ½	123 (58.3%)	9 (56.2%)	
more than ½	80 (37.9%)	6 (37.5%)	
2009 FIGO stage			
IA	114 (54.0%)	7 (43.8%)	**0.025**
IB	54 (25.6%)	1 (6.3%)	
II	14 (6.6%)	2 (12.5%)	
IIIA	7 (3.3%)	1 (6.3%)	
IIIB	5 (2.4%)	0 (0.0%)	
IIIC1	11 (4.7%)	2 (12.5%)	
IIIC2	0 (0.0%)	0 (0.0%)	
IVA	1 (0.5%)	1 (6.3%)	
IVB	5 (2.4%)	2 (12.5%)	

*chi-square test

Results

Between January 2008 and December 2014, 266 patients had uterine cancer diagnosed with D&C and/or HSC. 227 patients who had either HSC (N =

TABLE 2. Histologic characteristics of the patients with endometrial carcinoma (N = 227) who were diagnosed with hysteroscopy (HSC) or dilatation and curettage (D&C)

Histologic parameter	HSC (N = 144)	D&C (N = 83)	P value
Histologic type			
endometrioid adenocarcinoma	137 (95.1%)	74 (89.2%)	0.090*
non-endometrioid adenocarcinoma	7 (4.9%)	9 (10.8%)	
Tumour differentiation			
G1	90 (62.5%)	37 (45.1%)	**0.012***
G2	40 (27.8%)	27 (32.9%)	
G3	14 (9.7%)	18 (22.0%)	
Myometrial invasion			
none	5 (3.5%)	4 (4.8%)	0.726*
less than ½	82 (56.9%)	50 (60.2%)	
more than ½	57 (39.6%)	29 (34.9%)	
Lymphovascular invasion			
present	20 (13.9%)	14 (16.9%)	**0.545***
absent	124 (86.1%)	69 (83.1%)	
2009 FIGO stage			
IA	75 (52.1%)	46 (55.4%)	
IB	42 (29.2%)	13 (15.7%)	
II	10 (6.9%)	6 (7.2%)	
IIIA	1 (0.7%)	7 (8.4%)	
IIIB	3 (2.1%)	2 (2.4%)	**0.040***
IIIC1	9 (6.3%)	4 (4.8%)	
IIIC2	0 (0.0%)	0 (0.0%)	
IVA	1 (0.7%)	1 (1.2%)	
IVB	3 (2.1%)	4 (4.8%)	
Peritoneal cytology			
positive or suspicious	19 (13.2%)	10 (12.0%)	0.803*
negative	125 (86.8%)	73 (88.0%)	
Time from diagnosis to operation (days)	35.8 ± 13.8	32.8 ± 15.4	0.140**

*chi-square test
** independent sample t-test

144) or D&C (N = 83) as well as available information on peritoneal washings were included in the statistical analysis.

Two hundred and eleven (93.0%) patients had endometrioid endometrial carcinoma and 16 (7.0%) had non-endometrioid endometrial carcinoma. The differences between both groups regarding the differentiation, myometrial invasion and tumour stage are shown in Table 1. Significantly higher rate of poorly differentiated tumours (chi-square = 29.114; p < 0.001) and higher stages (chi-square = 16.019; p = 0.025) have been noted in the non-endometrioid group.

Overall, there was no significant difference in the incidence of positive or suspicious peritoneal washings regarding the procedure performed during the diagnostic evaluation (13.2% after HSC, 12.0% after D&C; chi-square = 0.062; p = 0.803).

The groups (HSC *vs.* D&C) did not differ in the prevalence of histologic types of the tumour, depth of myometrial invasion and lymphovascular invasion (Table 2). However, there were significant differences in tumour differentiation as well as FIGO stage, with more patients having FIGO stage I disease in the hysteroscopy group (Table 2). Due to this difference we conducted analysis only in the subgroup of patients with stage I disease. The HSC and D&C groups of stage I patients did not differ in tumour differentiation, the prevalence of histologic types of the tumour, the time from diagnosis to operation and in myometrial invasion. A separate evaluation of patients with stage I tumours showed that 12.8% in the HSC group and only 3.4% in the D&C group had positive peritoneal washings. This difference was statistically significant (chi-square = 2.422; p = 0.046) (Table 3).

One out of 15 FIGO stage I patients with positive peritoneal washings after hysteroscopy had disease recurrence by the end of April 2015 (mean follow-up 40.2 months). Neither of the two FIGO stage I patients with positive peritoneal washings after D&C had disease recurrence in the same period (mean follow-up 39.5 months).

Discussion

The possibility of microscopic intraperitoneal spread of endometrial cancer cells after hysteroscopy has been a subject of debate for more than a decade. In our study, we did not find an increased incidence of positive peritoneal washings after hysteroscopy in comparison to D&C in the overall study population of patients with endometrial carcinoma. Several other studies similarly found no association between hysteroscopy and an increased rate of positive peritoneal cytology.[5,6] On the other hand, Bradley *et al.*[7] reported a higher frequency of positive or suspicious peritoneal cytology after hysteroscopy compared to blind endometrial sampling using logistic regression controlling for confounders of grade and stage. They also reported a higher rate of disease upstaging (according to the 1988 FIGO staging system) after hysteroscopy attributed solely to the positive cytology. Similar results have been reported by Zerbe *et al.*[10] and Obermair *et al.*[11] In a study conducted at our institution in 2007[8], positive peritoneal cytology was present in 12.5% of patients after hysteroscopy and only in 1.6% after D&C. The difference

was statistically significant (chi-square = 4.2455; $p < 0.005$).

In a meta-analysis of nine trials including 1015 patients with confirmed endometrial carcinoma, Polyzos *et al.*[12] evaluated the rate of positive peritoneal washings after hysteroscopy in comparison to other diagnostic procedures or no diagnostic procedures. They concluded that the frequency of positive peritoneal washings was significantly higher after hysteroscopy. The analysis also revealed a higher rate of disease upstaging based only on the positive peritoneal cytology. A detailed literature search performed by Guralp and Kushner[13] revealed 0–83% of positive peritoneal cytology after hysteroscopy and 0–13.6% after D&C. However, the authors emphasized a number of unanswered questions regarding the type and volume of distension medium, intrauterine pressure during the procedure, time interval between hysteroscopy and definitive surgery, stage, grade of the disease and duration of the procedure.[13] Another meta-analysis by Chang *et al.*[14] also reported on higher rates of positive peritoneal cytology after hysteroscopy. Nevertheless, a detailed analysis of patients with stages I or II failed to show significantly higher rates of positive peritoneal cytology in patients who had hysteroscopy.

Interestingly, our results showed a significantly higher incidence of positive or suspicious peritoneal cytology in patients with stage I disease who were diagnosed with hysteroscopy compared to those diagnosed with D&C. This is an unexpected finding because the disease at this stage is confined to the uterus. For example, only 3.3% of patients with stage I and II endometrial cancer in a large retrospective analysis by Garg *et al.*[15] had positive peritoneal cytology. In our study, the rate of positive or suspicious peritoneal cytology in stage I disease was 3.3% in the D&C group but as much as 12.1% in the hysteroscopy group. Positive or suspicious peritoneal cytology was shown to be more frequent after hysteroscopy in endometrial carcinoma patients who would be staged as FIGO IA in the new staging system by Obermair *et al.*[11] On the other hand, Biewenga *et al.*[6] showed no association between hysteroscopy and the rate of positive peritoneal washings in stage I disease.

Saline solution was used as the distension medium in all our patients in the hysteroscopy group. Hysteroscopy with saline solution was specifically linked to a higher rate of positive peritoneal cytology in a meta-analysis by Polyzos *et al.*[12] In a meta-analysis by Chang *et al.* [14], the distension medium was either saline solution or 5% glucose solution.

TABLE 3. Histologic characteristics of the patients with stage 1 endometrial carcinoma (N = 187) who were diagnosed with hysteroscopy (HSC) or dilatation and curettage (D&C)

Stage 1	HSC (N = 117)	D&C (N = 59)	P value
Histologic type			
endometrioid adenocarcinoma	112 (95.7%)	56 (94.9%)	0.807*
non-endometrioid adenocarcinoma	5 (4.3%)	3 (5.1%)	
Tumour differentiation			
G1	75 (64.1%)	29 (49.2%)	0.156*
G2	32 (27.4%)	22 (37.3%)	
G3	10 (8.5%)	8 (13.5%)	
Myometrial invasion			
none	5 (4.3%)	3 (5.1%)	0.079*
less than ½	71 (60.7%)	45 (76.2%)	
more than ½	41 (35.0%)	11 (18.6%)	
Peritoneal cytology positive or suspicious negative	 15 (12.8%) 102 (87.2%)	 2 (3.4%) 57 (96.6%)	**0.046***
Time from diagnosis to operation (days)	35.6 ± 14.0	33.1 ± 12.7	0.255**

*chi-square test
** independent sample t-test

Neither of these two meta-analyses found a connection between intrauterine pressure during hysteroscopy higher than 100 mmHg and a higher incidence of positive peritoneal cytology.[12,14] In our study, the exact intrauterine pressure during hysteroscopy was not known for each patient individually due to the retrospective nature of the analysis.

The time interval between the diagnostic procedure and definitive surgery was similar in patients with positive and negative peritoneal cytology in our study. This is in line with evidence from another retrospective study in 196 patients with endometrial cancer diagnosed with hysteroscopy.[16]

Based on the data from our retrospective study, we cannot give a definite reason for the significantly higher incidence of positive peritoneal washings after HSC compared to D&C in stage I disease. We used saline solution as the distension medium, which has been previously associated with higher rates of positive peritoneal cytology.[12] Unfortunately, we do not have the exact information on the intrauterine pressure during HSC and the duration of the diagnostic procedure for each patient and therefore we cannot draw conclusions about the influence of these factors on peritoneal cytology.

Another important limitation of our study is the inclusion of suspicious peritoneal cytology in the

positive peritoneal cytology group. Even after immunostaining most of the cases without evident malignant cells remained cytologically suspicious because positive immune reaction was seen in only a small fraction of cells. However, it is not easy to differentiate positive from suspicious cytology because severe atypia of reactive mesothelial cells may be interpreted as suspicious. We are aware of this methodological limitation and should aim to lower the incidence of suspicious peritoneal cytology in the future firstly by obtaining sufficient amount of fluid for cytological analysis during final surgery and secondly with accurate cytological diagnosis. Some published research on this subject also included positive and suspicious cytology in the same group.[7,8,11]

Our data show that among patients with positive or suspicious peritoneal washings after hysteroscopy, in FIGO stage I patients, one out of 15 had local disease recurrence during follow-up of approximately 40 months, whereas neither of the two with positive washings after D&C had the recurrence. As these numbers are small, further research is necessary to draw relevant conclusions. Conflicting results exist in the literature regarding the prognostic significance of positive peritoneal cytology.[17,18] The updated FIGO staging system from 2009 excluded positive peritoneal cytology as a stage defining variable. Previously, all patients with positive peritoneal cytology were upstaged to stage IIIA.[19] In an analysis of 14,704 patients, Garg *et al.*[15] reported peritoneal cytology to be associated with survival in univariate analysis along with race, age, histology, grade and the number of removed lymph nodes. In multivariate analysis, positive peritoneal cytology remained an independent prognostic factor in stages I and II. Shiozaki *et al.*[20] studied the influence of positive peritoneal washings on the prognosis of 265 patients with stage I endometrial cancer. Progression-free survival was significantly lower in the group with positive peritoneal cytology. Other factors associated with progression-free survival in univariate analysis were lymph node dissection and vessel permeation, but positive peritoneal cytology was the most influential factor.[20] Disease-free survival has been shown to be 91% in FIGO stage I patients and 52.5% in those with FIGO stage II, III and IV.[21] In the study by Garg *et al.*, survival in patients with stage I endometrioid adenocarcinoma with positive peritoneal washings was significantly poorer than in patients with negative peritoneal washings (88.2% *vs.* 98.6%).[15]

In conclusion, the diagnostic procedure did not influence the overall incidence of positive peritoneal washings in our study. However, hysteroscopy was associated with a significantly higher rate of positive peritoneal cytology in stage I endometrial carcinoma. Although statistically significant, this finding must be interpreted with caution because of the small sample size of this subgroup. In addition, it is still not known whether iatrogenic dissemination of malignant cells bears the same influence on disease prognosis as spontaneous dissemination. Despite being excluded as the stage defining variable, peritoneal cytology should still be reported separately as requested by FIGO.[15] We believe that additional trials are needed to further clarify the prognostic value of positive peritoneal cytology after hysteroscopy, particularly in the early stages of endometrial cancer.

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research article

Implant-prosthetic rehabilitation after radiation treatment in head and neck cancer patients: a case-series report of outcome

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Background. Slovenia has a high burden of head and neck cancer. Patients are mostly treated with surgery followed by radiation therapy. Advanced surgical and prosthodontic techniques have expanded the rehabilitation options. The aim of the study was to review the outcome of implant-prosthetic treatment after radiation therapy.

Patients and methods. Twenty irradiated head and neck cancer patients who received a removable implant-supported denture at the University Medical Centre Ljubljana were included in the study. Kaplan-Meier survival analysis, Cox proportional hazard models and logistic regression were used to assess the implant survival and success rate.

Results. Twenty patients had 100 implants inserted. The estimated implant survival rate was 96% after 1 year and 87% after 5 years. Failures were mostly observed before loading (91.2%). Implants inserted in the transplanted bone were significantly more likely to fail. Out of 89 implants supporting the dentures, 79 implants (88.7%) were successful, meaning that they were functionally loaded and exhibited no pain, radiolucency or progressive bone loss. Prosthetic treatment was significantly less successful in older patients. The attachment system and the number of implants did not have a statistically significant influence on the success rate.

Conclusions. Implant-supported dentures have been shown to be a reliable treatment modality after head and neck cancer surgery and radiation therapy. Possible early failures should be communicated with the patients.

Key words: head and neck cancer; radiation therapy; dental implants; implant-supported dentures

Introduction

Slovenia is among the countries with the highest incidence of oral and oropharyngeal cancer.[1,2] In a population of 2 million people, approximately 450 cases are diagnosed per year.[3] Most tumours are regionally advanced and the patients are treated with radical resection and reconstruction followed by radiation therapy.[3] Afterwards, patients experience profound changes in the oral anatomy, function and facial appearance. Radiation therapy causes irreversible damage to both hard and soft tissues by creating a hypoxic, hypocellular and hypovascular environment which impedes wound healing and creates a risk for osteoradionecrosis.[4] A protocol involving hyperbaric oxygen treatment (HBO) has been proposed to enhance wound healing by increasing the tissue oxygenation.[5]

Following the radical procedures to eradicate cancer, the greatest problems perceived by irradi-

ated patients are swallowing, mouth opening, xerostomia and compromised aesthetics.[6] Prosthodontic treatment is advocated to regain lost oral functions, enhance the physical appearance and enable the patient to take part in normal daily activities with greater confidence. However, comprehensive prosthetic treatment after head and neck cancer is challenging, time-consuming and costly. Therefore, only 40% of such patients are treated following the postsurgical prosthetic protocol. Among them, 70% receive dentures supported by the residual teeth and bone and 30% receive implant-supported dentures.[7] Edentulous patients with head and neck cancer after radiation therapy are an especially vulnerable subgroup. Radiation treatment sequelae persist throughout the patient's lifetime and in the past, radiation therapy used to be an absolute contraindication to placing of dental implants.[8] Treatment options have therefore been limited to conventional complete dentures or no prosthetic rehabilitation at all.

Advanced surgical and prosthodontic techniques, such as 3D planning and guided implant surgery, have expanded the treatment options.[9] In addition to conventional ball- and bar-retained dentures, new attachments have been introduced to clinical practice. Locator attachment is commonly used in removable implant prosthodontics. It is a self-aligning system with relatively simple maintenance requirements.[10] Locator is, however, a non-rigid type of attachment and does not completely relieve the stress from the underlying mucosa. The nylon matrices and male parts are subjected to wear, which diminishes retention. Their replacement is one of the most frequent reasons for maintenance visits.[11-13]

To address these issues, a technically more advanced system of prefabricated double crowns on implants has been introduced.[14] The SynCone system (Dentsply, Germany) is indicated in unfavourable resection areas, where completely rigid constructions are necessary due to the anatomical constraints. Among the possible complications, debonding of the secondary crowns and the abutment screw loosening are reported.[15]

Both Locator and Syncone systems are commonly used at the University Medical Centre Ljubljana, Slovenia. These attachment systems have expanded the treatment possibilities for the rehabilitation of head and neck cancer patients, which is always a collaborative work between oral surgeons and prosthodontists. The aim of the study was to review the outcome of the implant-prosthetic rehabilitation of irradiated patients performed at the University Medical Centre Ljubljana.

Patients and methods

A retrospective chart review was performed for the patients who were treated jointly by the Department of oral and maxillofacial surgery and the Department of prosthodontics at the University Medical Centre Ljubljana in the time period from 2008 to 2014. Only patients who underwent resection of malignant tumours with a subsequent radiation treatment and received removable implant-supported dentures were included in the study. The recorded data included patient gender and date of birth, smoking status at the time of prosthetic rehabilitation, cancer type, surgical management, date and dose of radiation treatment, administration of hyperbaric oxygen treatment (HBO), timing to implant surgery and to functional loading, treated jaw, type of implant bed, number of implants supporting the dentures, implant system and the denture attachment system used. At University Medical Centre Ljubljana, HBO is provided according to the protocol suggested by Marx and Larsen.[4,5] Patients are scheduled for 20 sessions before and 10 sessions after the implant insertion, respectively. Each 90-minute session consists of exposure to 100% oxygen on 2.5 ATA (1.5 bar) with three breaks during which patients breathe normal air. The study was conducted according to the Helsinki Declaration. Each patient's informed consent was obtained and Institutional Review board approval was granted.

The implants were assessed for survival and success using the guidelines proposed by van Steenberghe *et al*.[16] The survival criteria included osseointegration and presence in the mouth. To be considered successful, the implant had to be functionally loaded, immobile, without persistent pain or inflammation of the periimplant tissue and without progressive bone loss evident from radiographs and probing depth at the recall.

The survival time was measured from the date of the implant insertion to the date of the implant failure or the last control of the implant.

The present series consisted of 20 patients (11 men and 9 women) with a median age of 57.6 years (range 46.7 to 77.2 years) at the time of the implant insertion. Seventeen patients (85%) had a history of squamous cell carcinoma. Mucoepidermoid, adenoid cystic and *origo ignota* metastatic cancer were diagnosed in 1 patient each. The most common sites of primary cancer were the tongue and the floor of the mouth (6 patients each), followed by pharynx and maxilla (2 patients each). Mandible, tonsilla, larynx and *origo ignota* metastases in lymph nodes

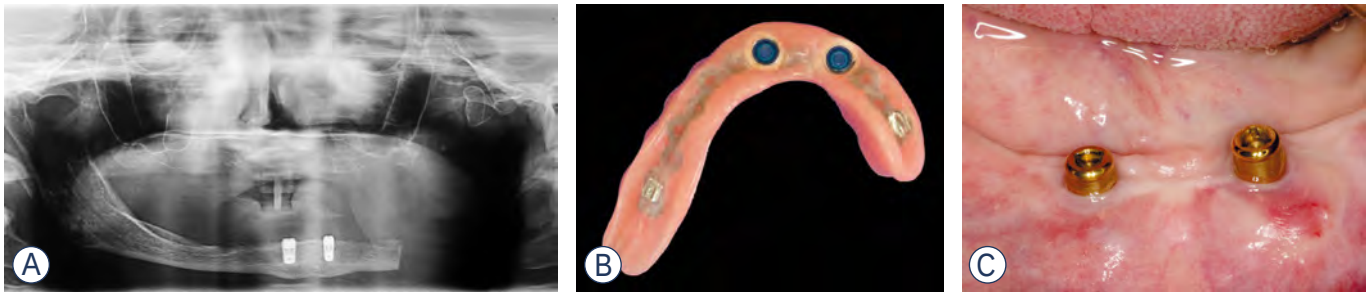

FIGURE 1. A patient after segmental resection of the left mandible body due to cancer, with two implants as seen on the radiograph **(A)**. The patient received an implant-supported lower denture **(B)**, where the retention was based on the Locator attachments **(C)**.

TABLE 1. Summary of the implant survival according to the factors of interest

Parameter	All implants (n = 100)	Survived implants (n = 88)	Failed implants (n = 12)
Patient's median age at implant insertion in years (range)	58.3 (46.7–77.2)	61.5 (46.7–77.2)	57.9 (46.7–77.2)
Median time interval between radiation therapy and implant insertion in years (range)	3.8 (1.1–38.1)	3.2 (1.1–38.1)	5.2 (2.4–12.8)
Patient's gender			
Female, n (%)	40 (40%)	32 (36%)	8 (67%)
Male, n (%)	60 (60%)	56 (64%)	4 (33%)
Smoking			
No, n (%)	66 (66%)	57 (65%)	9 (75%)
Yes, n (%)	34 (34%)	31 (35%)	3 (25%)
Implant system			
Astra, n (%)	18 (18%)	18 (20%)	0 (0%)
Straumann, n (%)	22 (22%)	17 (20%)	5 (42%)
Ankylos, n (%)	60 (60%)	53 (60%)	7 (58%)
Jaw			
Lower, n (%)	72 (72%)	63 (72%)	9 (75%)
Upper, n (%)	28 (28%)	25 (28%)	3 (25%)
Bone			
Native, n (%)	92 (92%)	85 (97%)	7 (58%)
Transplanted, n (%)	8 (8%)	3 (3%)	5 (42%)
HBO administered			
No, n (%)	19 (19%)	14 (16%)	5 (42%)
Yes, n (%)	81 (81%)	74 (84%)	7 (58%)

were encountered in one patient each. A segmental resection of the mandible was performed in 8 patients. In one patient, the tumour resection in the maxilla resulted in an oronasal communication. After surgery, all patients were subjected to the radiation therapy, with reported doses ranging from 54 to 66 Gy.

Reconstruction with bone and soft tissue grafts was accomplished in 3 patients. In two cases the fibular graft was used to reconstruct the mandible. In one patient the maxilla was reconstructed with the iliac crest bone graft.

The Kaplan-Meier method was utilized to estimate the implant survival rate. The association between the survival and the potential prognostic factors was analysed by fitting univariate Cox proportional hazards models. The association between the potential prognostic factors and implant success was analysed with univariate logistic regression models. The Holm-Bonferroni method was used to account for multiple comparisons and the level of significance was set to $\alpha = 0.05$. Statistical analyses were conducted with the statistical software package R.[17]

Results

As presented in Table 1, 100 implants of 3 different implant systems were included in the study: 18 Astra Tech implants (Dentsply, Mannheim, Germany), 22 Straumann implants (Institut Straumann AG, Basel, Switzerland) and 60 Ankylos implants (Dentsply, Mannheim, Germany). 28 implants were inserted in the maxilla and 72 in the mandible. 92 implants were inserted in native bone and 8 in transplanted bone. The median time between the end of the radiation therapy and the implant surgery was 3.8 years (range 1.1 to 38.1 years).

Prophylactic antibiotic therapy was prescribed to all patients and HBO was administered to 16 patients. Osteoradionecrosis was not observed in this study. All implants were inserted in edentulous jaws, with 5 patients receiving implants in both jaws, 14 only in the mandible and 1 only in the maxilla. Twelve patients were non-smokers and 8 patients were smokers at the time of the prosthetic rehabilitation. A two-stage implant insertion protocol was used in all cases. The median healing period between the implant insertion and functional loading was 15.1 months (range 4.3 to 54.3 months). Three different attachment systems for implant-supported dentures were used: 39 Locator attachments (Zest Anchors, Escondido, USA) (Figure 1), 40 prefabricated conical crowns (SynCone, Dentsply Friadent, Mannheim,

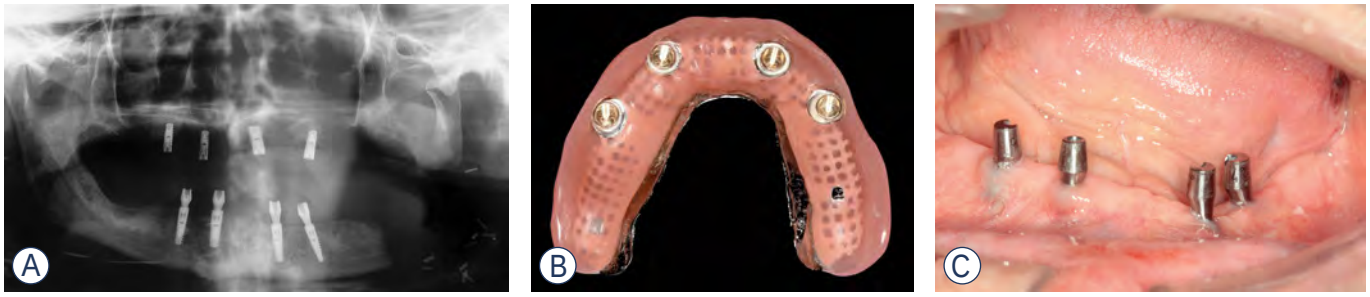

FIGURE 2. A patient after surgical treatment of oropharyngeal carcinoma. Segmental resection of the left mandible body is visible on the radiograph **(A)**. Patient received implant-supported lower and upper dentures **(B)** with retention based on the SynCone double crowns **(C)**.

TABLE 2. Predictors of the implant failure

Parameter	Hazard ratio (95% confidence interval)	*p*
Patient's age at implant insertion in years	1.05 (0.99–1.12)	1.0000
Time interval between the radiation therapy and the implant insertion in years	0.99 (0.92–1.07)	1.0000
Female gender	2.74 (0.82–9.10)	1.0000
Smoker	0.72 (0.19–2.66)	1.0000
Lower jaw	0.84 (0.23–3.09)	1.0000
Transplanted bone	12.37 (3.87–39.56)	**0.0003**
HBO administered	0.31 (0.10–0.98)	0.4753

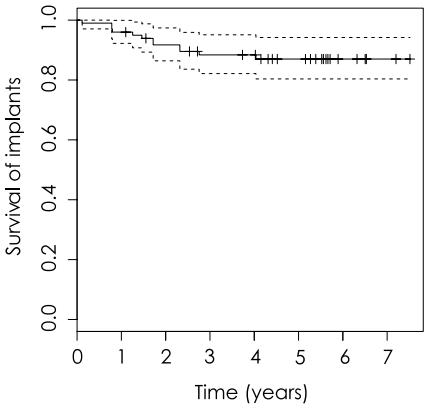

FIGURE 3. The Kaplan-Meier curve for the survival of the implants. 95% confidence intervals and censored data are included on the plot.

Germany) (Figure 2), and 10 custom designed bar-clip systems. Median follow up after implant insertion was 61.9 months (range 1.4 to 90.2 months).

The Kaplan-Meier estimated 1- and 5-year cumulative implant survival rates were 96% (95% confidence interval: 92.2%–99.9%) and 87.0% (95% confidence interval: 80.4–94.2%). The survival curve is shown on Figure 3. During the examination period, three patients died. Time of these 14 implants' service in the mouth was, as with other implants, registered from the date of the implant insertion to the date of the last follow-up examination. The median time of failure was 19.1 months (range 1.4 to 48.5 months) after implantation.

The crude survival rate in our sample was 88%, as 12 implants in 5 patients failed. Data for all the implants and for the subgroups of survived and failed implants are described in Table 1. Primary implant failure during the healing period before functional loading was recorded in 11 implants (91.2%). The causes of implant removal in our sample were incomplete osseointegration (4), persistent pain (4), and periimplantitis with recurrent soft tissue hyperplasia (3). The only implant that was lost after functional loading (secondary implant failure) was included in a bar-supported denture and had to be removed because of periimplantitis. Results of the analysis with the Cox regression models is presented in Table 2.

The survived and failed implants were comparable considering the patient's gender, age and smoking status, the time elapsed between the radiation therapy and the implant surgery, the jaw of the implant insertion and the administration of HBO. The results for the bone type indicated that the implants inserted in the transplanted bone were statistically significantly more likely to fail than those inserted in the native bone. The influence of the implant system on the survival could not be analysed because of the insufficient number of failure events among the three groups. As presented in Table 3, 89 implants were observed after functional loading.

Seventy-nine of those implants (88.7%) were successful, meaning that they were functionally loaded and exhibited no pain, radiolucency or progressive

TABLE 3. Summary of the loaded implants' success according to the factors of interest

Parameter	All loaded implants (n = 89)	Successful loaded implants (n = 79)	Unsuccessful loaded implants (n = 10)
Median patient's age at prosthetic rehabilitation in years (range)	59.1 (49.3–79.2)	58.9 (49.3–67.9)	71.7 (59.1–79.2)
Median healing time after the implant insertion in months (range)	15.1 (4.3–54.4)	15.2 (4.3–54.4)	13.0 (4.3–24.6)
Patient's gender			
Female, n (%)	33 (37%)	25 (32%)	8 (80%)
Male, n (%)	56 (63%)	54 (68%)	2 (20%)
Smoking			
No, n (%)	57 (64%)	49 (62%)	8 (80%)
Yes, n (%)	32 (36%)	30 (38%)	2 (20%)
Median number of the implants supporting the denture (range)	4 (2–5)	4 (2–5)	4
Implant denture system			
Bar, n (%)	10 (11%)	6 (8%)	4 (40%)
Locator, n (%)	39 (44%)	37 (47%)	2 (20%)
SynCone, n (%)	40 (45%)	36 (45%)	4 (40%)
Jaw			
Upper, n (%)	25 (28%)	21 (27%)	4 (40%)
Lower, n (%)	64 (72%)	58 (73%)	6 (60%)
Bone			
Native, n (%)	86 (97%)	76 (96%)	10 (100%)
Transplanted, n (%)	3 (3%)	3 (4%)	0 (0%)
HBO administered			
Yes	75 (84%)	65 (82%)	10 (100%)
No	14 (16%)	14 (18%)	0 (0%)

TABLE 4. Predictors of the loaded implants' success

Parameter	Odds ratio for the loaded implants' success (95% confidence interval)	*p*
Patient's age at prosthetic rehabilitation	0.66 (0.49–0.80)	**0.0075**
Healing time after the implant insertion	1.09 (0.53–2.73)	1.0000
Male gender	8.64 (1.99–60.09)	0.1456
Smoker	2.44 (0.57–16.95)	1.0000
Number of the implants supporting the denture	0.78 (0.24–1.94)	1.0000
Denture attachment system		
Locator *vs* bar	12.33 (1.98–104.98)	0.1456
SynCone *vs* bar	6.00 (1.15–32.68)	0.4416
SynCone *vs* Locator	0.48 (0.06–2.65)	1.0000
Upper jaw	0.54 (0.14–2.30)	1.0000

bone loss evident from radiographs and probing depth at yearly recall. Regardless of the appropriate osseointegration, prosthetic rehabilitation of 10 implants (11.2%) in four patients (20%) did not have a favourable outcome. Two patients with 4 implants experienced difficulties in adapting to dentures and did not wear them on regular basis. In addition, two further implants were considered unsuccessful because of persistent soft tissue discomfort reported by the patients. As shown in Table 4, patient age was a statistically significant predictor for the success. Gender, smoking status, healing time after implant insertion, the number of implants supporting the denture, the prosthetic system, jaw, bone type and administered HBO did not have a statistically significant effect on the success rate of loaded implants in this sample of irradiated patients.

Discussion

It has been shown in this study that the implant-supported denture is a reliable treatment modality for the head and neck cancer patients that undergo radiation therapy. When surgical and prosthodontic protocols are conducted appropriately, dental implants greatly enhance the stability of the dentures and improve the facial contours. According to the current guidelines, very few absolute contraindications exist for using dental implants in medically compromised patients.[18] Radiation therapy in the head and neck region is no longer a contraindication, as there is a growing number of reports that a high osseointegration rate and a predictable treatment outcome can be expected.[19,20] When considering prosthetic treatment options, the socio-economic status of patients should carefully be evaluated. Head and neck cancer is commonly associated with smoking and alcohol abuse. Both tobacco and alcohol are known as strong risk factors and when combined, their carcinogenic potential has been shown to be even more pronounced.[21,22] Many patients do not give up smoking and drinking after the initial cancer treatment, which puts them at risk for cancer recurrence and might also jeopardize the implant-prosthetic rehabilitation outcome.

In this case series of irradiated patients, the implant failures were rare and mostly confined to the healing period. The predominantly early implant loss is in accordance with the findings of Linsen *et al.*[23] Extended healing time should therefore be allowed after implantation and immediate loading protocols are not advised.[18]

The Kaplan-Meier estimated 1- and 5-year cumulative implant survival rates were 96% and 87%. This is in accordance with Buddula *et al.*[24] reporting implant survival rates of 98.9% and 89.9% af-

ter 1 and 5 years and Yerit *et al.*[25] reporting a 95% and 91% survival after 2 and 5 years, respectively. Due to the small number of failed implants, detailed statistical analysis of prognostic factors for implant failure is often not possible or lacks power. It is therefore difficult to draw meaningful conclusions from the results of single studies alone. In this study, some limited insight could be obtained regarding the survival of implants. There was a statistically significant higher failure rate in the transplanted bone. The reduced survival of implants in the transplanted bone may be explained by differences in bone quality, bone volume, and revascularization compared to the native bone.[9] Our findings are in agreement with Yerit *et al.*[25], where lower survival was also reported for the transplanted bone. In contrast, Buddula *et al.*[24] reported no difference between implant survival in the native and transplanted bone. They also reported no difference in survival between genders and considering the time span between radiation treatment and implant insertion, which is in accordance with this study. Their finding of the statistically significant higher hazard ratio for implants in the upper jaw could not be confirmed in this study.

The risk for implant failure is generally higher in smoking patients, as shown in the systematic review papers by Chambrone *et al*. and Moraschini and Barboza.[26,27] The smoking status was not a statistically significant predictor for the implant survival in this study, but the effect might have been detected with a larger sample size and more precise smoking classification. It is also notable that the risk for implant failure in smokers was suggested to be significantly elevated only for a limited time after surgery, presumably when tobacco smoke components impede bone healing.[27]

HBO, which is commonly used at the University Medical Centre Ljubljana for the head and neck cancer patients requiring surgery, was also not a statistically significant predictor for the implant survival in this study. Generally, there is no agreement on the HBO efficacy and value.[28]

Implant osseointegration and survival data provide valuable information about the success of the implant therapy. Nevertheless, the final judgement of the implant-prosthetic therapy should be made according the denture performance in the oral cavity. Successful implants enable patients to use the dentures and do not cause any persistent discomfort. To achieve a favourable clinical outcome, it is crucial to design a viable prosthetic plan early in the rehabilitation process. Head and neck cancer patients present severely altered and unfavourable tissue conditions, making it challenging to model rehabilitation using the optimal top-down approach. The fragile mucosa, xerostomia, limited mouth opening and jaw deviations are additional factors to consider. The possible locations, angulations and implant dimensions might not be ideal and should be discussed thoroughly between the oral surgeon and the prosthodontist.

The optimal number of implants should be carefully planned. There is a tendency to insert as few implants as possible in oncological patients, to facilitate bone healing. On the other hand increased number is often required to design rigid, implant-borne prosthetic constructions.[18] More implants also allow more flexibility in prosthetic treatment planning. Moreover, if some implants are lost, implant dentures can be successfully repaired and worn by the patients. It is currently thought that the number of implants is not critical for the success of the prosthetic treatment[29], but long-term clinical studies are lacking. In the present study the number of implants supporting the dentures was not a detectable factor in the success rate of the prosthodontic rehabilitation. Similarly, neither was the treated jaw or the healing time, which exceeded 4 months in all implants.

The loaded implants also exhibited similar success rates regardless of the denture attachment system used. While the bar-supporting implants experienced less success, the differences in comparison to the Locator attachments and SynCone systems were not significant.

Additional systemic and patient-related factors might play an important role in implant prosthetic rehabilitation. In this case series of irradiated patients, advanced age showed a negative prognostic value for the rehabilitation success, but not for implant survival. One of the possible limitations of this study was that the data on systemic diseases and alcohol consumption which might negatively affect implant performance were not included. After the implant-prosthetic rehabilitation, it is of utmost importance to enrol the patient in an appropriate supportive program, with regular recalls, cancer screening and maintenance of the peri-implant conditions. The recall program should meet the individual needs of the patients according to the overall risk profile. Some patients should be recalled every 3 months, while others may need to be checked once per year.[30]

This study has shown that favourable rehabilitation results can be obtained with implant-prosthetic treatment in irradiated patients. With proper collaboration between experienced surgeons and

prosthodontists, this treatment modality can be regarded a viable option for oral rehabilitation after head and neck cancer.

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research article

Determination of dosimetric parameters for shielded ^{153}Gd source in prostate cancer brachytherapy

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Background. Interstitial rotating shield brachytherapy (I-RSBT) is a recently developed method for treatment of prostate cancer. In the present study TG-43 dosimetric parameters of a ^{153}Gd source were obtained for use in I-RSBT.

Materials and methods. A ^{153}Gd source located inside a needle including a Pt shield and an aluminum window was simulated using MCNPX Monte Carlo code. Dosimetric parameters of this source model, including air kerma strength, dose rate constant, radial dose function and 2D anisotropy function, with and without the shields were calculated according to the TG-43 report.

Results. The air kerma strength was found to be 6.71 U for the non-shielded source with 1 GBq activity. This value was found to be 0.04 U and 6.19 U for the Pt shield and Al window cases, respectively. Dose rate constant for the non-shielded source was found to be 1.20 cGy/(hU). However, for a shielded source with Pt and aluminum window, dose rate constants were found to be 0.07 cGy/(hU) and 0.96 cGy/(hU), on the shielded and window sides, respectively. The values of radial dose function and anisotropy function were tabulated for these sources. Additionally, isodose curves were drawn for sources with and without shield, in order to evaluate the effect of shield on dose distribution.

Conclusions. Existence of the Pt shield may greatly reduce the dose to organs at risk and normal tissues which are located toward the shielded side. The calculated air kerma strength, dose rate constant, radial dose function and 2D anisotropy function data for the ^{153}Gd source for the non-shielded and the shielded sources can be used in the treatment planning system (TPS).

Key words: interstitial rotating shield brachytherapy; ^{153}Gd; TG-43 dosimetric parameters; Monte Carlo simulation

Introduction

Prostate cancer is the most common cancer in men. In 2013, it was reported that the number of 2,850,139 men were living with prostate cancer in the United States.[1] The number of new cases of prostate cancer from the year 2009 to 2013, was 129.4 per 100,000 men per year and the number of deaths caused by prostate cancer was 20.7 per 100,000 men per year.[1] This cancer is observed with a higher prevalence in African-American men than in white men.[2] Brachytherapy is one of the com-

mon therapeutic methods for treatment of prostate cancer. This approach has shown successful outcomes in treatment of this cancer. The success of brachytherapy is due to advantages such as simple performance and reduced side effects of treatment compared to external radiation therapy and surgical removal of the tumor.[3-6] Brachytherapy is a treatment in which one or a number of covered radioactive sources are inserted at a short distance from the target. Brachytherapy is performed either as intracavitary procedure, in which the brachytherapy source is placed inside the natural body cavity, adjacent to the tumor. The second method is interstitial brachytherapy, in which brachytherapy seeds are implanted directly inside the tumor mass. With this treatment method, the required amounts of radiation dose can be delivered to the tumor with the rapid dose fall off to the healthy tissues around the tumor.

Originally, brachytherapy treatments were performed with ^{226}Ra sources. Nowadays, the use of artificial radionuclides such as ^{125}I, ^{131}Cs, and ^{103}Pd is rapidly increasing.[7-9] Presently, brachytherapy sources are widely used for treatment of various types of cancer patients. ^{153}Gd is a medium energy brachytherapy source emitting photons in the range of 40 to 100 keV. This isotope has a low dose rate and its half-life is 242 days. Interstitial rotating shield brachytherapy (I-RSBT) is a type of brachytherapy in which a shield is used to spare normal tissues from radiation damage. The rotating capability enables the user to select an emission angle to direct the radiation to a specified tumor. It can also be used in sequencing the rotating shield in dynamic rotational shield brachytherapy. With I-RSBT it is possible to have a significant dose reduction in urethra while delivering higher prostate organ dose. The main reason for the use of ^{153}Gd in I-RSBT method is that it requires less shielding within the source than other higher energy isotopes such as iridium.

The main goal of any radiation therapy treatment is to maximize the dose to the tumor while limiting the dose to adjacent normal tissue and organs. Therefore, shielding the sensitive organs around the target volume has always been one of the important issues. In brachytherapy of prostate and cervix, the sensitive organs such as the rectum and bladder should be protected to receive the radiation dose lower than their tolerance level. For the purpose (I-RSBT), the use of special cylindrically shielded source can reach the aim. The shield there is a platinum sleeve with an aluminium gap leaking the narrow source beam. The difficulty of manufacturing of a shielded source is in its limited size. Namely, the cylindrical source should be capable to enter the hollow brachytherapy needle with the diameter *e.g.* 1.6 gauge. Energetic sources (Ir-192) need more shielding thickness which prevents them entering the small diameter brachytherapy needles.

The report by task group No. 43 (TG-43) from American Association of Physicists in Medicine is known as the most common formalism for calculation of the dose distribution around brachytherapy sources. According to this protocol, dosimetric parameters around brachytherapy sources are obtained by experimental measurement or simulation techniques using the Monte Carlo codes in a uniform water phantom.[10-11] Monte Carlo is a computational technique for solving problems in various fields including physical and mathematical sciences. This method is based on random sampling to achieve the required results. Different Monte Carlo codes are currently used in medical radiation physics: MCNP, EGSnrc, GEANT4, SIMIND, etc. Monte Carlo N-Particle (MCNP) is specially designed for nuclear application of radiation transport but it also can be used for other fields, for example in medical physics applications. Monte Carlo codes are of high capabilities in particle transport physics lacking applications in practical medical physics dosimetric problems.[12-14] These methods can have desired results to assess dosimetric parameters such as air kerma strength, dose rate constant, radial dose function and anisotropy function of brachytherapy sources.[15,16] In a number of previous studies, dosimetric parameters of hypothetical brachytherapy sources were calculated prior to their fabrications in order to assess the feasibility of its fabrication. One of these sources is ^{153}Gd that has been introduced by Enger *et al*.[17] Additionally, ^{153}Gd radionuclide has been recently used in nuclear medicine imaging systems *e.g.* linear scanners and quality assurance procedures such as phantom calibrations.

In radiotherapy the radiation dose destroys the tumor cells causing the healing of cancer and improving the patient's quality of life. There is a need in prostate brachytherapy to reduce the dose to radiosensitive organs such as rectum and bladder reducing the dose side effects to these sensitive organs. Therefore, in a proper patient treatment plan the amount of absorbed dose to critical organs should be exactly specified and controlled.[18]

Interstitial rotating shield brachytherapy is a new type of high dose rate brachytherapy in which the radiation dose is delivered by shielded

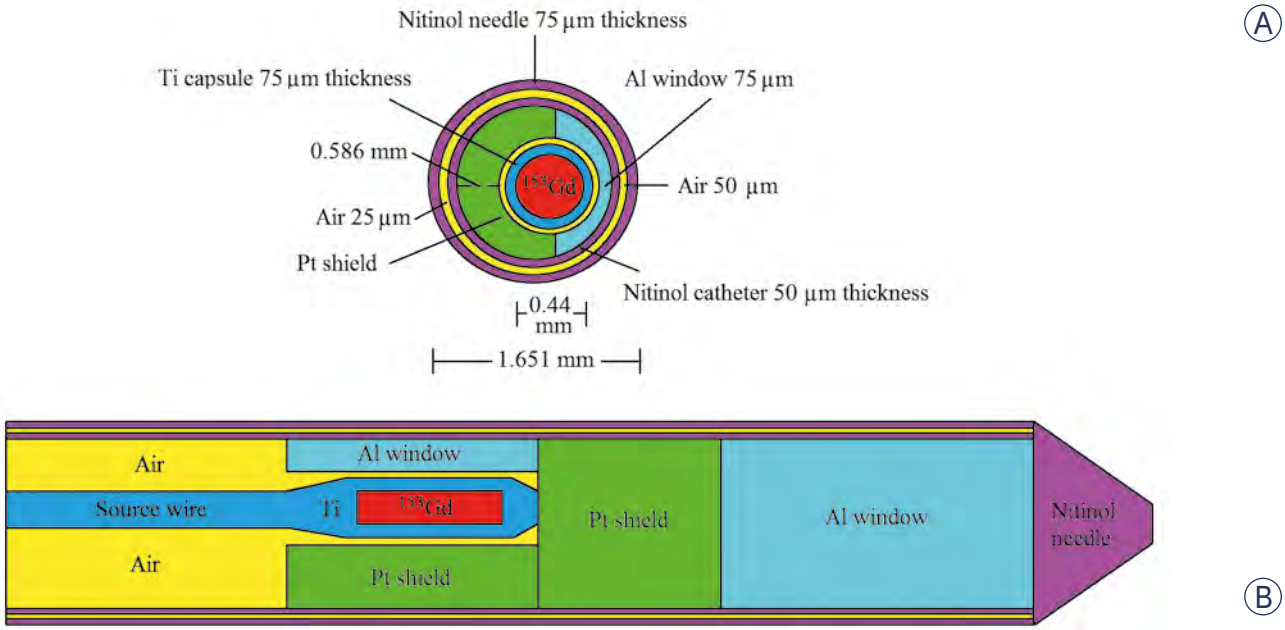

FIGURE 1. A schematic view of the simulated ^{153}Gd source, Nitinol needle including Pt shields and Al windows. **(A)** transverse view, **(B)** longitudinal view. This figure is not in to a real scale. Polar angles that were used to calculate the anisotropy function.

and rotating catheters. In I-RSBT a brachytherapy source has a well-defined shield with the capability of rotation around the source's longitudinal axis to spare normal tissues. With I-RSBT the limitation that dose distributions should be symmetric around a brachytherapy source is resolved. Therefore, with this method there is the potential to deliver unrivalled dose distributions.[19] In a recent study conducted by Adams *et al*.[19], the interstitial rotating shield brachytherapy (I-RSBT) method was introduced for brachytherapy of prostate. In that study the method was presented to reduce the dose to rectum, bladder and urethra in I-RSBT with a ^{153}Gd source. They have demonstrated that brachytherapy with rotational shield has the potential to reduce the dose to organs at risk compared to traditional brachytherapy. The isodose curves around the shielded ^{153}Gd source were compared with the isodose curves of an^{192}Ir source. Additionally, they introduced the data related to an anonymous patient in a treatment planning system and compared dose volume histograms from conventional brachytherapy with ^{192}Ir source with those from ^{153}Gd based I-RSBT. However, in that study TG-43 dosimetric parameters of the source with shield were not investigated. Enger *et al*.[17] introduced a hypothetical ^{153}Gd source and calculated TG-43 parameters for the source without rotational shield. In that study, the hypothetical ^{153}Gd source was introduced for potential use in I-RSBT. However, only TG-43 parameters were determined and the effect of rotational shield geometry on dosimetric aspects was not evaluated. In another study Ghorbani and Behmadi[20] calculated TG-43 parameters for the same hypothetical nonshielded ^{153}Gd source and compared them with dosimetric parameters of commercially available ^{192}Ir and ^{125}I sources. In the study by Ghorbani and Behmadi the effect of shielding was not considered, as it was not evaluated by the study of Enger *et al*.[7] In the aforementioned studies TG-43 parameters of a shielded ^{153}Gd source were not evaluated.

Consequently the present study evaluates a ^{153}Gd source for use in the I-RSBT and its dosimetric parameters are obtained according to TG-43 formulation for use in prostate brachytherapy.

Materials and methods

Geometry of ^{153}Gd source

In our study the design of a ^{153}Gd source, a needle including Pt shields and Al windows were adopted from the study by Adams *et al*.[19] The geometry of the ^{153}Gd source is designed so that the active part of this source is a cylinder with length of 10 mm and diameter of 440 µm. The source has an activity of 62 GBq. Having the activity of the source, it is possible to normalize a dosimetric quantity to that activity. For example by having this ^{153}Gd source

TABLE 1. The energy spectrum of ^{153}Gd radionuclide.[18] In these data, the energy values were rounded to two decimal places

Energy (keV)	Prevalence (%)
5.18	0.374
5.82	0.976
5.82	0.1341
5.85	8.86
6.44	0.559
6.46	5.64
6.57	0.9214
6.62	0.0845
6.84	1.8512
7.48	0.947
7.77	0.173
7.79	0.244
14.06	0.0183
21.20	0.0224
40.47	0.00953
40.90	35.29
41.54	63.516
46.90	6.2516
47.04	12.13
47.37	0.1848
48.25	4.001
48.39	1.597
54.19	40.01622
69.67	2.41923
75.42	0.078323
83.37	0.1964
89.49	0.0694
96.88	0.0022
97.43	29
103.18	21.1123
118.11	0.000121
166.56	0.00033
172.30	0.00022
172.85	0.036017

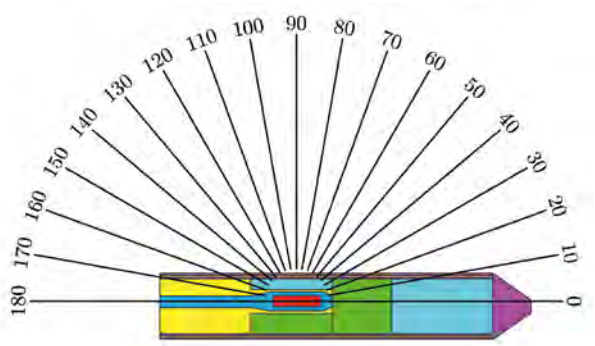

FIGURE 2. Polar distribution of angles in calculation of anisotropy function in the shielded mode.

with 62 GBq activity and by dividing a quantity of this source to 62 GBq it is feasible to calculate that quantity per GBq. The active core is surrounded by 75 μm titanium capsule with a density of 4.506 g/cm^3. At one side of the titanium capsule there is a window of pure aluminum with 2.70 g/cm^3 density and the thickness of 75 μm. On the other side of the source construction, there is a Pt shield which is an alloy of 90% Pt and 10% Ir expressed in weight percentages. The density of this shield is 21.45 g/cm^3. The radioactive source is within Nitnol catheter and a Nitinol needle (Figure 1). These are composed of Ni and Ti with 55.6% and 44.4% percentage weight fractions, respectively. The density of the catheter and needle was considered as 6.54 g/cm^3. The outer diameter of the needle is 1.651 mm. Figure 1 illustrates transverse and longitudinal views of the ^{153}Gd source, catheter, and the needle including the Pt shield and Al windows. It should be noticed that some of the dimensions in this Figure are based on the study by Adams *et al.*[19], while the others are based on assumptions. In other words, we assumed all the dimensions which were not directly reported in the study by Adams *et al.* The photon spectrum of the ^{153}Gd radionuclide is listed in Table 1.[21]

Calculation of dosimetric parameters

According to TG-43U1 report[10], dose distribution around a brachytherapy source can be obtained using the following equation:

$$\dot{D}(r,\theta) = S_K \Lambda \frac{G(r,\ \theta)}{G(r_0,\theta_0)}\ g(r)F(r,\theta) \qquad [1]$$

In this equation the S_K, Λ, $G(r, \theta)$, $g(r)$ and $F(r, \theta)$, are air kerma strength, dose rate constant, geometry function, radial dose function and anisotropy function.

The TG-43U1 quantities agenda are as follows:

MCNPX (version 2.6.0) Monte Carlo simulation code was used to calculate the TG-43 dosimetric parameters of the ^{153}Gd source. The parameters including air kerma strength, dose rate constant, radial dose function and anisotropy function were calculated for the source with the shield and without it.

To calculate the air kerma strength in the non-shielded mode, the source was assumed to be inside a vacuum sphere with a radius of 100 cm. Then, the spherical tally cells were placed at distances ranging from 1 to 40 cm from the source center at 1 cm intervals. These spheres were made of air and their radii were defined according to a joint report from AAPM and European Society for Therapeutic Radiology and Oncology (ESTRO).[22] The amount of air kerma was scored by F6 tally (in terms of MeV/g). Throughout this project the energy cut off for photons and electrons was defined as 1 keV. Each simulation was performed for 1.4×10^9 particles and the maximum type A uncertainty of Monte Carlo simulation in these calculations was 3.08%. The uncertainty quantity in tally calculation is normally listed in the output file of a Monte Carlo program. For the shielded source, the implementation of the program and the calculation of air kerma strength was similar to the non-shielded source. In calculation of air kerma strength for the Pt shield case, the scoring spheres were defined on the Pt shield side. In calculation for the Al window case, the scoring spheres were defined on the Al window side. The maximum type A uncertainties of Monte Carlo simulation calculations in the calculation of air kerma strength for the Pt shield and Al window cases were 32.78% and 3.24%, respectively.

Calculation of dose rate constant was performed based on the formalism presented in the TG-43 report. In the non-shield mode, the ^{153}Gd source was defined in a water sphere with 100 cm radius and a spherical water tally cell with 0.005 cm radius was defined at 1 cm distance from the source. Then, *F4 tally was calculated in combination with mass energy absorption coefficient in order to score the energy deposition per mass. Mass energy absorption coefficients of water listed in Table 4 of National Institute of Standards and Technology (NIST) webpage were used.[23] This program was run for 1.2×10^9 particles and the type A Monte Carlo uncertainty was obtained as 0.34% that is related to statistical fluctuation of the simulation data. By dividing the absorbed dose rate to air kerma strength, the dose rate constant was achieved. In the shielded source

TABLE 2. Radial dose function values for the non-shield and shield modes. The shield mode is related to the Pt and Al window sides

g(*r*)			
r (cm)	Without shield	With shield	
		Pt shield side	Al window side
0.5	0.90	0.52	0.93
1	1.00	1.00	1.00
1.5	1.06	1.39	1.04
2	1.11	1.73	1.04
2.5	1.13	2.03	1.07
3	1.18	2.22	1.07
3.5	1.14	2.44	1.07
4	1.19	2.72	1.05
5	1.17	2.65	1.02
8	1.01	3.01	0.91
10	0.89	2.63	0.80
12	0.77	2.44	0.71

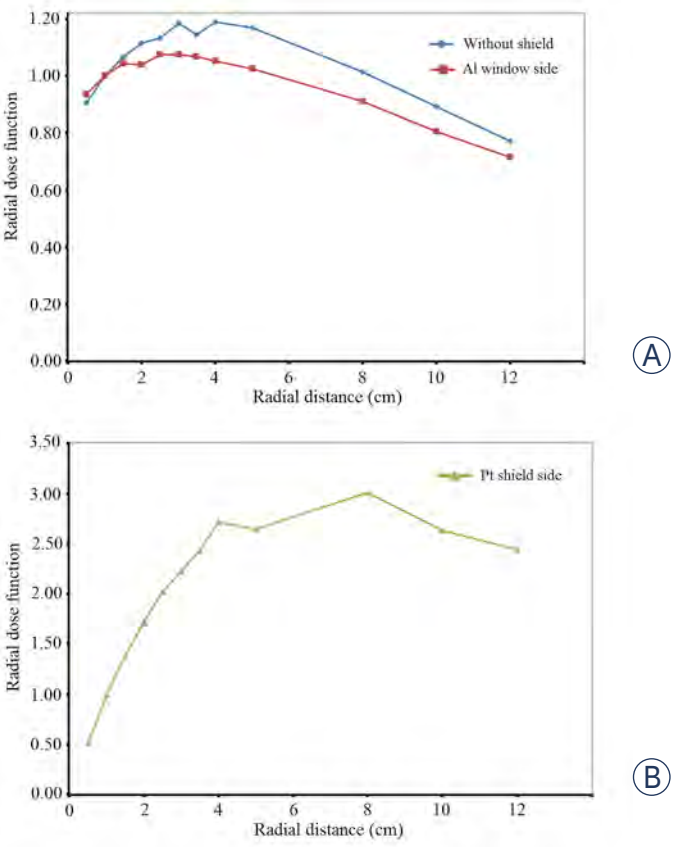

FIGURE 3. (A) Radial dose function for the ^{153}Gd source without the shield and with the shield on Al window side **(B)** radial dose function for the shielded source at Pt shield side.

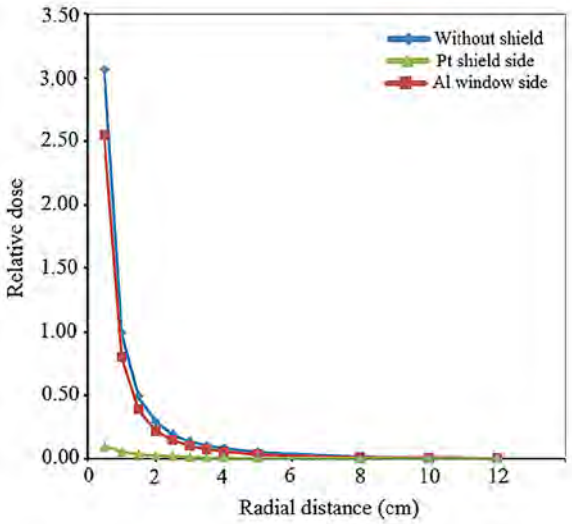

FIGURE 4. Relative dose in non-shielded mode, Pt shield side and aluminum window side in the shielded mode of the source. The normalization point is at 1 cm for the non-shielded case.

cases the calculations were performed for both sides of source. In the calculation of dose rate constant in each case, the dose rate at 1 cm was divided by the air kerma strength for the non-shielded case. The type A uncertainties in Monte Carlo simulations were 0.37% and 1.50% for the calculation at Al window and Pt shield sides, respectively. The other details of the simulations for the shielded source were the same as the simulation details for the non-shielded source.

Radial dose function for the non-shielded source was calculated by selecting a spherical water phantom with 100 cm radius and placing spherical water tally cells at distances ranging from 0.5 cm to 12 cm with 0.5 cm intervals, relative to the source center. The radii of these tally cells were defined according to the report by AAPM and ESTRO[22], in other words, for distances less than 1 cm the radius of the spheres was considered as 0.005 cm, and for distances 1 cm ≤ r<5 cm the radius was considered as 0.025 cm and for distances 5 cm ≤ r< 10 cm the radius was considered 0.05 cm and finally for distances more than 10 cm the radius was considered to be 0.1 cm. In this program *F4 tally was scored and the tally outputs in various energy bins were multiplied by mass energy absorption coefficients using DE and DF cards in MCNP code. Based on the MCNP manual, "DE" and "DF" stand for dose energy and dose function, respectively. In DE cards, energy bins are defined, while in DF card mass energy absorption coefficients are introduced. The program was run for 1.2 × 10^9 particles and the maximum type A Monte Carlo uncertainty was 1.81%. A similar program was defined for the shielded case so that the source was defined in a water sphere with 100 cm radius and also the same intervals and water spheres were defined. Unlike the previous case in this program, spheres were defined on both sides of the source. In this program the output data were obtained using *F4 tally and "DE" and "DF" cards. This program was run for 1.2 × 10^9 particles. The maximum Monte Carlo uncertainty in these investigations in the non-shielded case was observed as 2.07% and in the case of the Pt shield it was observed as 4.49%. It can be considered that the values of these uncertainties are different at different parts of the simulation, and it is because the uncertainty depends on the complexity of geometry, tally type used, number of particle histories, etc.

To calculate the anisotropy function in the case of non-shielded source, a water sphere with 100 cm radius was defined as the phantom. In order to obtain anisotropy function, spheres were defined at different radial distances and angles. These spheres were defined at distances of 0.5, 1, 2, 3, 5, 10 and 12 cm. The angles were ranging from 0 to 180 degrees with 10 degrees intervals. The energy flux was scored in these spheres using *F4 tally. In the calculation of this program mass energy absorption coefficient was utilized. The radius of each sphere was considered according to the report by AAPM and ESTRO. This program had been run for 2 × 10^9 photons and the maximum uncertainty in the Monte Carlo calculation was observed as 1%. The calculations defined for the shielded source were performed similar to the non-shielded source. The difference was that the spheres were on both sides (Pt shield side and Al window side). The uncertainty in the calculation in this case was observed less than 5%. Polar angles that were used to calculate the anisotropy function of the Pt shield case are illustrated in Figure 2. Based on this Figure zero angle begins from the tip of the source for the Pt shield case. For the rest of the cases (nonshielded source and the Al window case) the polar angles are likewise.

Results

Air kerma strength per source activity (per GBq) for the non-shielded mode was obtained equal to 6.71 $cGyh^{-1}cm^2GBq^{-1}$. Based on this value for the nonshielded ^{153}Gd source, 1 U is equal to 4.03 mCi. This value was found to be 0.04 $cGyh^{-1}cm^2GBq^{-1}$

TABLE 3. Anisotropy function values for the ^{153}Gd source at different distances for the non-shielded mode

θ (degrees)	r (cm)					
	0.5	1	2	3	5	10
0	0.71	0.59	0.48	0.41	0.29	-
10	0.75	0.63	0.58	0.51	0.44	0.75
20	0.82	0.76	0.69	0.64	0.63	0.81
30	0.86	0.83	0.77	0.75	0.76	0.86
40	0.90	0.85	0.85	0.84	0.87	0.91
50	0.95	0.91	0.90	0.90	0.93	0.94
60	0.95	0.98	0.94	0.94	0.96	0.96
70	1.00	0.96	0.97	0.98	0.98	0.97
80	0.99	0.99	0.97	0.98	1.01	1.00
90	1.00	1.00	1.00	1.00	1.00	1.00
100	0.97	0.97	0.99	1.00	1.01	1.00
110	1.01	0.95	0.97	0.97	0.96	0.97
120	0.97	0.95	0.92	0.94	0.96	0.97
130	0.95	0.93	0.89	0.90	0.93	0.94
140	0.92	0.87	0.85	0.84	0.87	0.90
150	0.88	0.80	0.78	0.76	0.79	0.87
160	0.83	0.74	0.68	0.65	0.63	0.81
170	0.76	0.65	0.56	0.50	0.45	0.75
180	0.69	-	-	-	-	-

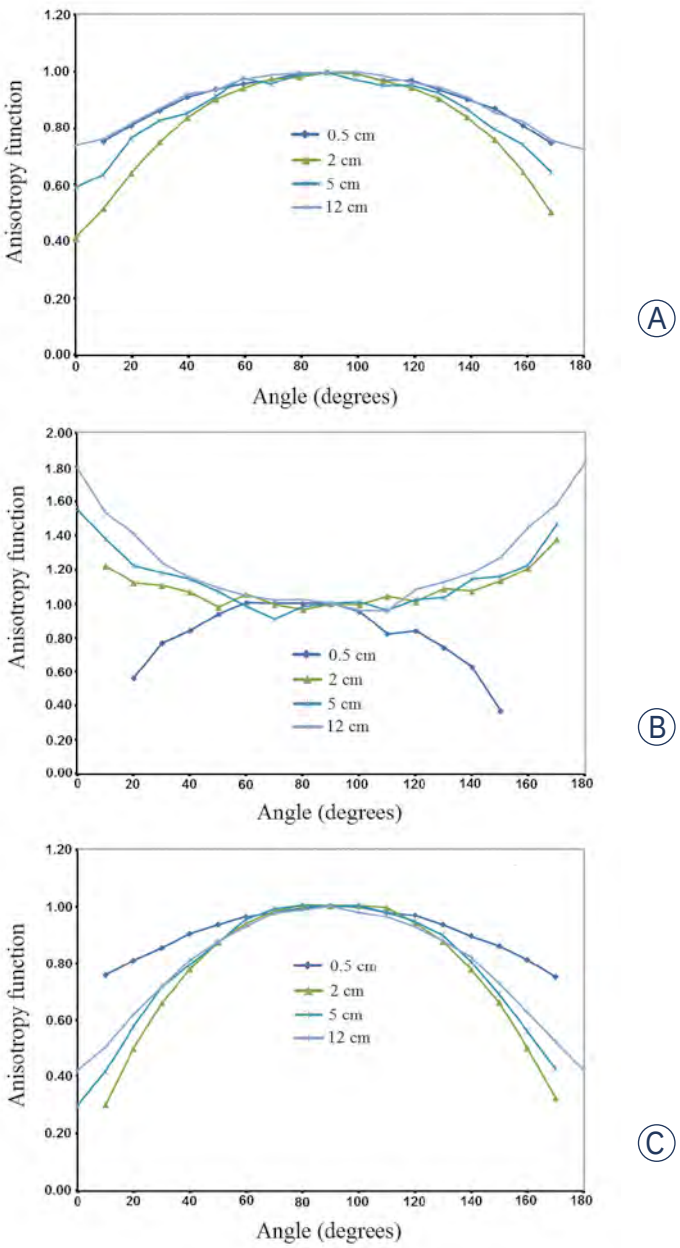

FIGURE 5. Anisotropy function for the ^{153}Gd source: **(A)** non-shielded mode, **(B)** shielded mode, Pt shield side, **(C)** shielded mode, Al window side.

and 6.19 cGyh^{-1}cm^{2}GBq^{-1} for the Pt shield and Al window cases, respectively. The values of dose rate constant for the non-shielded mode was obtained as 1.20 cGyh^{-1}U^{-1} and for the shielded mode for the Pt shield and Al window sides were achieved as 0.07 cGyh^{-1}U^{-1} and 0.96 cGyh^{-1}U^{-1}, respectively. It should be noted that in calculation of dose rate constant for these three cases the dose rate at the reference point (at 1 cm and 90 degrees angle) in each case was divided by the air kerma strength value for the non-shielded source. It can be noted that in the shielded case the dose rate constant is reduced.

Radial dose function values for ^{153}Gd source are listed in Table 2. The first column there refers to the source without shield, the second column refers to the Pt shield side of the source and the third column refers to the Al window side of the source. The radial dose function values are plotted in the Figure 3B for the Pt shield side. Figure 4 shows the relative dose for the non-shielded mode of the source, the Pt shield and Al window sides of the shielded source. In this graph, for all three cases, the dose values have been normalized to the dose at 1 cm distance of the non-shielded source.

Anisotropy function values for the ^{153}Gd source were calculated for 0.5, 1, 2, 3, 5, 10 and 12 cm distances from the source at 19 different angles (ranging from 0 to 180 degrees) with angular intervals of 10 degrees. The results of anisotropy function are presented in Table 3 and Table 4 for the non-shielded and shielded cases, respectively. Figure 5A also shows anisotropy function values at different distances from the source for non-shielded mode. In

TABLE 4. Anisotropy function values for the ^{153}Gd source at different distances for the shielded mode at the Pt and Al windows sides

θ (degree)	r (cm)													
	Pt shield side							Al window side						
	0.5	1	2	3	5	10	12	0.5	1	2	3	5	10	12
0	0.42	0.39	0.30	-	-	-	-	1.80	1.78	1.56	-	-	-	-
10	0.51	0.52	0.42	0.35	0.31	0.29	0.76	1.54	1.57	1.38	1.32	1.22	0.35	-
20	0.62	0.61	0.58	0.51	0.50	0.55	0.81	1.41	1.36	1.23	1.21	1.12	0.88	0.38
30	0.72	0.71	0.72	0.66	0.66	0.70	0.85	1.24	1.31	1.18	1.17	1.10	0.87	0.56
40	0.81	0.81	0.79	0.77	0.78	0.81	0.90	1.15	1.23	1.14	1.07	1.07	1.05	0.77
50	0.88	0.88	0.87	0.86	0.87	0.90	0.93	1.09	1.14	1.07	1.08	0.98	0.86	0.84
60	0.93	0.91	0.95	0.92	0.94	0.97	0.96	1.05	1.07	0.98	1.06	1.05	0.91	0.94
70	0.98	0.96	0.99	0.97	0.98	1.01	0.98	1.02	1.07	0.91	1.04	0.99	1.03	1.00
80	0.99	0.99	1.00	1.00	1.00	1.06	0.99	1.02	1.01	0.98	0.99	0.96	1.00	1.00
90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
100	0.98	0.99	1.00	0.98	1.00	1.02	1.00	0.96	1.01	1.01	1.04	0.99	1.03	1.00
110	0.96	0.97	0.98	0.97	0.99	0.97	0.98	0.96	1.07	0.96	1.01	1.04	0.99	0.95
120	0.93	0.93	0.95	0.92	0.94	0.96	0.97	1.08	1.09	1.02	1.07	1.01	1.08	0.82
130	0.88	0.89	0.90	0.88	0.88	0.93	0.93	1.13	1.24	1.03	1.07	1.09	0.95	0.84
140	0.82	0.83	0.81	0.77	0.78	0.84	0.89	1.18	1.21	1.14	1.12	1.07	1.04	0.74
150	0.73	0.70	0.69	0.67	0.66	0.71	0.86	1.27	1.29	1.16	1.15	1.13	0.87	0.63
160	0.63	0.62	0.56	0.52	0.50	0.54	0.81	1.45	1.53	1.23	1.33	1.21	0.89	0.37
170	0.53	0.49	0.43	0.36	0.33	0.30	0.75	1.59	1.57	1.47	1.51	1.38	1.06	-
180	0.43	0.41	-	-	-	-	-	1.83	1.89	-	-	-	-	-

TABLE 5. Mass attenuation coefficient (μ/ρ) of Pt, Al and Ti in the 40 keV-100 keV energy range[21]

Energy (keV)	Pt	Al	Ti
40	12.45	0.57	2.21
50	6.95	0.37	1.21
60	4.34	0.28	0.77
80	8.73	0.20	0.41
100	4.99	0.17	0.27

Figure 5A anisotropy function for the non-shielded source is presented, in Figure 5B anisotropy function for the shielded source at Pt shield side and in Figure 5C anisotropy function for the shielded source at Al window side of the source is presented. In Figure 6 anisotropy function for three modes is presented. There are four graphs for distances of the evaluation point of the anisotropy function from the source: (A) 0.5 cm, (B) 2 cm, (C) 5 cm and (D) 12 cm distance.

Isodose curves (%) for the non-shielded source and the shielded source (Pt shield side) are plotted in Figure 7. In these plots the dose distributions were normalized to the dose at 1 cm distance from the source at transverse plane.

Discussion

In this study TG-43 dosimetric parameters for a 153Gd source with Pt shield were calculated for use in I-RSBT of prostate brachytherapy. Air kerma strength value for the non-shielded 153Gd source was found to be 6.71 U per 1 GBq activity.

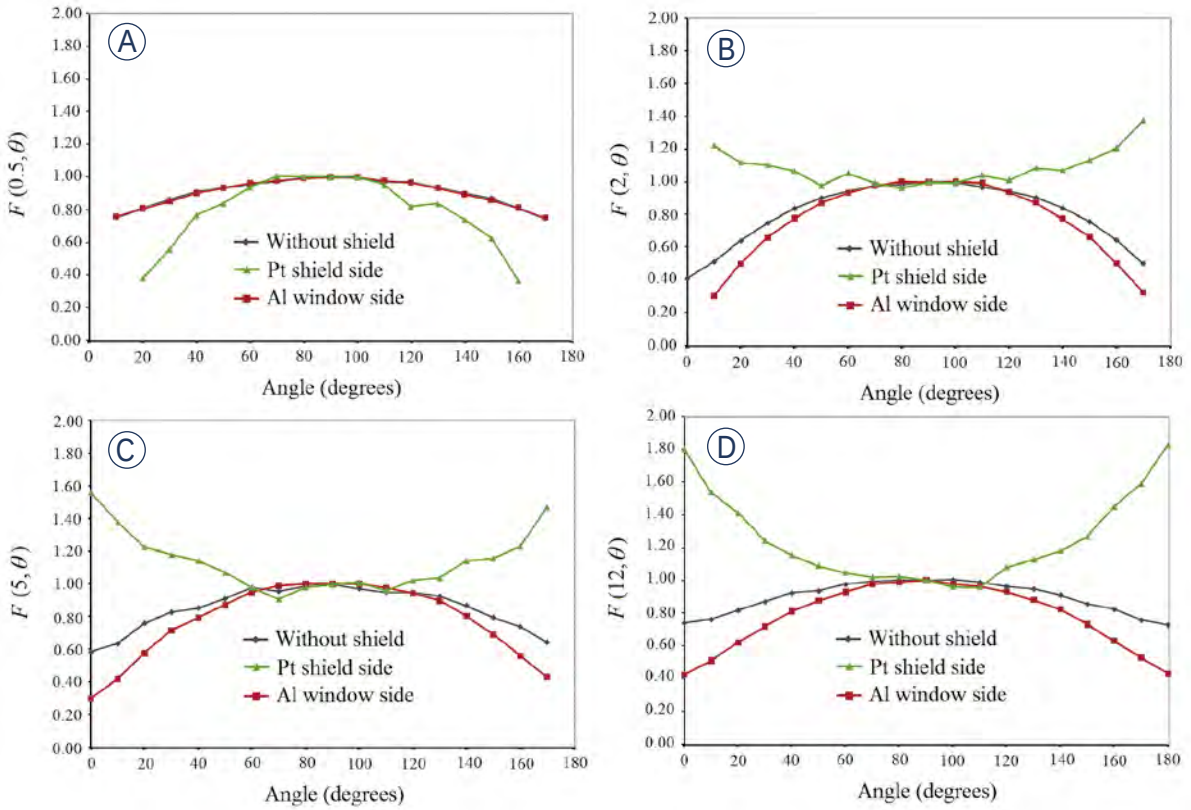

FIGURE 6. Comparison of anisotropy function for non-shielded mode, Pt shielded mode, Al window mode. All modes are presented for distances: **(A)** 0.5 cm, **(B)** 2 cm, **(C)** 5 cm, **(D)** 12 cm.

This means that 6.71 U of ^{153}Gd is equal to 27 mCi activity and 1U is equal to 4.03 mC activity. The results obtained in this study reveal that the ^{153}Gd dose rate constant is higher compared to the dose rate constant of ^{125}I source. This is due to the fact that average photon energy of ^{153}Gd is higher than that of ^{125}I (53.70 keV versus 28.37 keV). We found also that the simulated dose rate constant for non-shielded source is higher compared to the dose rate constant of shielded source. The reason for a higher dose rate constant in this case is the lower level of beam attenuation in non-shielded source compared to the shielded one. Furthermore I-RSBT source is two-part shielded (Pt shield and Al window Figure 1) where radiation attenuation at the Pt shield is higher compared to Al window. This effect can be explained by the values of the mass energy attenuation coefficients for Pt and Al, which are listed in Table 5. In the 153Gd photon energy range (40 keV to 100 keV), mass attenuation coefficient of Al is lower than Pt. Additionally there are also attenuations of the photons in the catheter and needle materials (Nitinol alloy of Ni and Ti elements) in the beam's path (Figure 1). Finally the radiation beam attenuation on the Al window side of the source is lower compared to the other Pt shield side of the source which is the essence of the I-RSBT.

In Figure 3A it can be seen that the trend of radial dose function is falling with distance. According to this chart, at distances less than 1 cm, the red graph which corresponds to existence of Al window is higher than the blue graph (related to the source without shield). Beyond the 1 cm distance the value of radial dose function for the non-shielded mode is higher compared to Al window case. It is due to lower attenuation of photons in Al window material. The calculated treatment dose should normally include a factor accounting for the attenuation of the material. In a treatment situation the rotating source with Al gap is "directed" to the tumor side which means that effective emission dwell times are longer in that situation. Consequently the dose received by the tumor is relatively higher compared to the healthy tissue. As it can be seen in Figure 3B, radial dose function is increasing with radial distance, and this is due to the density of platinum. The distance could be plotted

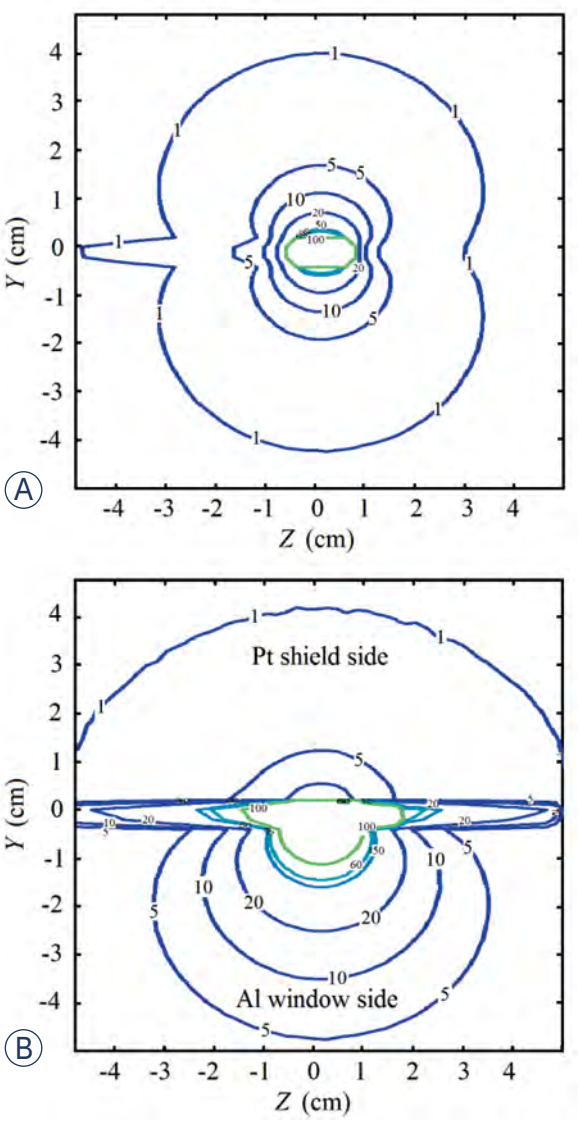

FIGURE 7. Isodose curves (%): **(A)** non shielded source, **(B)** shielded source. The dose distributions were normalized to the dose at 1 cm distance from the source at transverse plane. The *Z* axis is on the longitudinal axis of the source.

in terms of *r*×Pt density and the data be normalized to the dose rate at 1 cm physical distance from source center for the density corrected value. That way the trend of radial dose function may not be increasing.

In the calculation of radial dose function in Figure 3, each case was normalized to the dose value at 1 cm of itself. Based on Figure 3A the radial dose function in the Al window side is lower. This is due to higher photon attenuation in Al than in the water. Figure 3B, which corresponds to the Pt shield side, the radial dose function at the Pt shield side has increasing trend up to the distance of about 8 cm. In Figure 4 all the obtained dose values in three cases (non-shielded, Pt shield, Al window) were normalized to dose at 1 cm distance of the non-shield mode. With this calculation, there is evidence to have more accurate comparison. The graph in Figure 4 reveals the dependence of the source distance to the point of absorbed dose in the phantom. By increasing that distance the dose becomes lower for all three cases. Secondly, the dose in the case of Pt shield side is lower than at the Al window side. Additionally the dose at the Al widow source is lower than at the non-shielded source. These two effects are related to different absorption coefficients in Pt, Al and phantom. Pt has higher absorption coefficient than Al and Al has higher absorption coefficient than phantom (*i.e* water).

Figure 5A is related to the non-shielded source. Figure 5B is related to the Pt window source. In case of Pt window that side of the source receive lower dose. It indicates that Pt side should be turned to the side of organs at risk which means that central areas of the organs will receive lower dose. According to Figure 5A and C it can be mentioned that anisotropy at Al window mode is lower than at the non-shielded mode of the I-RSBT source. This is due to the attenuation of radiation in Al. This effect is similar for the comparison of anisotropy of Pt shield side versus non-shielded case. In directions other than 90º, beam traverses through longer path inside the needle and source, therefore the beam attenuation is more pronounced. The higher beam attenuation results to lower doses at these angles. Calculating the anisotropy function we normalized it to the dose at 90º. Thus the lower isotropy at these angles can be explained. The lower isotropy in I-RSBT source is like a disadvantage, because it causes non-uniformity of tumor dose. The peripheral parts of the tumor receive systematically lower doses than the central ones. In the Figure 6, anisotropy function is further analyzed. We compared different shielding effects of the I-RSBT source at four different distances in the phantom. We compared the source without shield, Pt shielded source and the source with Al window. They were compared at each of the four distances (0.5 cm, 2 cm, 5 cm, and 12 cm). As it can be concluded from the charts, at 0.5 cm from the source the anisotropy functions for the non-shielded mode and Al Window mode are relatively the same. This is because of the similar magnitude of self-absorption in both modes. Increasing the distance from the source there is a change of energy spectrum of photons in the phantom. The differences become greater by increasing the distance

from the source. The reason for anisotropy function equality at a distance of 0.5 cm for these two modes is their almost equal self-absorption.

In Figure 7, isodose curves are plotted for both non-shielded and shielded cases. As it can be seen in the shielded source considerable dose reductions occur at the Pt shield side. Thus the organs at risk located near the shield side of the source receive significantly lower dose. Organs that are located at 1 cm distance from the source (Pt shield side) receive 5% dose. This relative dose value is reduced further to 1% when the distance is approaching 4 cm. It should be noted that the shield should be turned to the side of organs at risk, such as the rectum and bladder. In prostate cancer the total organ volume is tumor which is covered with many irradiation source dwell positions. Thus the optimization of treatment plan can compensate eventual cold spots from the individual source dwell positions. As it was mentioned in the study of Adams *et al.*[19], by adjusting the number of needles and dwell positions as well as the number of rotations per dwell position, prostate dose can be controlled. It seems that in I-RSBT treatment of prostate cancer of Adams *et al.*[19] the radiation sensitive organs at risk are protected receiving the relative lower dose compared to tumor dose. As a consequence some parts of the tumor can receive lower dose which has to be compensated extending the affected irradiation dwell times in the treatment plan calculations. Overall radiation time is thus prolonged using this irradiation technique. The I-RSBT could be promising also for some other sites in brachytherapy like gynecology or breast brachytherapy. It is interesting as a subject of further research.

Additionally, the dose distribution around the source in shielded mode is less symmetrical compared to unshielded source which could be accounted as a disadvantage (Figure 7). However this can be compensated in treatment planning. This leads to "Intensity Modulated Brachytherapy" which is considered as an innovation at this time. Only the future will show us the real possibilities of these new techniques. As it was aforementioned in the text, Adams *et al.*[19] have introduced the interstitial rotating shield brachytherapy using a ^{153}Gd source for brachytherapy of prostate. In the study they used Computed Tomography imaging to offer clinical data for the shielded source positioning and rotation in the treatment planning process. They used 19 needles and 16 rotational angles per dwell position of the ^{153}Gd source rotating shield for annonimized prostate patient. The dose-volume histograms from conventional brachytherapy with ^{192}Ir source were compared with those from I-RSBT with ^{153}Gd source. Our study continues the scientific efforts adding TG-43 simulated dose parameters which enable Treatment Planning Systems to calculate the I-RSBT doses in phantoms and tissues.

Conclusions

The calculated TG-43 dosimetric parameters in this study for a combination of the ^{153}Gd source and the shielded needle could be used in treatment planning system. Dose rate constant and radial dose function with the shielded sources are considerably different than the non-shielded sources. Further studies will be required to justify our Monte Carlo simulation parameters as dose calculation tools on a real human RANDO phantom. From clinical point of view there are some issues relating the tumor dose uniformity which is related to dose volume histograms. The I-RSBT source has lower dose isotropy which can lead to lack of uniformity distribution in the tumor. This can be compensated with new Treatment Planning Systems based on the live Monte Carlo calculations. These are topics for the future research. In our opinion I-RSBT technique has realistic chances in the "Intensity Modulated" development of future Brachytherapy.

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research article

Optimal planning strategy among various arc arrangements for prostate stereotactic body radiotherapy with volumetric modulated arc therapy technique

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Jin Beom Chung and Tae Suk Suh contribution equally to this work

Backgorund. The aim of this study was to determine the optimal strategy among various arc arrangements in prostate plans of stereotactic body radiotherapy with volumetric modulated arc therapy (SBRT-VMAT).

Patients and Methods. To investigate how arc arrangements affect dosimetric and biological metrics, SBRT-VMAT plans for eighteen patients were generated with arrangements of single-full arc (1FA), single-partial arc (1PA), double-full arc (2FA), and double-partial arc (2PA). All plans were calculated by the Acuros XB calculation algorithm. Dosimetric and radiobiological metrics for target volumes and organs at risk (OARs) were evaluated from dose-volume histograms.

Results. All plans were highly conformal (CI<1.05, CN=0.91) and homogeneous (HI=0.09-0.12) for target volumes. For OARs, there was no difference in the bladder dose, while there was a significant difference in the rectum and both femoral head doses. Plans using 1PA and 2PA showed a strong reduction to the mean rectum dose compared to plans using 1FA and 2FA. Contrastively, the $D_{2\%}$ and mean dose in both femoral heads were always lower in plans using 1FA and 2FA. The average tumor control probability and normal tissue complication probability were comparable in plans using all arc arrangements.

Conclusions. The use of 1PA had a more effective delivery time and produced equivalent target coverage with better rectal sparing, although all plans using four arc arrangements showed generally similar for dosimetric and biological metrics. However, the $D_{2\%}$ and mean dose in femoral heads increased slightly and remained within the tolerance. Therefore, this study suggests that the use of 1PA is an attractive choice for delivering prostate SBRT-VMAT.

Key words: stereotactic body radiotherapy; volumetric modulated arc therapy; prostate cancer; arc arrangement; dosimetric and biological metrics

Introduction

Prostate cancer is the most common cancer in men, accounting for over one fifth of male cancer diagnoses, with the number of prostate cancer patients rapidly increasing. Various radiotherapy techniques for treating prostate cancer have been considered effective noninvasive treatment options,

especially for elderly patients and those unfit for surgery.[1-3]

Radiation therapy options for prostate cancer include external beam radiation therapy (EBRT) and brachytherapy. Volumetric modulated arc therapy (VMAT) and intensity-modulated radiation therapy (IMRT) for EBRT is widely used as the standard treatment for prostate cancer. VMAT and IMRT permit dose optimization, in which the dose to the prostate can be increased while reducing toxicities and doses to the rectum and bladder, resulting in improved local control and reduced complications when compared to three-dimensional conformal radiation therapy (3DCRT).[4-7] In addition, VMAT can produce equivalent or even better target coverage and normal tissue sparing compared to fixed-field IMRT while taking advantage of more efficient monitor unit (MU) and reducing the delivery times.

The use of SBRT to irradiate primary or metastatic tumors in several anatomical sites is becoming the standard treatment.[8–13] SBRT with VMAT (SBRT-VMAT) is a radiotherapy method, which very precisely delivers a high dose of radiation to the target area in either a single dose or a small number of fractions. It is an attractive approach to dose escalation. Recently, researchers using SBRT, including the linear accelerator (Linac) and CyberKnife (CK), have achieved promising results in the treatment of prostate cancer.[14–17] To our knowledge, there is very little information regarding the optimal planning for various arc arrangements of prostate SBRT-VMAT.[18,19] Moreover, there is no study, which investigates the radiobiological effect of prostate SBRT-VMAT plans.

The aim of this study is to determine the optimal treatment planning approach under the different arc arrangements by analyzing the dosimetric and radiobiological impact in plans for prostate SBRT-VMAT.

Patients and methods

Patient selection and contouring

For this retrospective study, we chose 18 patients diagnosed with prostate cancer that had previously been treated in our department from September 2013 to October 2015. All prostate cancer patients were enrolled in our SBRT planning study, which was approved by the institutional review board of Seoul National University Bundang Hospital. (IRB No. B-1501/284-107).

A computer tomography (CT; The Brilliance CT Big Bore, Philips, Eindhoven, Netherlands) simulation was performed with the patients, who were placed in a supine position on a flat bench and stabilized with Knee-fix™ and Feet-fix™ (CIVCO Medical Solutions, Coralville, IA, USA). Prior to the CT simulation, the patients were asked to drink 300 ml of water 1 h before the simulation to ensure that the bladder was completely filled. An endorectal balloon (ERB) was inserted into the rectum and filled with 70 cc of air. After 1 min, the ERB catheter was placed at the pre-marked position and the inflated ERB was immobilized above the anal sphincter. A detailed description of the patient setup was given in our previous study.[20,21] The CT scans were acquired with a 3 mm slice thickness. The prostatic bed was delineated as the clinical target volume (CTV), and the planning target volume (PTV) was defined as the CTV plus a treatment margin of 7 mm posteriorly and 10 mm in all other directions. The relevant normal tissue including rectum, bladder, and femoral head were delineated as OARs. The rectum was defined as extending from the sigmoid flexure to the bottom of the ischium.

Planning strategy for SBRT

Prostate SBRT using VMAT plans were created by the Eclipse™ Treatment Planning System (ver. 11.0.34, Varian Medical Systems). The SABR-VMAT planning system was commissioned for a Truebeam™ Linac (Varian Medical Systems) with a high definition multileaf collimator (HD MLC). Dose distributions were calculated using a 10-MV flattening-filter-free (FFF) beam and the Acuros XB (AXB) dose calculation algorithm with inhomogeneity correction. The calculation grid size was 2.5-mm.

The SBRT-VMAT plans were set up with four arc arrangements such as single-full arc (181° to 179°; 1FA), single-partial arc (240° to 120°; 1PA), double-full arcs (181° to 179° and 179° to 181°; 2FA), and double-partial arcs (240° to 120° and 120° to 240°; 2PA). The arc arrangements for SBRT-VMAT plans are presented in Figure 1 A to D. The collimator rotation angles were 30°.

The prescription dose was 42.7 Gy and was administered in seven fractions. Compared to 78 Gy in 39 fractions, which is the standard prostate fractionation, this delivers a higher biologically effective dose (BED) to the prostate (216.3 Gy vs. 182.0 Gy; α/β 1.2 Gy) but an equivalent dose to late responding tissues (129.5 Gy vs. 130.0 Gy; α/β 3.0 Gy). For all cases, a minimum of 95% of the prescription dose (40.6 Gy) was assigned to cover 95% of the PTV ($V_{95\%} \geq 95\%$). No OAR constraints for

prostate SBRT have yet been reported. Therefore, we used the modified constraints for the OARs derived from those reported by Murray *et al*.[22] that were suitable for our planning study. The OAR constraints for this planning study are listed in Table 1.

Evaluation of dosimetric and biological parameters

The mean, maximum, and minimum doses for PTV were measured from cumulative dose-volume histograms (DVH) of plans using four arc arrangements for all patients. In order to investigate the target coverage, $V_{100\%}$ for CTV and PTV were evaluated.

The conformity index (CI) was defined as follows:

$$CI = \frac{V_{RI}}{TV}$$

The ideal conformity is defined as CI = 1. A value of CI > 1 indicated that healthy tissue has been irradiated.[23] The conformation number (CN) takes into consideration the irradiation of healthy tissue. It is the product of two fractions, TV_{RI}/TV and TV_{RI}/V_{RI}, where TV is the volume of the PTV, TV_{RI} is the volume of the PTV covered by the reference isodose line, and V_{RI} is the volume enclosed by the reference isodose line. TV_{RI}/TV is the quality of the target coverage and TV_{RI}/V_{RI} is the volume of healthy tissue irradiated with the reference isodose (95% of prescribed dose) or more.[24]

The dose homogeneity index (HI) was determined as follows:

$$HI = \frac{(D_{5\%} - D_{95\%})}{D_{50\%}}$$

where $D_{5\%}$ is the maximum dose received by 5% of PTV, $D_{95\%}$ is the minimum dose received by 95% of PTV, and $D_{50\%}$ is the dose received by 50% of PTV. A lower HI represents a more homogeneous plan, because $D_{5\%}$ and $D_{95\%}$ were surrogate markers of maximum dose and minimum dose in the PTV, respectively.

The near-to-maximum dose ($D_{2\%}$) and mean dose for the OARs were evaluated. In addition, we conducted a detailed analysis of the rectum and bladder volumes that received at least 95% ($V_{95\%}$), 80% ($V_{80\%}$), 50% ($V_{50\%}$), and 20% ($V_{20\%}$) of the prescription dose; these values represent very high, high, intermediate, and low doses, respectively. In addition, total monitor units (MUs) were compared in each plan between single arc and double arc arrangements.

TABLE 1. Dose volume constraints adopted for planning study

Volume	Rectum	Bladder	Femoral heads
Constraints	$V_{42.7\ Gy\ (100\%)}$ < 5%	$V_{42.7\ Gy(100\%)}$ < 10%	$V_{29.9\ Gy\ (70\%)}$ < 50%
	$V_{38.4\ Gy\ (90\%)}$ < 15%	$V_{34.7\ Gy\ (81\%)}$ < 25%	D_{max} <29.9 Gy
	$V_{32.0\ Gy\ (75\%)}$ < 35%	$V_{29.9\ Gy(70\%)}$ < 50%	
	$V_{28.0\ Gy\ (65\%)}$ < 45%		
	$V_{24.8\ Gy\ (58\%)}$ < 70%		
	$V_{20.0\ Gy\ (47\%)}$ < 80%		

D_{max} = the maximum dose; $V_{xx\ Gy}$ = the volume receiving dose of xx Gy (x% of the prescription dose)

For the radiobiological model evaluation, we utilized the MATLAB program to calculate the Niemierko's equivalent uniform dose (EUD)-based tumor control probability (TCP) and normal tissue complication probability (NTCP).[25,26] According to Niemierko's phenomenological model, the EUD is defined as follows:

$$\mathrm{EUD} = \left(\sum_i (v_i EQD_i^{\alpha})\right)^{\frac{1}{\alpha}}$$

where α is a tissue-specific parameter describing the volume effect, and is the partial tumor volume, which receives dose D in Gy. For tumors, α takes negative values; for serial-like structures, α takes large positive values; and for parallel-like structures, α takes values close to 1.

The prostate TCP was calculated with Niemierko's EUD-based TCP. The equation is defined as follows:

$$\mathrm{TCP} = \frac{1}{1 + \left(\frac{TCD_{50}}{EUD}\right)^{\gamma_{50}}}$$

where TCD_{50} is the tumor dose to control 50% of the tumors when the tumor is homogeneously irradiated, and the γ_{50} is the slope of dose response at a TCP of 50%. NTCP for OARs were calculated using Niemierko's EUD-based NTCP with the following equation:

$$\mathrm{NTCP} = \frac{1}{1 + \left(\frac{TD_{50}}{EUD}\right)^{\gamma_{50}}}$$

where TD_{50} is the tolerance dose for a 50% complication rate at a specific time interval (*e.g.*, 5 years in the Emami *et al.* normal tissue tolerance data[27]) when the whole organ of interest is homogenously irradiated. The γ_{50} is specific to the normal structure of interest and describes the slope of the dose-response curve.

Table 2 lists parameters used to calculate Niemierko's EUD-based TCP and NTCP. These

TABLE 2. Parameters used to calculated Niemierko's EUD-based TCP and NTCP

Tissue	100%dpf (Gy)	Fraction (#)	α	γ_{50}	TD_{50} (Gy)	TCD_{50} (Gy)	dpf (Gy)	α/β (Gy)
Prostate	6.1	7	-10	1.0		28.34	2	1.20
Rectum	6.1	7	8.33	4	80		2	3.90
Bladder	6.1	7	2	4	80		2	8.00
Femur	6.1	7	4	4	65		2	0.85

$\frac{\alpha}{\beta}$ = Alpha-beta ratio; 100%dpf = 100% dose per fraction; dpf = Parameters' source data's dose per fraction

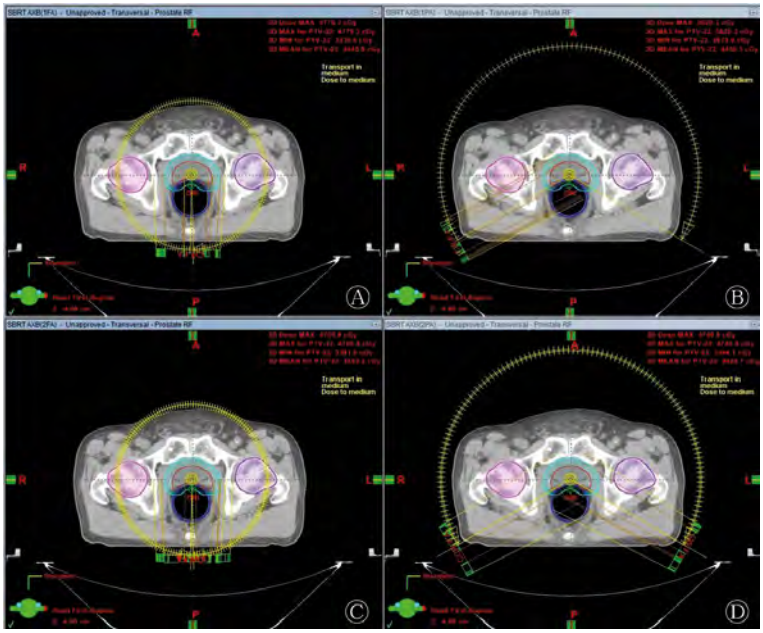

FIGURE 1. An arrangement of **(A)** one full arc (1FA), **(B)** one partial arc (1PA), **(C)** two full arcs (2FA), and **(D)** two partial arcs (2PA) in prostate SBRT-VMAT for the same patient.

parameters come from data reported in previous study[28] and were suitably modified for our study.

Results

Figure 2A to 2D show the dose distributions achieved with four arc arrangements for the same patient. There were small differences in the dose distributions and corresponding DVHs among each arc arrangement. Average cumulated DVHs of the PTV, rectum, bladder, and left and right femoral head are presented in Figure 3A to 3E for each of the four arc arrangements.

Tables 3 and 4 show the average (range) values for the dosimetric results of target volumes and several OARs (*i.e.*, bladder, rectum, and left and right femoral head). The resulting PTV and CTV of the four arc arrangements of all patients were very similar as shown in Table 3 and Figure 3A. In four arc arrangements, the volume of the CTVs that received 100% of prescription dose was greater than 97.6% (range 96.7–98.4%), which indicated very good coverage the CTV in the VMAT plan. All plans were highly conformal with CI < 1.05 and CN = 0.91, and the doses were homogeneous (HI = 0.09±0.12).

The doses to bladder tissue showed no difference among all arc arrangements. However, there were significant differences in the doses of the rectum, left and right femoral head (Table 4, Figure 3 A, C, D, E). Compared to 1FA and 2FA, 1PA and 2PA arrangements resulted in a significant reduction of the mean dose ($V_{50\%}$) of the rectum. These arc arrangements resulted in a significant increase in the near-to-maximum dose $D_{2\%}$ and mean dose of the left and right femoral head.

Table 5 summarizes the MUs and delivery time for the prostate SBRT plans using the four arc arrangements. The average MU was 1575 ± 63, 1607 ± 56, 1646 ± 97, and 1660 ± 98 for plan using 1FA, 1PA, 2FA, and 2PA, respectively. Plans using 1FA required lower MUs than those using other arc arrangements. The average delivery time was 1.01 ± 0.02, 0.69 ± 0.01, 2.00 ± 0.01, and 1.36 ± 0.02 for 1FA, 1PA, 2FA, and 2PA, respectively. The ratio of delivery time was 1.46, 2.90, and 1.97 for 1FA, 2FA, and 2PA compared to 1PA.

The average TCP of prostate tumors and the average NTCP of OARs in the four arc arrangement plans are shown in Table 6. TCP and NTCP values were comparable for all arc arrangements.

Discussion

Much has been published regarding the use of VMAT in prostate cancer, but little regarding the use of SBRT using VMAT for prostate cancer. Most of the previous studies for prostate SBRT employed the CK technique. The CK technique is an

accurate image guided method for delivering radiation to a precisely targeted area using multiple nonisocentric beams with steep surrounding dose gradient.[16,29]

In the previous study reported by Chow and Jiang[30], the dosimetry and radiobiological model variation was investigated in prostate VMAT plans using the single- and double-full arc technique. The authors reported that the double-arc technique could lower the dose-volume criteria of the rectum and bladder but increase the rectal NTCP.

In addition to the study above, we included the single and double-partial arc (1PA and 2PA), which avoided irradiation of the rectum for the optimal prostate SBRT-VMAT plan. This study focused on arc arrangements as a key preparatory step in facilitating clinical planning studies.

The prostate SBRT-VMAT plans for all arc arrangements generated conformal dose distributions for target volumes (PTV and CTV). We found that the dose distributions in the anterior and posterior direction were lower when the partial arc arrangements (1PA and 2PA) were used instead of the full arc (1FA and 2FA). In contrast, the dose distributions in the left and right direction for full arc arrangements were lower than those of the partial arc (Figure 2). This was due to the application of arc angles, which avoided the direct irradiation of the rectum.

With respect to the dose to the OARs, the effect of the dose difference on bladder tissue was negligible for the different arc arrangements. The differences between the partial and full arc arrangements were observed in several dose-volume criteria (*e.g.*, $V_{20\%}$ and $V_{50\%}$) and were slightly lower in plans using the partial arc arrangements than the full arc arrangements. For the rectum, the partial arc arrangements showed relatively strong reductions of the mean dose compared to the full arc arrangements while all plans using four arc arrangements showed no dramatic differences in the high and low doses. For the left and right femoral head in Table 3, Figures 3D,E, we found that the $D_{2\%}$ and mean dose were always lower when the full arc arrangements were used compared to the partial arc arrangements. We also observed better sparing in the right femoral head compared to the left femoral head.

The clinical delivery time of prostate SBRT-VMAT plans using the one arc arrangements (1FA and 1PA) was approximately half compared to that of the two arc arrangements (2FA and 2PA). The reduced delivery time by using one arc arrangements has the potential to decrease the effects of intrafractional motion because prostate motion is

TABLE 3. Dosimetric results for target volumes in prostate SABR-VMAT plans using four arc arrangements

	Avg ±SD (range)			
	1FA	1PA	2FA	2PA
CTV coverage				
$D_{50\%}$ (Gy)	44.9±0.4 (44.4-45.4)	44.6±0.5 (44.1-45.1)	44.5±0.5 (44.0-45.3)	44.4±0.3 (44.0-44.7)
$D_{5\%}$ (Gy)	46.0±0.1 (45.9-46.1)	45.9±0.1 (45.7-46.0)	45.9±0.1 (45.8-46.0)	45.8±0.2 (45.7-45.9)
$D_{95\%}$ (Gy)	43.5±0.5 (43.0-44.2)	43.1±0.4 (42.8-43.5)	43.1±0.6 (42.6-43.9)	42.8±0.2 (42.7-43.1)
$V_{100\%}$ (%)	98.4±1.8 (95.5-100.0)	97.9±0.0 (95.0-99.8)	97.8±3.8 (95.2-100.0)	97.6±2.0 (94.2-99.9)
PTV coverage				
D_{mean}	44.3±0.2 (44.2-44.6)	44.2±0.1 (44.0-44.4)	44.1±0.3 (44.0-44.6)	44.3±0.1 (44.2-44.5)
D_{max}	48.8±0.8 (47.8-49.8)	49.5±0.9 (48.4-50.5)	48.5±1.1 (47.2-50.0)	49.0±1.4 (47.4-50.5)
Dmin	33.0±0.4 (32.4-33.2)	32.3±0.0 (31.9-33.7)	32.8±0.8 (32.1-33.9)	33.7±0.3 (33.4-34.1)
$D_{50\%}$ (Gy)	44.6±0.3 (44.2-45.0)	44.5±0.2 (44.3-44.8)	44.4±0.4 (44.1-45.0)	44.5±0.2 (44.3-44.8)
$D_{5\%}$ (Gy)	46.1±0.2 (45.9-46.3)	46.1±0.1 (45.9-46.2)	46.1±0.2 (45.9-46.3)	46.0±0.1 (45.9-46.2)
$D_{95\%}$ (Gy)	41.5±0.3 (41.1-41.8)	41.4±0.2 (41.2-41.8)	41.3±0.3 (41.0-41.7)	41.7±0.1 (41.6-41.9)
$V_{100\%}$ (%)	87.5±1.9 (85.1-90.2)	86.6±1.2 (85.0-87.8)	87.7±1.5 (85.8-89.3)	88.1±1.0 (87.1-89.9)
Conformity index	1.05±0.00 (1.02-1.03)	1.04±0.01 (1.02-1.04)	1.03±0.01 (1.02-1.04)	1.05±0.01 (1.02-1.03)
Conformation number	0.91±0.01 (0.90-0.92)	0.91±0.01 (0.09-0.92)	0.91±0.01 (0.91-0.92)	0.91±0.01 (0.90-0.93)
Homogeneity index	0.10±0.01 (0.09-0.12)	0.11±0.00 (0.10-0.11)	0.11±0.01 (0.10-0.12)	0.10±0.00 (0.09-0.10)

Avg. = average; $D_{5\%}$ = the dose received at least 5% volume; D_{mean} = the mean dose; D_{max} = the maximum dose; D_{min} = the minimum dose; $D_{50\%}$ = the dose received at least 50% volume; $D_{95\%}$ = the dose received at least 95% volume; SD = the standard deviation; $V_{100\%}$ = the volume received 100% of prescription dose.

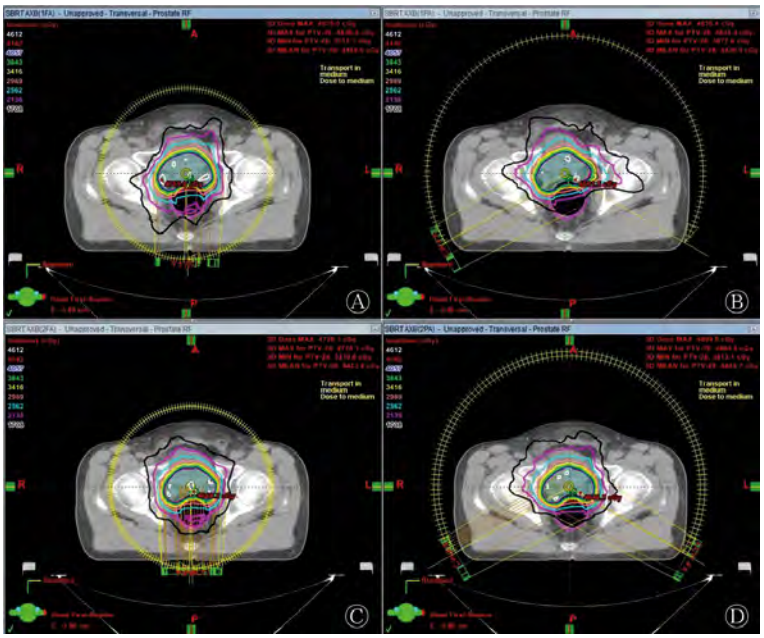

FIGURE 2. An example of the dose distributions achieved with **(A)** one full arc (1FA), **(B)** one partial arc (1PA), **(C)** two full arcs (2FA), and **(D)** two partial arcs (2PA) arrangement in prostate SBRT-VMAT for the same patient.

TABLE 4. Dosimetric results for organs at risk in prostate SABR-VMAT plans using four arc arrangements

	Avg SD (range)			
	IFA	1PA	2FA	2PA
Organs at risk				
Rectum D_{mean} (Gy)	25.2±1.7 (23.1-27.2)	23.3±2.0 (21.4-25.8)	23.6±1.8 (21.3-26.0)	23.4±1.9 (21.3-26.4)
Rectum $D_{2\%}$ (Gy)	44.2±0.3 (43.7-44.5)	44.6±0.4 (44.0-45.1)	44.1±0.4 (43.8-44.7)	44.5±0.2 (44.3-44.9)
Rectum $V_{95\%}$ (%)	9.0±1.4 (6.6-10.3)	10.1±1.3 (8.1-11.5)	8.3±1.3 (7.0-10.2)	9.8±1.5 (7.6-11.6)
Rectum $V_{80\%}$ (%)	19.1±3.0 (15.1-23.2)	19.7±3.2 (15.2-23.8)	18.0±2.8 (15.2-21.9)	19.4±3.4 (14.8-24.3)
Rectum $V_{50\%}$ (%)	71.0±8.8 (61.1-80.1)	53.9±9.4 (41.9-63.7)	60.0±11.1 (46.9-77.4)	54.1±9.0 (42.5-66.7)
Rectum $V_{20\%}$ (%)	90.2±2.5 (87.7-93.4)	90.0±2.7 (87.1-94.1)	89.6±3.0 (85.6-93.5)	90.0±2.8 (87.3-94.0)
Bladder D_{mean} (Gy)	19.0±6.9 (13.4-31.1)	18.7±7.1 (13.2-31.0)	18.5±6.1 (15.7-29.0)	18.3±6.9 (12.9-30.4)
Bladder $D_{2\%}$ (Gy)	45.6±0.2 (45.5-45.8)	45.6±0.2 (45.3-45.9)	45.5±0.3 (45.0-45.8)	45.6±0.4 (45.3-46.4)
Bladder $V_{95\%}$ (%)	21.2±11.1 (10.5-39.3)	21.4±11.1 (10.6-39.4)	20.9±10.5 (10.3-37.7)	21.4±11.3 (10.7-39.9)
Bladder $V_{80\%}$ (%)	25.6±11.9 (16.6-46.4)	26.2±11.9 (16.5-46.3)	25.7±11.1 (16.6-44.6)	26.0±12.0 (16.3-46.2)
Bladder $V_{50\%}$ (%)	40.4±13.6 (29.4-64.1)	38.6±12.6 (28.5-60.5)	39.5±12.4 (29.2-60.7)	38.2±12.9 (27.5-60.6)
Bladder $V_{20\%}$ (%)	54.4±17.0 (37.2-79.1)	52.5±16.1 (36.4-76.5)	54.1±17.4 (37.0-79.4)	52.1±16.1 (36.2-76.1)
Left femoral head D_{mean} (Gy)	12.8±1.8 (10.5-15.1)	18.7±2.3 (16.9-22.7)	12.7±2.4 (9.3-14.9)	13.9±1.4 (11.7-15.3)
Left femoral head $D_{2\%}$ (Gy)	19.7±2.2 (16.9-21.8)	23.5±4.4 (15.6-26.1)	18.3±3.0 (13.4-20.7)	20.1±2.0 (16.7-21.8)
Right femoral head D_{mean} (Gy)	10.8±0.9 (9.2-11.3)	13.3±2.5 (9.2-15.5)	12.4±1.7 (10.5-14.2)	13.7±2.1 (10.7-16.5)
Right femoral head $D_{2\%}$ (Gy)	16.4±1.3 (14.3-18.0)	20.6±3.7 (14.3-23.6)	18.1±1.9 (15.8-20.1)	19.7±2.3 (16.2-22.5)

Avg. = average; $D_{5\%}$ = the dose received at least 5% volume; D_{mean} = the mean dose; D_{max} = the maximum dose; D_{min} = the minimum dose; $D_{50\%}$ = the dose received at least 50% volume; $D_{95\%}$ = the dose received at least 95% volume; SD = the standard deviation; $V_{100\%}$ = the volume received 100% of prescription dose.

TABLE 5. Average monitor unit and delivery time for prostate SBRT-VMAT plans using four arc arrangements

Beams	1FA (Mean ± SD)	1PA (Mean ± SD)	2FA (Mean ± SD)	2PA (Mean ± SD)
MU	1575±63	1627±56	1646±97	1660±98
Delivery time (min)	1.01±0.02	0.69±0.01	2.00±0.01	1.36±0.02

TABLE 6. TCP of prostate tumor and NTCP of OARs for four arc arrangement plans

Beams	1FA (Mean±SD)	1PA (Mean±SD)	2FA (Mean±SD)	2PA (Mean±SD)
TCP (%)				
Prostate	93.35±0.08	99.28±0.06	93.32±0.08	93.36±0.08
NTCP (%)				
Rectum	0.44±0.23	0.61±0.21	0.40±0.23	0.56±0.22
Bladder	0.01±0.02	0.01±0.03	0.00±0.01	0.00±0.02
LT Femur	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
RT Femur	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

time dependent. The delivery time for partial arc and full arc in single or double arc arrangements was similar because the delivery duration is limited by the gantry rotation speed and leaf speed, not the dose rate.

The MUs were observed to significantly increase with the number of arcs used in an SBRT-VMAT plan. The MU value of the 2FA and 2PA was up to 5% greater than that of the 1FA. This was in line with the previous study[20], which reported the incensement of MUs in plans using double arcs compared to those with single arcs. However, the plan using 1FA for this study required lower MUs than the plan using 1PA. The ratio of delivery time for 1FA, 2FA, and 2PA compared to 1PA was between 1.5 and 3. The use of 1PA appeared to reduce the treatment delivery time, which has obvious benefits for SBRT.

In this study, we compared the radiobiological impact among four arc arrangements within SBRT-VMAT plans for prostate cancer. There were no obvious differences in the TPC and the NTCP for plans using four arc arrangements, excluding the NTCP value of the rectum. The NTCP difference of rectum was also small (within 0.2%) among the four arc arrangements as shown in Table 6. These mean that radiobiological outcomes have no difference relative to four arc arrangements. Furthermore, the TCP increase/decrease was correlated with D_{mean}, which is related to the mean dose. The mean dose of PTV for four arc arrangements showed no difference (Table 3). The NTCP of the rectum for the partial arc arrangements (1PA and 2PA) was higher than that of the full arc arrangements (1FA and 2FA). The reason for this is that the rectum includes the high-dose region with higher mean and maximum doses in plans using partial arc arrangements. Nevertheless, such an increased NTCP of the rectum is still within the acceptable range.

Limitation of this study is that there are no definitive clinical data on short- and long-term outcomes. This study focused mainly on the investigation of optimal treatment planning in prostate SBRT-VMAT. Therefore, future follow-up studies are required to evaluate the clinical outcome and toxicity for practice application of this technique.

Conclusions

Prostate SBRT-VMAT plans using four arc (1FA, 1PA, 2FA, and 2PA) arrangements offered high conformity for target volumes. This study demonstrated that prostate SBRT-VMAT using 1PA (240°

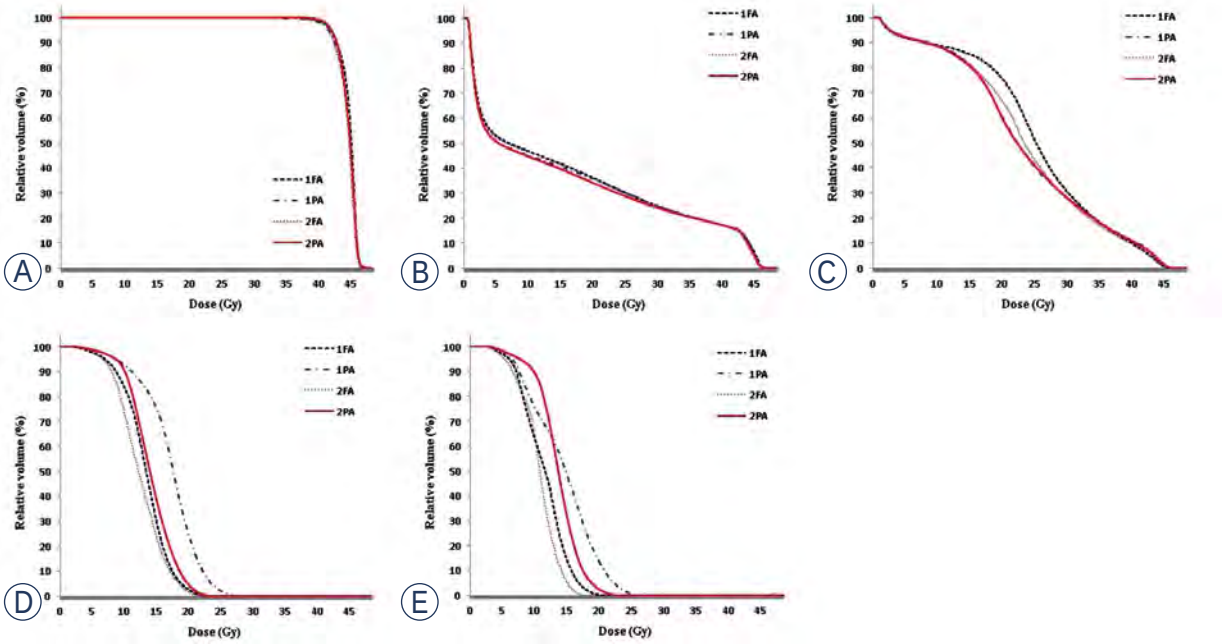

FIGURE 3. Average dose-volume histograms of the **(A)** PTV, **(B)** bladder, **(C)** rectum, **(D)** left femoral head, and **(E)** right femoral head from prostate SBRT-VMAT plans for four arc arrangements (1FA, 1PA, 2FA, and 2PA).

to 120°) showed reasonably fast delivery time and produced equivalent target coverage and better rectum sparing, although the near-to-maximum dose and mean dose of the left and right femoral heads increased slightly. However, the doses in both femoral heads remained well within the clinical normal tissue tolerance. For evaluating the radiobiological metrics, all plans using four arc arrangements produced comparable TCP for prostate tumors and NTCP for OARs. Therefore, it was concluded that the use of 1PA was an attractive choice for treating prostate cancer using SBRT-VMAT.

Author Contribution

Conception and design of the study: JBC TSS. Coordination of the study: SWK JSK JWL. Case selection, radiation treatment planning, and analysis: KYE CHS JSK JYK JBC. Manuscript preparation: JBC SWK. Manuscript revision and editing: JBC JSK IAK TSS. Manuscript approval: all authors.

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Primarni pljučni horiokarcinom

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Izhodišča. Primarni pljučni horiokarcinom je redek in visoko maligen pljučni tumor s slabo napovedjo poteka bolezni. Namen raziskave je bil ugotoviti, ali obstajajo različne klinične oblike primarnega pljučnega horiokarcinoma, ki zahtevajo različen diagnostični pristop in primernejšo izbiro zdravljenja.

Bolniki in metode. S pomočjo iskalnika PubMed smo naredili pregled literature predhodno objavljenih primerov primarnega pljučnega horiokarcinoma. Vključitveni dejavniki za raziskavo so bili bolniki, ki so imeli histološko potrjeno diagnozo pljučnega horiokarcinoma in temeljito preiskavo reproduktivnih organov za izključitev morebitnega horiokarcinoma v gonadah. Predstavili smo tudi bolnika zdravljenega v naši ustanovi.

Rezultati. Vključili smo 55 primerov (17 moških) s srednjo starostjo 34 let. Ženske z anamnezo gestacijskega dogodka so imele daljšo dobo preživetja kot ženske brez gestacijskega dogodka. Bolniki, zdravljeni s kombinacijo kirurgije in kemoterapije, so imeli daljšo dobo preživetja kot bolniki, ki niso bili zdravljeni s kombinacijo kirurgije in kemoterapije. Z multivariatno analizo smo prikazali, da je zdravljenje s kombinacijo kirurgije in kemoterapije neodvisni napovedni dejavnik. Z univariatno in multivariatno analizo pa smo prikazali, da ima velikost tumorja statistično pomemben vpliv na izid bolezni.

Zaključki. Primarni pljučni horiokarcinom je izjemno redka bolezen z različnimi kliničnimi oblikami in izidom. Pomembno je, da bolnike zajamemo in začnemo zdraviti v zgodnjih fazah bolezni. Ženske z anamnezo gestacijskega dogodka imajo daljšo dobo preživetja, kar nam lahko s pomočjo genetske analize pomaga oceniti napoved poteka bolezni pri bolnicah. Kirurško zdravljenje z adjuvantno kemoterapijo predstavlja najboljšo izbiro zdravljenja primarnega pljučnega horiokarcinoma.

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PET/CT slikovna tehnika pri revmatični polimialgiji: predsramnično kopičenje z ^{18}F označene fluorodeoksiglukoze je povezano z entezitisi in tenosinovitisi mišic grebenke in dolge pritezalke stegna

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Izhodišča. V diagnostiki revmatične polimialgije (RPM), ki velja za eno najpogostejših revmatičnih bolezni, ima PET/CT s ^{18}F označeno fluorodeoksiglukozo (^{18}F-FDG PET/CT) vse večjo vlogo. Poleg kopičenj ^{18}F-FDG na drugih lokacijah se pri nekaterih bolnikijh povečano kopiči ^{18}F-FDG tudi v predsramničnem področju. Pri tem pa povečano predsramnično kopičenje ^{18}F-FDG še vedno ni natančno opisano predvsem pa ne patofiziološko razloženo. Namen naše raziskave je bil dokazati zmanjšano predsramnično kopičenje ^{18}F-FDG kot odgovor na zdravljenje pri bolnikih z RP ter opisati potencialne povezave z drugimi ^{18}F-FDG PET/CT značilnostmi. Poleg tega pa smo želeli osvetliti patološki vidik predsramničnega kopičenja ^{18}F-FDG.

Bolniki in metode. V retrospektivno študiji smo pregledali bolnike s RPM obravnavane med feburajem 2010 in marcem 2016. Tiste bolnike, pri katerih smo ugotovili predsramnično kopičenje ^{18}F-FDG smo natančno analizirali vizualno in semi-kvantitativno z določanjem razmerja med predsramničnim kopičenjem in kopičenjem v jetrih. Pri teh bolnikih smo sistematično smo opisali tudi kopičenje ^{18}F-FDG v ostalih področjih (ramenskem, kolčnem in sternoklavikularnem sklepu, sedničnozadnjični burzi in medtrnastimi prostori).

Rezultati. Pri triindvajsetih od 89 pregledanih bolnikov (26 %) je bilo vidno predsramnično kopičenje ^{18}F-FDG. Pri petnajstih od 23 bolnikov smo naredili tudi dodatno ^{18}F-FDG PET/CT preiskavo med samim spremljanjem poteka bolezni. Pri petih od petnajstih bolnikov je bilo vidno kopičenje ^{18}F-FDG tudi v velikih arterijah, kot znak velikoceličnega arteritisa. Med ^{18}F-FDG PET/CT preiskavo ob spremeljanju petnajstih bolnikov smo, kot posledica zdravljenja, ugotovili zmanjšano kopičenje ^{18}F-FDG v vseh prvotno pozitivnih področjih. Poleg tega nismo našli novih kopičenj ob sklepih ali zunaj njih ali v tarčnih velikih žilah. Pri vseh petnajstih biolnikih se je po zdravljanju zmanjšalo tudi predsramnično kopičenje ^{18}F-FDG.

Zaključki. Povečano predsramnično kopičenje ^{18}F-FDG pri bolnikih s RPM je pogosto in ga je potrebno med diagnostičnim postopkom revmatičnih ali malignih obolenj sistematično proučiti. Predsramnična vnetja so verjetno v povezana z entesitisom in tenosinovitisom grebenske mišice in dolge pritezalke stegna pred samo sramno zrastjo.

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Ehokardiografija in srčni biološki označevalci pri bolnikih z nedrobnoceličnim pljučnim rakom zdravljenih s kemoterapijo na osnovi platine

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Izhodišča. Nedrobnocelični pljučni rak (NSCLC) je v svetu eden izmed glavnih razlogov za smrt. Namen raziskave je bil oceniti kardiotoksičnost pri bolnikih z NSCLC zdravljenih s kemoterapijo na osnovi platine ter ugotoviti, kateri bolniki imajo večje tveganje za nastanek kardiotoksičnosti.

Bolniki in metode. V prospektivno, opazovalno raziskavo smo vključili bolnike z začetnim in napredovalim NSCLC, ki smo jih zdravili s kemoterapijo na osnovi platine. Pri bolnikih smo ugotavljali kardiotoksičnost, ki smo jo opredelili kot zvišanje ultrasenzitivnega troponina T, N-terminalnega natriuretičnega propeptida tipa B ali zmanjšanje iztisnega deleža levega prekata (LVEF) pred in po zaključku kemoterapije ter 6 mesecev po zaključku kemoterapije.

Rezultati. Vključili smo 41 bolnikov (povprečna starost 61 let ± 9 let, 54 % moških, 68 % z napredovalo boleznijo) z NSCLC zdravljenih s kemoterapijo na osnovi platine (v povprečju s 4 krogi). Med raziskavo so umrli 3 bolniki, pri nobenem od njih nismo ugotovili srčnega popuščanja. Povprečne vrednosti bioloških označevalcev in LVEF se niso razlikovale ($p > 0,2$). Kljub temu pa je 10 bolnikov (25 %) imelo kardiotoksičnost, ki je bila neodvisno povezana z znano ishemično boleznijo srca ($p = 0.026$).

Zaključki. Pri bolnikih z NSCLC zdravljenih s kemoterapijo na osnovi platine, ki imajo ishemično bolezen srca in pri tistih z dolgo pričakovano življenjsko dobo, bi bilo smiselno razmisliti o kardiološki obravnavi in modifikaciji življenjskega sloga.

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Diagnostika in staging tumorjev želodca – doprinos difuzijsko obremenjenega slikanja z magnetno resonanco in primerjava z računalniško tomografsko preiskavo

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Izhodišča. Primerjava diagnostične učinkovitosti magnetnoresonančnega difuzijskega slikanja *(angl. Diffusion-Weighted Imaging – DWI)* in računalniške tomografije (*angl.* CT) pri določanju TNM stadija raka želodca.

Bolniki in metode. Pri 51 bolnikih z rakom želodca smo predoperativno uporabili T2 obtežene slike in DWI (b-0,400 in b--800s/mm2). Opravili smo tudi CT slikanje. Iskali smo zvišanje signala na sekvencah DWI. Ocenjevali smo globino vraščanja tumorja (T), prizadetost regionalnih bezgavk (N) in prisotnost ali odsotnost oddaljenih metastaz (M) na sekvencah DWI in CT preiskavi. Izračunali smo občutljivost, specifičnost, pozitivno in negativno napovedno vrednost DWI in CT preiskave v primerjavi s patološkim izvidom kirurgije, ki velja za zlati standard. Ujemanje smo ocenili s koeficientom ujemanja Kapa.

Rezultati. Senzitivnost in specifičnost preiskavav DWI in CT pri ocenjevanju prizadetosti regionalnih bezgavk sta bili sledeči: N1: DWI: 75 %, 84,6 %; CT: 66,7 %, 82 %; N2: DWI: 79,3 %, 77,3 %; CT 69%, 68,2 %; N3: DWI: 60%, 97,6 %; CT 50 %, 90.2 %. DWI slikanje se je pri ocenjevanju regionalnih bezgavk izkazalo za bolj primerljivo z zlatim standardom (kirurškim patološkim izvidom) kot CT preiskava. Pri ocenjevanju globine vraščanja tumorja (T) so bili rezultati preiskav DWI in CT boljši kot zlati standard pri višjem stadiju T, vendar se DWI ni izkazal za boljšega od CT preiskave. Pri določanju oddaljenih metastaz (M) sta senzitivnost in specifičnost obeh preiskovalnih metod bili 100 %.

Zaključki. Diagnostična natančnost slikanja DWI pri TNM klasifikaciji raka želodca je primerljiva s CT slikanjem in dodatna uporaba DWI pri rutinskem protokolu lahko izboljša diagnostično natančnost kadar ocenjujemo metastatsko spremenjene bezgavke.

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Elektroprenos plazmidne DNA radiosenzibilzira tumorje B16F10 preko aktivacije imunskega odgovora

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Izhodišča. Obsevanje tumorjev v kombinaciji z različnimi žilno ciljanimi ali imunskimi dodatnimi zdravili je predmet številnih sodobnih raziskav. Genski elektroprenos terapevtskih plazmidov je eden izmed primernih pristopov. V predstavljeni raziskavi nas je zanimalo, ali lahko z genskim elektroprenosom plazmidne DNA z zapisom za shRNA za utišanje endoglina radiosenzibiliziramo tumorje melanoma B16F10.

Materiali in metode. Poskuse smo izvedli na mišjem melanomskem tumorskem modelu B16F10. Tumorje smo inducirali na hrbtu miši C57Bl/6 in jih zdravili s trikratnim genskim elektroprenosom terapevtskih plazmidov in obsevanjem. Protitumorsko učinkovitost smo ovrednotili z določanjem zaostanka v rasti tumorjev in števila popolnih odgovorov. Dodatno smo za določanje mehanizmov delovanja izvedli še histološko analizo tumorjev (določili smo nekrozo, apoptozo, proliferacijo, ožiljenje, prisotnost hipoksije in infiltracijo imunskih celic).

Rezultati. Rezultati raziskave so pokazali, da ima genski elektroprenos plazmida za utišanje endoglina predvsem žilno ciljan učinek; zdravljenje je povzročilo značilen zaostanek v rasti tumorjev in 44 % pozdravljenih miši. Obsevanje je imelo manjši učinek na ta radiorezistenten melanomski model, saj smo zabeležili le 11% ozdravitev. Kombinirana terapija pa je bila zelo uspešna; zabeležili smo kar 88 % ozdravljenih miši, podoben odziv pa smo opazili tudi po kombinirani terapiji s plazmidom brez terapevtskega gena. Histološka analiza tumorjev po kombinirani terapiji je pokazala podoben mehanizem delovanja plazmida za utišanje endoglina in plazmida brez terapevtskega gena, namreč preko indukcije imunskega odziva.

Zaključki. Rezultati naše raziskave nakazujejo, da lahko obsevanje pri sicer radiorezistentem melanomskem tumorskem modelu deluje kot aktivator imunskega odziva proti tumorskim antigenom sproščenim iz obsevanih celic. Ta aktiviran protitumorski imunski odgovor lahko dodatno pospešimo z genskim elektroprenosom plazmidov z ali celo brez terapevtskega gena, kar smo potrdili z visoko radisenzibilizacijo, ki se je kazala v 88 % popolnih ozdravitev in zaostanku v rasti tumorjev. Poleg tega ima genski elektroprenos terapevtskega plazmida za utišanje endoglina tudi direkten učinek na tumorsko žilje in tumorske celice, ki pa po kombinirani terapiji z obsevanjem ni bil viden, saj je bil zakrit z močnim imunskim odzivom.

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Primerjava krioablacije in ireverzibilne elektroporacije jeter v zajcih neposredno ob žolčniku

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Izhodišča. Ablacija jetrnih tumorjev v bližini žolčnika lahko povzroči komplikacije. Namen študije je bil primerjati učinkovitost in varnost ablacije jeter v bližini žolčnika s krioablacijo in ireverzibilno elektroporacijo.

Materiali in metode. Krioablacijo in ireverzibilno elektroporacijo smo naredili na 12 novozelandskih zajcih, na jetrih v bližini žolčnika, tako da je bil zajet tudi ta organ. Spremljali smo serumske aminotrasferaze, pred in po terapiji. Tkivne poškodbe smo spremljali histopatološko v ablativni coni, 7. dan po terapiji.

Rezultati. Po 7. dneh so bile vse živali žive. Ireverzibilna elektroporacija ni povzročila perforacije žolčnika, le nekrozo mukoze in edem. Nasprotno je krioablacija povzročila perforacijo žolčnika pri 4. zajcih. Zabeležili smo povišane serumske aminotransferase 3. dan po terapiji tako pri ireverzibilni elektroporaciji, kot pri krioablaciji, vendar so za tem upadle. Povišanje aminotrasnferaz in bilirubina je bilo večje po krioablaciji kot po ireverzibilni elektroporaciji. Obe metodi ablacije sta povzročili nekrozo jetrnega parenhima od ablacijskega centra proti žolčniku. Kriobalacije je povzročila nekrozo stene žolčnika, medtem ko ireverzibilna elektroporacije tega ni povzročila, le granulacijo in hiperplazijo smo opazili.

Zaključki. Za ablacijo jetrnega tkiva v bližini žolčnika je primernejša ireverzibilna elektroporacija kot krioablacija, saj je varnejša, ker ne povzroča perforacije žolčnika in je učinkovitejša, sa povzroči popolno nekrozo s hitro regeneracijo tkiva.

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Breme raka v Sloveniji z oceno časovnih trendov

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Izhodišče. Z raziskavo smo želeli opisati breme raka v Sloveniji in oceniti časovne trende za vse rake skupaj, kot tudi za posamezne pogoste lokacije in nekatere redke rake.

Bolniki in metode. Osnovni vir podatkov je bil populacijski Register raka Republike Slovenije. Breme raka je predstavljeno z incidenco in prevalenco za obdobje 1950–2013 ter z umrljivostjo za obdobje 1985–2013. Pri časovnih trendih smo ocenjevali delež letne spremembe z Jointpoint regresijsko analizo. Za oceno incidence leta 2016 smo uporabili metodo po Dybi in Hakulinenu, za projekcijo števila novo zbolelih v letu 2025 pa program Globocan.

Rezultati. V zadnjem obdobju v Sloveniji letno za rakom zboli okrog 14.000 ljudi, nekaj več kot 6.000 jih umre, med nami pa živi 94.000 ljudi, ki so bili že obravnavani zaradi rakave bolezni. Pet najpogostejši rakov pri nas – nemelanomski kožni rak, raki debelega črevesa in danke, pljučni rak, rak dojke in rak prostate – obsega skoraj 60 % vseh novih primerov rakavih bolezni. Incidenca pogostih rakov se veča povprečno letno za 3,0 %, medtem ko je incidenca redkih rakov stabilna.

Zaključek. Rak je bolezen starejših in ker se številčna povojna generacija hitro stara, lahko pričakujemo, da bo breme raka v Sloveniji raslo še naprej, kljub pričakovanim uspehom primarne in sekundarne preventive.

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Leiomiosarkom ledvične vene. Analiza preživetja in napovednih dejavnikov do sedaj objavljenih 67 primerov bolnikov

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Izhodišča. Leiomiosarkom je redek maligen mezenhimski tumor. V svetovni literaturi so opisali več primerov leiomiosarkoma ledvične vene, analiza preživetja in napovednih dejavnikov pa še ni bila opravljena. Namen raziskave je bil opisati leiomiosarkom ledvične vene ter glede na dosedanje objave primerov opredeliti celokupno preživetje, preživetje brez lokalne ponovitve bolezni, preživetje brez oddaljene ponovitve bolezni in napovedne dejavnike zanje.

Bolniki in metode. Objavljenim primerom leiomiosarkoma ledvične vene iz literature smo dodali naš primer.

Rezultati. Zbrali smo 67 primerov bolnikov, srednja starost je bila 56,6 let, 76,1 % je bilo žensk. Srednja velikost tumorja je bila 8,9 cm, pri 68,7 % bolnikov je bil tumor na levi strani. Tumorski trombus se je širil v lumen spodnje votle vene pri 13,4 %. Operirani so bili vsi bolniki razen enega (98,5 %). Celokupno preživetje je bilo 79,5 %, srednji čas sledenja 24 mesecev. Preživetje brez lokalne ponovitve bolezni je bilo 83,5 %, srednji čas sledenja 21,5 meseca, preživetje brez oddaljene ponovitve bolezni pa je bilo 76,1 %, srednji čas sledenja 22 mesecev. V univariatni analizi je bil napovedni dejavnik za celokupno preživetje status kirurških robov, za preživetje brez lokalne ponovitve bolezni pa širjenje tumorja v lumen spodnje votle vene in gradus. Napovednih dejavnikov za preživetje brez oddaljene ponovitve bolezni nismo uspeli opredeliti. V multivariatni analizi nismo uspeli opredeliti napovednih dejavnikov za nobenega od parametrov.

Zaključki. Leiomiosarkom ledvične vene zraste običajno v hilusu ledvice. Nanj naj bi pomislili v diferencialni diagnozi ledvičnih in retroperitonealnih tumorjev, še posebaj pri ženskah starejših od 40 let, če je tumor na levi strani in ob odsotnosti hematurije. Opraviti moramo histološko biopsijo tumorja. Bolniki naj bodo napoteni v obravnavo k multidisciplinarnemu timu za sarkome. Tumor naj bo odstranjen z negativnimi kirurškimi robovi.

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Napovedni pomen vrednosti uPA/PAI-1, statusa HER2 in tradicionalnih histoloških dejavnikov za preživetje pri bolnicah z rakom dojk brez zasevkov v bezgavkah

Fokter Dovnik N, Takač I

Izhodišča. Urokinazni aktivator plazminogena (uPA) in inhibitor aktivatorja plazminogena 1 (PAI-1) sta povezana s statusom HER2, zaradi česar se postavlja vprašanje, ali nosi vrednost uPA/PAI-1 dodatno klinično pomembno napovedno informacijo neodvisno od statusa HER2. Namen naše raziskave je bil primerjati napovedno moč vrednosti uPA/PAI-1, statusa HER2 in tradicionalnih napovednih dejavnikov za preživetje pri bolnicah z rakom dojk brez zasevkov v bezgavkah.

Bolniki in metode. Opravili smo retrospektivno analizo 858 bolnic z rakom dojk brez zasevkov v bezgavkah, zdravljenih v Univerzitetnem kliničnem centru Maribor v letih 2000-2009. Podatke smo pridobili iz zdravstvene dokumentacije bolnic. Srednji čas sledenja je bil 100 mesecev. Univariatne in multivariatne analize preživetja brez ponovitve bolezni in celokupnega preživetja smo opravili s pomočjo Coxove regresije in Coxovega modela sorazmernih tveganj.

Rezultati. V univariatnih analizah so bili s preživetjem brez ponovitve bolezni povezani starost, velikost tumorja, diferenciacija, limfovaskularna invazija, status HER2 in vrednost uPA/PAI-1, s celokupnim preživetjem pa starost, velikost tumorja, diferenciacija in vrednost uPA/PAI-1. V multivariatnem modelu so bili s preživetjem brez ponovitve bolezni najbolj povezani dejavniki starost, status estrogenskih receptorjev in vrednost uPA/PAI-1, s celokupnim preživetjem pa starost bolnic in diferenciacija tumorja. V primerjavi z bolnicami z nizkim uPA in PAI-1 so imele bolnice z visoko eno ali obema vrednostma razmerje tveganj za smrt iz kateregakoli razloga v multivariatnem modelu 1,98 (95 % interval zaupanja 0,83–4,76).

Zaključki. Vrednost uPA/PAI-1 ima pri bolnicah z rakom dojk brez zasevkov v bezgavkah jasno napovedno vrednost ne glede na status HER2, zato bi jo pri teh bolnicah lahko uporabljali kot dodaten napovedni dejavnik pri odločanju o dopolnilnem sistemskem zdravljenju.

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Varnost in učinkovitost, superabsorbentnih polimernih kroglic eluiranih z doksirubicinom za zdravljenje jetrnih zasevkov nevroendokrinih tumorjev. Preliminarni rezultati

Bonne L, Verslype C, Laenen A, Cornelissen S, Deroose C, Prenen H, Vandecaveye V, Van Cutsem E, Maleux G

Izhodišča. Namen retrospektivne raziskave je bil oceniti nadzor nad simptomi, tumorski odgovor na zdravljenje in stopnjo zapletov pri bolnikih z metastatskimi nevroendokrinimi tumorji jeter. Bolnike smo zdravili s transarterijsko kemoembolizacijo in uporabo superabsorbentnih polimernih (SAP) mikrokroglic, eluiranih z doksorubicinom.

Bolniki in metode. V raziskavo smo vključili bolnike z jetrnimi metastazami nevroendokrinih tumorjev, ki smo jih zdravljeni s transarterijsko kemoembolizacijo in uporabo mikrokroglic SAP (delci 50–100 um Hepasphere / Quadrasphere Microsphere®, Merit Medical, South Jordan, Utah, ZDA), eludiranih z doksirubicinom. Bolnike smo slikali pred in po postopku zdravljenja, tako smo ocenjevali kratkotrajni in dolgoročni odgovor tumorjev z uporabo kriterijev RECIST. Ovrednotili smo stopnjo zmanjšanja simptomov in zaplete povezane z zdravljenjem.

Rezultati. Skupno 27 postopkov embolizacije smo izvedli pri 17 bolnikih. Dvanajst od 17 bolnikov (70 %) je bilo simptomatskih, vključno s karcinoidnim sindromom (n = 8) in hudo, neobvladljivo hiperglikemijo (n = 4). Pri 8 od 12 bolnikov (67 %) smo ugotovili popolno izginotje simptomov, pri ostalih 4 (33 %) pa delno izboljšanje. En bolnik (6 %) je razvil ishemični holecistitis, sicer pa nismo zaznali nobenih drugih hepatobiliarnih zapletov. Kratkoročno sledenje bolnikov (> 3 mesece) s pomočjo slikanja je bilo na voljo pri 15 bolnikih in je pokazalo delni odgovor pri 14 bolnikih, pri enem bolniku pa napredovanje bolezni. Srednjeročno sledenje bolnikom (< 3 mesece) je bilo na voljo pri 12 bolnikih. Pri 7 bolnikoh (58 %) smo našli delen odgovor na zdravljenje, pri 3 (25 %) stabilno stanje bolezni in pri 2 (17 %) napredovanje bolezni.

Zaključki. Kemoembolizacija s kroglicami SAP, eluiranimi z doksorubicinom, je varna in učinkovita metoda za zdravljenje jetrnih metastaz neuroendokrinih tumorjev in ima malo zapletov. Pomembno je, da klinično ni bilo zaslediti zapletov, kot so nekroza jeter ali žočnih vodov.

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Dolgoročni izidi visokodoznega zdravljenja in transplantacije krvotvornih matičnih celic pri zdravljenju folikularnega limfoma in limfoma plaščnih celic. Izkušnje posamičnega centra

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Izhodišča. Napredovali folikularni limfom in limfom plaščnih celic sta neozdravljivi bolezni, če jih zdravimo konvencionalno. Visokodozno zdravljenje in transplantacija krvotvornih matičnih celic predstavljata možnost podaljšanja časa brez napredovanja bolezni ter celokupnega preživetja. V raziskavi smo ugotavljali preživetje brez dogodka in celokupno preživetje pri bolnikih s folikularnim limfomom in limfomom plaščnih celic, zdravljenih s transplantacijo.

Bolniki in metode. Analizirali smo 17 bolnikov s folikularnim limfomom in 29 bolnikov z limfomom plaščnih celic, ki smo jih zdravili s transplantacijo. 15 bolnikov je bilo v remisiji po zdravljenju drugega reda in 24 v remisiji po zdravljenju prvega reda. Vse bolnike smo zdravili z obsevanjem celega telesa in z visokim odmerkom ciklofosfamida med letoma 2006 in 2014. Vsi so s transplantacijo prejeli matične celice, ki smo jih pridobili iz periferne krvi.

Rezultati. Ocenjeno 5-letno celokupno preživetje je bilo v skupini folikularnega limfoma 87,8 % (95 % interval zaupanja [CI] 59,5–96,8 %) in v skupini limfoma plaščnih celic 79,3 % (95 % CI 56,1–91,1 %). Ocenjeno 5-letno preživetje brez dogodka je bilo v skupini folikularnega limfoma 76,0 % (95 % CI 48,0–90,3 %) in v skupini limfoma plaščnih celic 69,8 % (95 % CI 45,5–84,8 %). V času analize podatkov nismo beležili nobenega sekundarnega hematološkega obolenja.

Zaključki. Avtologna transplantacija krvotvornih matičnih celic je dobra terapevtska možnost za dolgoročna preživetja bolnikov s folikularnim limfomom in limfomom plaščnih celic z relativno nizko stopnjo poznih zapletov in sekundarnih hematoloških malignomov.

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Pogostnost pozitivnih peritonealnih izpirkov pri bolnicah z endometrijskim rakom po histeroskopiji ali abraziji maternične votline

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Izhodišča. Namen raziskave je bil primerjati pogostnost pozitivnih peritonealnih izpirkov pri bolnicah z rakom endometrija, ki smo jim v diagnostičnem postopku naredili histeroskopijo ali abrazijo maternične votline.

Metode. Opravili smo retrospektivno analizo 227 bolnic z rakom endometrija, ki smo jim naredili histeroskopijo (N = 144) ali abrazijo maternične votline (N = 83) v Univerzitetnem kliničnem centru Maribor med januarjem 2008 in decembrom 2014. V vsaki skupini smo določili pogostnost pozitivnih peritonealnih izpirkov.

Rezultati. Med obema skupinama nismo ugotavili razlik v pogostnosti pozitivnih peritonealnih izpirkov (histeroskopija 13,2 %; abrazija maternične votline 12,0 %; p = 0,803). Natančnejša analiza bolnic s stadijem I pa je razkrila statistično značilno več pozitivnih izpirkov pri bolnicah po histeroskopiji (histeroskopija 12,8 %; abrazija maternične votline 3,4 %; p = 0.046). Med temi bolnicami ni bilo razlik v histološkem tipu tumorja (hi-kvadrat = 0,059; p = 0,807), diferenciaciji tumorja (hi-kvadrat = 3,709; p = 0,156), času med diagnozo in operacijo (t = 0,930; p = 0,357) in miometrijski invaziji (hi-kvadrat = 5,073; p = 0,079).

Zaključki. Kljub temu, da v celotni populaciji ni bilo razlik v pogostnosti pozitivnih peritonealnih izpirkov po histeroskopiji ali po abraziji, smo pri bolnicah s stadijem I po histeroskopiji ugotovili statistično značilno pogostejše pozitivne peritonealne izpirke v primerjavi z abrazijo.

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Izid implantatno-protetične rehabilitacije po obsevanju zaradi raka glave in vratu

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Izhodišča. V Sloveniji je visoka pojavnost raka glave in vratu. Zdravljenje najpogosteje vključuje kombinacijo kirurške obravnave in obsevanja. Možnosti kasnejše protetične rehabilitacije smo izboljšali ob uporabi sodobnih kirurških tehnik in zobnih vsadkov. Namen prispevka je prikazati izid implantatno-protetične oskrbe obsevanih bolnikov na Univerzitetnem kliničnem centru Ljubljana.

Bolniki in metode. V raziskavi smo preverili izid zdravljenja 20 bolnikov po operaciji in obsevanju zaradi raka glave in vratu, ki smo jih oskrbeli z implantatno podprtimi protezami. Preživetje in uspešnost zobnih vsadkov smo statistično ovrednotili z metodo Kaplan-Meier, Coxovimi modeli sorazmernih tveganj in logistično regresijo.

Rezultati. 20 bolnikov je skupno prejelo 100 zobnih vsadkov. Ocenjena stopnja preživetja vsadkov je bila 96 % po 1 letu in 87 % po 5 letih. Do odpovedi vsadkov je večinoma prišlo pred obremenitvijo (91,2 %). Vsadki v presajeni kosti so imeli statistično značilno slabše preživetje. Izmed 89 obremenjenih vsadkov jih je bilo 79 (88,7 %) uspešnih, kar je pomenilo funkcionalno uporabo protez in odsotnost bolečin ali napredujoče izgube kostnine. Opazili smo statistično značilno slabšanje uspeha z napredujočo starostjo, medtem ko izbor protetičnega sidra in število vsadkov pod protezo na uspeh rehabilitacije nista imela statistično značilnega vpliva.

Zaključki. Implantatno podprte proteze so zanesljiv način protetične oskrbe po obsevanju zaradi raka glave in vratu. Bolnike je potrebno seznaniti z možnostjo zgodnje odpovedi vsadkov.

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Določitev dozimetričnih parametrov za ščiten vir ^{153}Gd pri brahiterapiji raka prostate

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Izhodišča. Intersticijska brahiterapija z rotirajočo zaščito (I-RSBT) je nedavno razvita metoda za zdravljenje raka prostate. V raziskavi smo želeli določiti dozimetrične parametre TG-43 vira ^{153}Gd za uporabo v I-RSBT.

Materiali in metode. Simulacijo vira ^{153}Gd, umeščenega znotraj igle in zaščitenega s platino in aluminijevim oknom, smo naredili s kodo Monte Carlo MCNPX. Dozimetrične parametre za ta model vira smo izračunali v skladu s poročilom TG-43: moč zračne kerme, konstanta hitrosti doze, funkcija radialne doze in 2D funkcija anizotropije z in brez ščitenja.

Rezultati. Moč zračne kerme je bila 6,71 U za neščiten vir z aktivnostjo 1 GBq. Za primere z zaščito iz platine in z aluminijevim oknom je bila ta vrednost 0,04 U in 6,19 U. Konstanta hitrosti doze za neščiten vir je bila 1,20 cGy/(hU); za vir z zaščito iz platine in z aluminijevim oknom na strani zašite 0,07 cGy/(hU); oziroma in na straneh z okni 0,96 cGy/(hU). Za te vire smo izdelali tabele za vrednosti funkcije radialne doze in anizotropije. Poleg tega smo za oceno učinka zaščite na razporeditev doze za vire z in brez zaščite izrisali izodozne krivulje.

Zaključki. Dodatek zaščite iz platine lahko močno zniža dozo na kritične organe in normalna tkiva, ki ležijo na ščiteni strani. Izračunane vrednosti za moč zračne kerme, konstante hitrosti doze, funkcije radialne doze in podatki o funkciji 2D anizotropije za neščiten in ščiten vir ^{153}Gd lahko uporabljamo v sistemih za načrtovanje obsevanj (TPS).

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Optimalna strategija načrtovanja različnih ureditev lokov pri stereotaktičnem obsevanju prostate z volumsko modulirano ločno tehniko

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Izhodišča. Cilj te raziskave je bil ugotoviti optimalno strategijo različnih ureditev lokov pri stereotaktičnem obsevanju prostate z volumsko modulirano ločno tehniko (SBRT-VMAT).

Bolniki in metode. Da bi raziskali, kako različne ureditve lokov vplivajo na dozimetrične in biološke podatke, smo SBRT-VMAT načrte za osemnajst bolnikov naredili z ureditvijo z enim polnim lokom (1PL), enim delnim lokom (1DL), dvojnim polnim lokom (2PL), in dvema delnima lokoma (2DL). Vse načrte smo izračunali s pomočjo algoritma Acuros XB. Dozimetrične in radiobiološke podatke za tarčne volumne in rizične organe smo ovrednotili iz dozno volumskih histogramov.

Rezultati. Vsi načrti so bili zelo konformni (CI <1.05, CN = 0,91) in homogeni (HI = 0,09-0,12) za tarčne volumne. Pri rizičnih organih ni bilo razlike v dozi na mehur, medtem ko je bila velika razlika v dozi na rektum in na glavico stegnenice. Načrti, ki so uporabljali 1DL in 2DL so pokazali močno zmanjšanje srednje doze na rektum v primerjavi z načrti, ki so uporabljali 1PL in 2PL. D2% in srednja doza na obe glavici stegnenice pa so bile vedno nižje v načrtih z uporabo 1PL in 2PL. Povprečna verjetnost nadzora tumorja in verjetnost zapletov normalnih tkiv so bili primerljivi v vseh načrtih z uporabo različnih ločnih ureditev.

Zaključki. Uporaba 1DL je omogočala krajši čas obsevanja ob enaki pokritosti tarče z boljšim varovanjem rektuma, čeprav so vsi načrti, ki so uporabljali štiri različne ločne ureditve, v splošnem pokazali podobne dozimetrične in biološke rezultate meritev. D2% in povprečni odmerek na glavico stegnenice se je nekoliko povečal, vendar je ostal v okviru dovoljenega odstopanja. Ta študija kaže, da je uporaba 1DL privlačna izbira za zdravljenje prostate s SBRT-VMAT.

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materialno spodbujati in poglabljati raziskovalno
dejavnost v onkologiji.

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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the first quarter of 2017

Doc. dr. Josip Cholewa Foundation for cancer research and education continues with its planned activities in the first quarter of 2017 and is commencing to prepare for the activities the whole year. Its primary focus remains the provision of grants and scholarships and other forms of financial assistance for basic, clinical and public health research in the field of oncology. An analysis of the ongoing activities in the last year was made in order to make an assessment of the impact of Foundation's activities, thus providing a basis for developing new strategies and approaches in its scope of fight against cancer.

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

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

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

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NOVA INDIKACIJA[1]

Vectibix® + FOLFIRI v 1. liniji zdravljenja bolnikov z mKRR in nemutiranim genom *RAS*

Zdravilo Vectibix® je sedaj indicirano za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mKRR) in nemutiranim genom RAS:

- v 1. liniji zdravljenja v kombinaciji s FOLFOX ali FOLFIRI
- v 2. liniji zdravljenja v kombinaciji s FOLFIRI pri bolnikih, ki so v prvi liniji zdravljenja prejemali kemoterapijo, ki je vključevala fluoropirimidin (vendar ni vključevala irinotekana)
- kot monoterapija po neuspehu shem kemoterapije, ki so vključevale fluoropirimidin, oksaliplatin in irinotekan.

Vectibix® 20 mg/ml koncentrat za raztopino za infundiranje (sterilni koncentrat) (panitumumab) – SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA
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SESTAVA ZDRAVILA: 1 ml koncentrata vsebuje 20 mg panitumumaba. 1 viala vsebuje 100 mg panitumumaba v 5 ml ali 400 mg panitumumaba v 20 ml koncentrata. **TERAPEVTSKE INDIKACIJE:** Zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mKRR) z divjim tipom *RAS* in sicer v prvi liniji zdravljenja v kombinaciji s FOLFOX ali FOLFIRI, v drugi liniji zdravljenja v kombinaciji s FOLFIRI pri bolnikih, ki so v prvi liniji zdravljenja prejemali kemoterapijo, ki je vključevala fluoropirimidin (vendar ni vključevala irinotekana), ter kot monoterapija po neuspehu shem kemoterapije, ki so vključevale fluoropirimidin, oksaliplatin in irinotekan. **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje z zdravilom Vectibix® mora nadzirati zdravnik z izkušnjami pri uporabi terapije proti raku. Pred začetkom zdravljenja z zdravilom Vectibix® mora biti potrjeno, da gre za stanje divjega tipa *RAS (KRAS* in *NRAS)*. Mutacijsko stanje mora ugotoviti izkušen laboratorij z uporabo validiranih testnih metod za detekcijo mutacij *KRAS* (eksoni 2, 3 in 4) in *NRAS* (eksoni 2, 3 in 4). Priporočeni odmerek zdravila Vectibix® je 6 mg/kg telesne mase enkrat na dva tedna. V primeru hudih (≥ 3. stopnja) dermatoloških reakcij je lahko potrebna prilagoditev odmerka zdravila Vectibix®. *Bolniki z okvaro ledvic ali jeter:* Varnost in učinkovitost zdravila Vectibix® nista raziskani. *Starejši bolniki:* Ni kliničnih podatkov, ki bi podprli prilagoditev odmerka. *Pediatrična populacija:* Zdravilo Vectibix® nima relevantne uporabe za indikacijo zdravljenja kolorektalnega raka. Zdravilo Vectibix® morate aplicirati v intravenski (i.v.) infuziji z infuzijsko črpalko. Če se pojavijo z infundiranjem povezane reakcije, je lahko potrebna upočasnitev infundiranja zdravila Vectibix®. Zdravila Vectibix® ne smete injicirati z intravenskim vbrizganjem ali v bolusu. **KONTRAINDIKACIJE:** Anamneza hude ali smrtno nevarne preobčutljivosti na zdravilno učinkovino ali katero koli pomožno snov, intersticijski pnevmonitis, pljučna fibroza. Pri bolnikih z mKRR z mutantnim *RAS* in tistih bolnikih z mKRR, pri katerih stanje *RAS* ni znano, je kontraindicirana kombinacija zdravila Vectibix® in kemoterapije, ki vključuje oksaliplatin. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** *Dermatološke reakcije in toksičnost mehkih tkiv:* Skoraj pri vseh bolnikih (približno 90%), zdravljenih z zdravilom Vectibix®, se pojavijo dermatološke reakcije, ki so farmakološki učinek, opažen pri zaviralcih EGFR. Če se pri bolniku pojavijo dermatološke reakcije 3. ali višje stopnje (po CTCAE verzija 4.0) ali dermatološke reakcije, ocenjene kot neznosne, je priporočljiva naslednja prilagoditev odmerka: *Prvi pojav kožnih simptomov ≥ 3. stopnje:* Zadržite 1 ali 2 odmerka zdravila Vectibix®. Če se po tem simptomi izboljšajo (< 3. stopnja), nadaljujte infundiranje s 100 % odmerka, ki ste ga aplicirali pred pojavom kožnih simptomov; če izboljšanja ni, prekinite uporabo zdravila Vectibix®. *Ob drugem pojavu kožnih simptomov ≥ 3. stopnje:* Zadržite 1 ali 2 odmerka zdravila Vectibix®. Če se simptomi izboljšajo (< 3. stopnja), nadaljujte infundiranje s 80 % odmerka, ki ste ga aplicirali pred pojavom kožnih simptomov; če izboljšanja ni, prekinite uporabo zdravila Vectibix®. *Ob tretjem pojavu kožnih simptomov ≥ 3. stopnje:* Zadržite 1 ali 2 odmerka zdravila Vectibix®. Če se simptomi izboljšajo (< 3. stopnja), nadaljujte infundiranje s 60 % odmerka, ki ste ga aplicirali pred pojavom kožnih simptomov; če izboljšanja ni, prekinite uporabo zdravila Vectibix®. *Ob četrtem pojavu kožnih simptomov ≥ 3. stopnje:* Prekinite uporabo. Bolnike s hudimi dermatološkimi reakcijami ali toksičnostjo mehkih tkiv ali poslabšanjem reakcij med uporabo zdravila Vectibix® morate nadzirati zaradi možnih vnetnih ali infekcijskih posledic (vključno s celulitisom in nekrotizirajočim fasciitisom) ter jim nemudoma uvesti ustrezno zdravljenje, če se pojavijo. Če se pojavi dermatološka toksičnost ali toksičnost mehkih tkiv, povezana s hudimi ali življenjsko ogrožujočimi vnetnimi ali infekcijskimi zapleti, zadržite ali prekinite zdravljenje z zdravilom Vectibix®. *Zapleti na pljučih:* V primeru akutnega nastanka ali poslabšanja pljučnih simptomov morate zdravljenje z zdravilom Vectibix® prekiniti in simptome takoj raziskati. Če diagnosticirate intersticijsko pljučno bolezen, morate zdravljenje z zdravilom Vectibix® za stalno prekiniti in bolnika ustrezno zdraviti. Pri bolnikih z anamnezo intersticijskega pnevmonitisa ali pljučne fibroze je potrebno skrbno razmisliti o koristih zdravljenja s panitumumabom v primerjavi s tveganjem za zaplete na pljučih. *Elektrolitske motnje:* Bolnike je treba pred uvedbo zdravljenja, med zdravljenjem in še 8 tednov po končanem zdravljenju z zdravilom Vectibix®, redno spremljati glede hipomagneziemije in spremljajoče hipokalciemije. Priporočljivo je dodajanje magnezija, kot je ustrezno. Opažali so tudi druge elektrolitske motnje, vključno s hipokaliemijo. Priporočljivo je spremljanje, kot opisano zgoraj in dodajanje teh elektrolitov, kot je ustrezno. *Z infundiranjem povezane reakcije:* Če se kadarkoli med ali po infundiranju pojavi huda ali smrtno nevarna reakcija (npr. z bronhospazmom, angioedemom, hipotenzijo, potrebo po parenteralnem zdravljenju ali anafilaksijo), je treba uporabo zdravila Vectibix® za stalno prekiniti. Bolnikom, ki se jim pojavi blaga ali zmerna (1. in 2. stopnja po CTCAE verzija 4.0) z infundiranjem povezana reakcija, je treba hitrost infundiranja za čas infuzije zmanjšati. To manjšo hitrost infundiranja je priporočljivo ohraniti tudi pri vseh nadaljnjih infuzijah. Opisane so preobčutljivostne reakcije, ki so se pojavile več kot 24 ur po infundiranju, vključno s smrtnimi primeri angioedema, ki so se pojavili več kot 24 ur po infundiranju. Bolnike je potrebno opozoriti na možnost reakcij s poznim nastankom in jim naročiti, naj se obrnejo na svojega zdravnika, če se jim pojavijo simptomi preobčutljivostne reakcije. *Akutna odpoved ledvic:* Opisana je akutna odpoved ledvic pri bolnikih, ki se jim pojavi huda driska in dehidracija. Bolnikom, ki se jim pojavi huda driska, je treba naročiti, da se takoj posvetujejo z zdravnikom. *Zdravilo Vectibix® v kombinaciji s kemoterapijo na podlagi oksaliplatina pri bolnikih z mKRR z mutantnim RAS oz. bolnikih z mKRR, pri katerih stanje RAS tumorja ni znano:* Kombinacija zdravila Vectibix® s kemoterapijo, ki vključuje oksaliplatin, je kontraindicirana pri bolnikih z mKRR z mutantnim *RAS* ali pri katerih stanje *RAS* ni znano. Če je predvidena uporaba zdravila Vectibix® v kombinaciji s FOLFOX, je priporočljivo, da mutacijsko stanje določi laboratorij, ki sodeluje v programu Eksterno zagotavljanje kakovosti *RAS*, ali da se stanje divjega tipa potrdi z dupliciranjem preiskave. *Očesni toksični učinki:* Bolnike, ki se jim med prejemanjem zdravila Vectibix® pojavijo znaki in simptomi, ki kažejo na keratitis, kot so akutni pojav ali poslabšanje: vnetja očesa, solzenja, občutljivosti na svetlobo, zameglienega vida, bolečine v očesu in/ali rdečih oči, je priporočljivo takoj napotiti k specialistu oftalmologu. Če je potrjena diagnoza ulcerativnega keratitisa, je treba zdravljenje z zdravilom Vectibix® začasno ali trajno prekiniti. Če je diagnosticiran keratitis, je treba skrbno pretehtati koristi in tveganja nadaljevanja zdravljenja. Zdravilo Vectibix® morate uporabljati previdno pri bolnikih z anamnezo keratitisa, ulcerativnega keratitisa ali zelo suhih oči. *Bolniki z zmogljivostnim stanjem 2 po ECOG, zdravljeni z zdravilom Vectibix® v kombinaciji s kemoterapijo:* Pri teh bolnikih je pred uvedbo zdravila Vectibix® v kombinaciji s kemoterapijo za zdravljenje mKRR priporočljivo oceniti koristi in tveganje, saj pri njih pozitivno razmerje med koristnostjo in tveganjem ni bilo zabeleženo. *Starejši bolniki:* V celoti niso ugotovili razlik v varnosti ali učinkovitosti med starejšimi bolniki (starimi ≥ 65 let), ki so prejemali monoterapijo z zdravilom Vectibix®. *Drugi previdnostni ukrepi:* Zdravilo vsebuje manj kot 0,150 mmol natrija (kar je 3,45 mg natrija) na mililiter koncentrata. To je treba upoštevati pri bolnikih, ki potrebujejo prehrano z nadzorovano količino natrija. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Podatki študije o medsebojnem delovanju zdravil, ki je vključevala zdravili Vectibix® in irinotekan, pri bolnikih z mKRR kažejo, da se med sočasno uporabo zdravil farmakokinetika irinotekana in njegovega aktivnega metabolita SN-38 ne spremeni. Rezultati primerjave v navzkrižni študiji so pokazali, da sheme z irinotekanom (IFL ali FOLFIRI) ne vplivajo na farmakokinetiko panitumumaba. Zdravila Vectibix® se ne sme uporabljati v kombinaciji s kemoterapijo IFL ali kemoterapijo, ki vključuje bevacizumab. Kombinacija zdravila Vectibix® s kemoterapijo, ki vključuje oksaliplatin, je kontraindicirana pri bolnikih z mKRR z mutantnim *RAS* ali pri katerih stanje *RAS* ni znano. **NEŽELENI UČINKI:** Neželeni učinki, zabeleženi v kliničnih študijah in v spontanih poročilih v obdobju trženja zdravila pri bolnikih z mKRR, ki so dobivali panitumumab kot edino zdravilo ali v kombinaciji s kemoterapijo, so: Zelo pogosti (≥ 1/10): paronihija, anemija, hipokaliemija, anoreksija, hipomagneziemija, nespečnost, konjunktivitis, dispneja, kašelj, driska, navzea, bruhanje, bolečine v trebuhu, stomatitis, zaprtost, akneiformni dermatitis, izpuščaj (vključuje splošne termine kožne toksičnosti, eksfoliacije kože, eksfoliativnega izpuščaja, papuloznega izpuščaja, pruritičnega izpuščaja, eritematoznega izpuščaja, generaliziranega izpuščaja, makularnega izpuščaja, makulo-papuloznega izpuščaja, kožne lezije), eritem, srbenje, suha koža, fisure na koži, akne, alopecija, bolečine v hrbtu, utrujenost, pireksija, astenija, vnetje sluznice, periferni edemi, paronihija, zmanjšanje telesne mase; Pogosti (≥ 1/100 do < 1/10): pustulozen izpuščaj, celulitis, okužba sečil, folikulitis, lokalizirana okužba, levkopenija, preobčutljivost, hipokalciemija, dehidracija, hiperglikemija, hipofosfatemija, anksioznost, glavobol, omotica, blefaritis, rast trepalnic, močnejše solzenje, očesna hiperemija, suho oko, srbenje oči, draženje oči, tahikardija, globoka venska tromboza, hipotenzija, hipertenzija, zardevanje, pljučna embolija, epistaksa, krvavitev iz danke, suha usta, dispepsija, aftozni stomatitis, heilitis, gastroezofagealna refluksna bolezen, sindrom palmarno-plantarne eritrodizestezije, kožni ulkus, krasta, hipertrihoza, lomljenje nohtov, bolezni nohtov, hiperhidroza, dermatitis, bolečine v okončini, bolečine v prsih, bolečina, mrzlica, znižanje magnezija v krvi; Občasni (≥ 1/1.000 do < 1/100): okužba oči, okužba vek, draženje vek, keratitis, cianoza, bronhospazem, suhost nosu, razpokane ustnice, sube ustnice, angioedem, hirzutizem, vraščanje nohta, oniholiza, z infundiranjem povezane reakcije; Redki (≥ 1/10.000 do < 1/1.000): anafilaktična reakcija, ulcerativni keratitis, nekroza kože, Stevens-Johnsonov sindrom, toksična epidermalna nekroliza; Pogostnost neznana (pogostnosti ni mogoče oceniti iz razpoložljivih podatkov): intersticijska pljučna bolezen. **FARMACEVTSKI PODATKI:** Shranjujte v hladilniku (2 °C - 8 °C). Ne zamrzujte. Zdravilo Vectibix® ne vsebuje nobenih antimikrobnih konzervansov ali bakteriostatikov. Zdravilo je treba uporabiti takoj po razredčenju. Razredčene raztopine ne smete zamrzniti. Zdravilo Vectibix® je namenjeno za enkratno uporabo. **NAČIN IN REŽIM PREDPISOVANJA TER IZDAJE ZDRAVILA:** Predpisovanje in izdaja zdravila je le na recept s posebnim režimom – H. **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:** November 2016. **DATUM PRIPRAVE INFORMACIJE:** Februar 2017. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu.

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AMGEN®

Vectibix® panitumumab | INDIVIDUALIZIRANO TARČNO ZDRAVLJENJE.

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

Iclusig- oglas- 01/jun- 2016

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

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

CYRAMZA™
(ramucirumab)

UKREPAJTE ZDAJ

USPOSOBLJENI ZA SPREMEMBE, ZA NEPRIMERLJIVE IZKUŠNJE

Skrajšan povzetek glavnih značilnosti zdravila

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

Cyramza 10 mg/ml koncentrat za raztopino za infundiranje

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

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

Pomembno obvestilo:

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

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

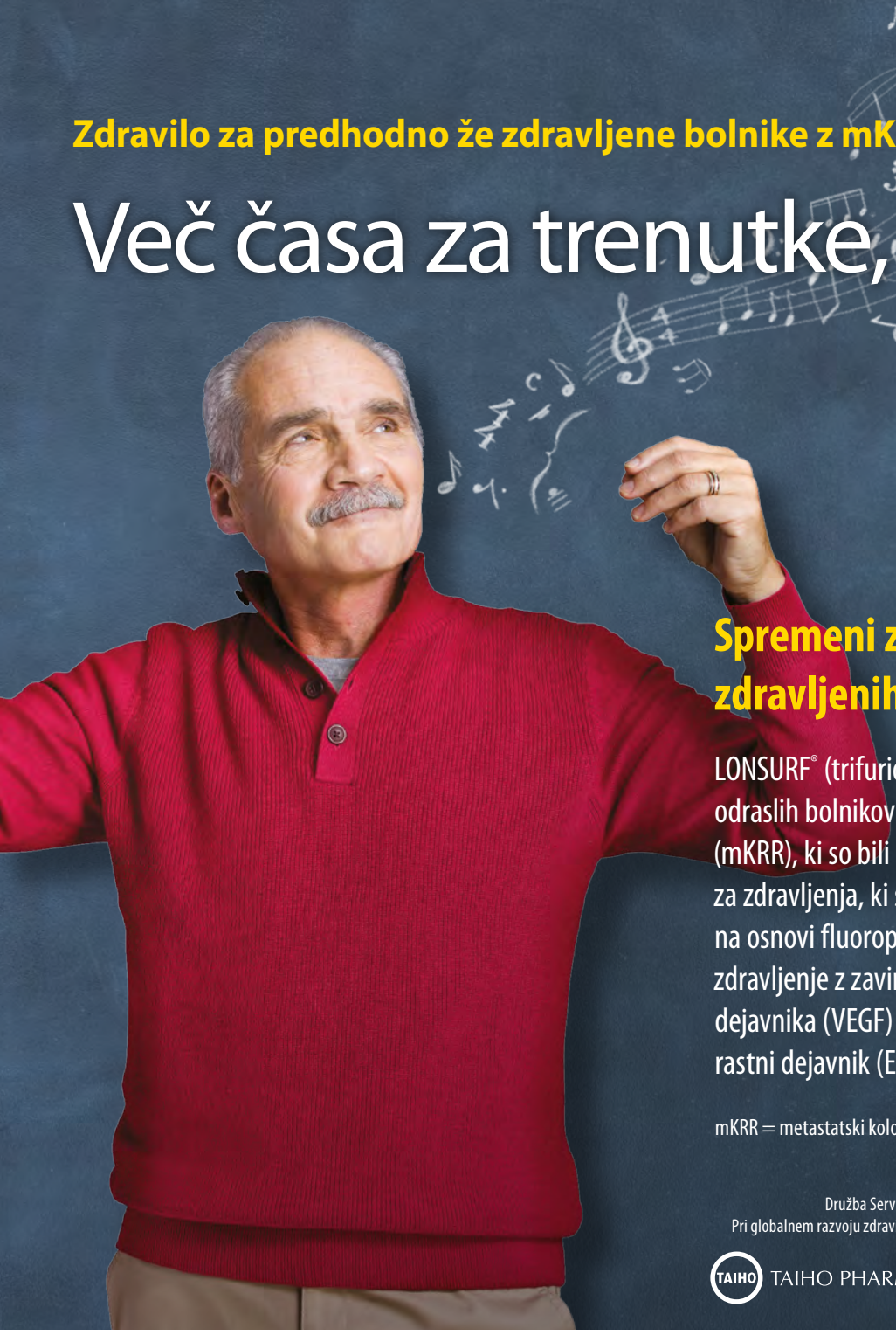
Referenca: 1. Cyramza, Povzetek glavnih značilnosti zdravila, zadnja odobrena verzija.

EERAM00010a, 12.02.2016.

Lilly

Zdravilo za predhodno že zdravljene bolnike z mKRR

Več časa za trenutke, ki štejejo



Lonsurf®
trifluridin/tipiracil

Spremeni zgodbo predhodno že zdravljenih bolnikov z mKRR

LONSURF® (trifuridin/tipiracil) je indiciran za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (mKRR), ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF) in zaviralci receptorjev za epidermalni rastni dejavnik (EGFR).

mKRR = metastatski kolorektalni rak

Družba Servier ima licenco družbe Taiho za zdravilo Lonsurf®.
Pri globalnem razvoju zdravila sodelujeta obe družbi in ga tržita v svojih določenih področjih.

TAIHO PHARMACEUTICAL CO., LTD.

SERVIER

Skrajšan povzetek glavnih značilnosti zdravila: Lonsurf 15 mg/6,14 mg filmsko obložene tablete in Lonsurf 20 mg/8,19 mg filmsko obložene tablete

▼Za to zdravilo se izvaja dodatno spremljanje varnosti. **SESTAVA*: Lonsurf 15 mg/6,14 mg:** Ena filmsko obložena tableta vsebuje 15 mg trifluridina in 6,14 mg tipiracila (v obliki klorida). **Lonsurf 20 mg/8,19 mg:** Ena filmsko obložena tableta vsebuje 20 mg trifluridina in 8,19 mg tipiracila (v obliki klorida). **TERAPEVTSKE INDIKACIJE*:** Zdravilo Lonsurf je indicirano za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom, ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF – Vascular Endothelial Growth Factor) in zaviralci receptorjev za epidermalni rastni dejavnik (EGFR – Epidermal Growth Factor Receptor). **ODMERJANJE IN NAČIN UPORABE*:** Odmerjanje: Priporočeni začetni odmerek zdravila Lonsurf pri odraslih je 35 mg/m2/odmerek peroralno dvakrat dnevno na 1. do 5. dan in 8. do 12. dan vsakega 28-dnevnega cikla zdravljenja, najpozneje 1 uro po zaključku jutranjega in večernega obroka. Odmerjanje, izračunano glede na telesno površino, ne sme preseči 80 mg/odmerek. Možne prilagoditve odmerka glede na varnost in prenašanje zdravila: dovoljena so največ 3 zmanjšanja odmerka na najmanjši odmerek 20 mg/m2 dvakrat dnevno. Potem ko je bil odmerek zmanjšan, povečanje ni dovoljeno. **KONTRAINDIKACIJE*:** Preobčutljivost na zdravilni učinkovini ali katero koli pomožno snov. **OPOZORILA IN PREVIDNOSTNI UKREPI*:** Supresija kostnega mozga: Pred uvedbo zdravljenja, pred vsakim ciklom zdravljenja in po potrebi je treba pregledati celotno krvno sliko. Zdravljenja ne smete začeti, če je absolutno število nevtrofilcev < 1,5 x 109/l, če je število trombocitov < 75 x 109/l ali če se je pri bolniku zaradi predhodnih zdravljenj pojavila klinično pomembna nehematološka toksičnost 3. ali 4. stopnje, ki še traja. Bolnike je treba skrbno spremljati zaradi morebitnih okužb, uvesti je treba ustrezne ukrepe, kot je klinično indicirano. Toksičnost za prebavila: Potrebna je uporaba antiemetikov, antidiaroikov ter drugih ukrepov, kot je klinično indicirano. Če je potrebno, prilagodite odmerke. Ledvična okvara: Zdravilo Lonsurf ni primerno za uporabo pri bolnikih s hudo ledvično okvaro ali končno stopnjo ledvične okvare. Bolnike z zmerno ledvično okvaro je treba zaradi hematološke toksičnosti bolj pogosto spremljati. Jetrna okvara: Uporaba zdravila Lonsurf pri bolnikih z zmerno ali hudo jetrno okvaro ni priporočljiva. Proteinurija: Pred začetkom zdravljenja in med njim je priporočljivo spremljanje proteinurije z urinskimi testnimi lističi. Pomožne snovi: Vsebujejo laktozo. **INTERAKCIJE*:** Zdravila, ki medsebojno delujejo z nukleozidnimi prenašalci CNT1, ENT1 in ENT2, zaviralci OCT2 ali MATE1, substrati humane timidin-kinaze (npr. zidovudinom), hormonskimi kontraceptivi. **PLODNOST*, NOSEČNOST IN DOJENJE*:** Ni priporočljivo. **KONTRACEPCIJA*:** Ženske in moški morajo uporabljati učinkovito metodo kontracepcije med zdravljenjem in do 6 mesecev po zaključku zdravljenja. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA S STROJI*:** Med zdravljenjem se lahko pojavijo utrujenost, omotica ali splošno slabo počutje. **NEŽELENI UČINKI*:** *Zelo pogosti:* nevtropenija, levkopenija, anemija, trombocitopenija, zmanjšan apetit, diareja, navzea, bruhanje, utrujenost. *Pogosti:* okužba spodnjih dihal, okužba zgornjih dihal, febrilna nevtropenija, limfopenija, monocitoza, hipoalbuminemija, nespečnost, disgevzija, periferna nevropatija, omotica, glavobol, vročinski oblivi, dispneja, kašelj, bolečina v trebuhu, zaprtje, stomatitis, bolezni ustne votline, hiperbilirubinemija, sindrom palmarne plantarne eritrodisestezije, izpuščaj, alopecija, pruritus, suha koža, proteinurija, pireksija, edem, vnetje sluznice, splošno slabo počutje, zvišanje jetrnih encimov, zvišanje alkalne fosfataze v krvi, zmanjšanje telesne mase. *Občasni:* septični šok, infekcijski enteritis, pljučnica, okužba žolčevoda, gripa, okužba sečil, vnetje dlesni, herpes zoster, tinea pedis, kandidiaza, bakterijska okužba, okužba, bolečina zaradi raka, pancitopenija, granulocitopenija, monocitopenija, eritropenija, levkocitoza, dehidracija, hiperglikemija, hiperkaliemija, hipokaliemija, hipofosfatemija, hipernatriemija, hiponatriemija, hipokalciemija, protin, anksioznost, nevrotoksičnost, disestezija, hiperestezija, hipoestezija, sinkopa, parestezija, pekoč občutek, letargija, zmanjšana ostrina vida, zamegljen vid, diplopija, katarakta, konjunktivitis, suho oko, vrtoglavica, neugodje v ušesu, angina pektoris, aritmija, palpitacije, embolija, hipertenzija, hipotenzija, pljučna embolija, plevralni izliv, izcedek iz nosu, disfonija, orofaringealna bolečina, epistaksa, hemoragični enterokolitis, krvavitev v prebavilih, akutni pankreatitis, ascites, ileus, subileus, kolitis, gastritis, refluksni gastritis, ezofagitis, moteno praznjenje želodca, abdominalna distenzija, analno vnetje, razjede v ustih, dispepsija, gastroezofagealna refluksna bolezen, proktalgija, bukalni polip, krvavitev dlesni, glositis, parodontalna bolezen, bolezen zob, siljenje na bruhanje, flatulenca, slab zadah, hepatotoksičnost, razširitev žolčnih vodov, luščenje kože, urtikarija, preobčutljivostne reakcije na svetlobo, eritem, akne, hiperhidroza, žulj, bolezni nohtov, otekanje sklepov, artralgija, bolečina v kosteh, mialgija, mišično-skeletna bolečina, mišična oslabelost, mišični krči, bolečina v okončinah, občutek teže, ledvična odpoved, neinfektivni cistitis, motnje mikcije, hematurija, levkociturija, motnje menstruacije, poslabšanje splošnega zdravstvenega stanja, bolečina, občutek spremembe telesne temperature, kseroza, zvišanje kreatinina v krvi, podaljšanje intervala QT na elektrokardiogramu, povečanje mednarodnega umerjenega razmerja (INR), podaljšanje aktiviranega parcialnega tromboplastinskega časa (aPTČ), zvišanje sečnine v krvi, zvišanje laktatne dehidrogenaze v krvi, znižanje celokupnih proteinov, zvišanje C-reaktivnega proteina, zmanjšan hematokrit. *Post-marketinške izkušnje:* pri bolnikih, zdravljenih z zdravilom Lonsurf na Japonskem, so poročali o primerih intersticijske bolezni pljuč. **PREVELIKO ODMERJANJE*:** Neželeni učinki, o katerih so poročali v povezavi s prevelikim odmerjanjem, so bili v skladu z uveljavljenim varnostnim profilom. Glavni pričakovani zaplet prevelikega odmerjanja je supresija kostnega mozga. **FARMAKODINAMIČNE LASTNOSTI*:** *Farmakoterapevtska skupina:* zdravila z delovanjem na novotvorbe, antimetaboliti, oznaka ATC: L01BC59. Zdravilo Lonsurf sestavljata antineoplastični timidinski nukleozidni analog, trifluridin, in zaviralec timidin-fosforilaze (TPaze), tipiracilijev klorid. Po privzemu v rakave celice timidin-kinaza fosforilira trifluridin. Ta se v celicah nato presnovi v substrat deoksiribonukleinske kisline (DNA), ki se vgradi neposredno v DNA ter tako preprečuje celično proliferacijo. TPaza hitro razgradi trifluridin in njegova presnova po peroralni uporabi je hitra zaradi učinka prvega prehoda, zato je v zdravilo vključen zaviralec TPaze, tipiracilijev klorid. **PAKIRANJE*:** 20 filmsko obloženih tablet. **NAČIN PREDPISOVANJA IN IZDAJANJA ZDRAVILA:** Rp/Spec. **Imetnik dovoljenja za promet z zdravilom:** Les Laboratoires Servier, 50, rue Carnot, 92284 Suresnes cedex, Francija. **Številka dovoljenja za promet z zdravilom:** EU/1/16/1096/001 (Lonsurf 15 mg/6,14 mg), EU/ 1/16/1096/004 (Lonsurf 20 mg/8,19 mg). *** Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila. Datum zadnje revizije besedila: april 2016. Celoten povzetek glavnih značilnosti zdravila in podrobnejše informacije so na voljo pri: Servier Pharma d.o.o., tel: 01 563 48 11, www.servier.si.**

Datum priprave informacije: januar 2017.
LNF16/17C1AD2 Samo za strokovno javnost.

COTELLIC® kobimetinib | Zelboraf® vemurafenib

DVOJNO ZAVIRANJE. VEČJA UČINKOVITOST.

Skrajšan povzetek glavnih značilnosti zdravila COTELLIC:

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

Ime zdravila: Cotellic 20 mg filmsko obložene tablete. **Kakovostna in količinska sestava:** Ena filmsko obložena tableta vsebuje kobimetinibijev hemifumarat, kolikor ga ustreza 20 mg kobimetiniba. Pomožna snov z znanim učinkom: Ena filmsko obložena tableta vsebuje 36 mg laktoze monohidrata. **Terapevtske indikacije:** Zdravilo Cotellic je v kombinaciji z vemurafenibom indicirano za zdravljenje odraslih bolnikov z neoperabilnim ali metastatskim melanomom, ki ima mutacijo BRAF V600. **Odmerjanje in način uporabe:** Zdravljenje z zdravilom Cotellic v kombinaciji z vemurafenibom sme uvesti in nadzorovati le usposobljen zdravnik, ki ima izkušnje z uporabo zdravil proti raku. Pred začetkom zdravljenja je treba z validirano preiskavo potrditi, da ima bolnik melanom z mutacijo BRAF V600. Odmerjanje: Vsak odmerek obsega tri 20-mg tablete (60 mg) in ga je treba vzeti enkrat na dan 21 dni zapored, temu sledi 7-dnevni premor. Zdravljenje z zdravilom Cotellic je treba nadaljevati, dokler bolniku ne koristi več oziroma do pojava nesprejemljive toksičnosti. Če bolnik izpusti odmerek, ga lahko vzame do 12 ur pred naslednjim odmerkom, da ohrani shemo enkrat na dan. Če bolnik po uporabi zdravila Cotellic bruha, tisti dan ne sme vzeti dodatnega odmerka, temveč mora zdravljenje nadaljevati naslednji dan, kot je predpisano. *Splošne prilagoditve odmerka:* Odločitev za zmanjšanje odmerka zdravila Cotellic ali vemurafeniba mora temeljiti na zdravnikovi oceni varnosti in prenašanja pri posameznem bolniku. Ko je bil odmerek enkrat zmanjšan, se ga kasneje ne sme več povečati. *Nasvet za prilagoditev odmerka v primeru disfunkcije levega prekata:* Če so srčni simptomi posledica zdravila Cotellic in se po prehodni prekinitvi njegove uporabe ne izboljšajo, je treba razmisliti o trajnem prenehanju zdravljenja z zdravilom Cotellic. *Nasvet za prilagoditev odmerka zdravila Cotellic med uporabo z vemurafenibom: Jetrna laboratorijska odstopanja:* Stopnja 3: Zdravilo Cotellic je treba nadaljevati v predpisanem odmerku. Odmerek vemurafeniba je mogoče zmanjšati, kot je klinično primerno. Stopnja 4: Zdravljenje z zdravilom Cotellic in zdravljenje z vemurafenibom je treba prekiniti. Za dodatna navodila glejte celotni povzetek glavnih značilnosti zdravila Cotellic. *Zvišanja kreatin-fosfokinaze (CPK):* Za obvladovanje nesimptomatskih zvišanj CPK odmerka zdravila Cotellic ni treba prilagoditi ali prekiniti. *Fotosenzibilnost:* Fotosenzibilnost stopnje ≤ 2 je treba obvladovati s podpornim zdravljenjem. Fotosenzibilnost stopnje 2 ali stopnje ≥ 3: zdravili Cotellic in vemurafenib je treba prekiniti, dokler se ne zmanjša na stopnjo ≤ 1. Zdravljenje je mogoče znova začeti brez spremembe odmerka zdravila Cotellic. Odmerjanje vemurafeniba je treba zmanjšati, kot je klinično primerno; za dodatne informacije glejte povzetek glavnih značilnosti vemurafeniba. *Izpuščaj:* Izpuščaj se lahko pojavi tako med zdravljenjem z zdravilom Cotellic kot med zdravljenjem z vemurafenibom. Odmerek zdravila Cotellic in/ali vemurafeniba je mogoče začasno prekiniti in/ali zmanjšati, kot je klinično primerno. Za dodatna navodila glejte celotni povzetek glavnih značilnosti zdravila Cotellic. *Podaljšanje intervala QT:* Če interval QTc med zdravljenjem preseže 500 ms, prosimo, glejte povzetek glavnih značilnosti vemurafeniba. Spremenba odmerkov zdravila Cotellic ni potrebna, kadar se ta uporablja v kombinaciji z vemurafenibom. Posebne populacije bolnikov: Bolnikom, starim ≥ 65 let, odmerka ni treba prilagoditi. Pri bolnikih s hudo okvaro ledvic je treba zdravilo Cotellic uporabljati previdno. Bolnikom z okvaro jeter odmerka ni treba prilagoditi. Bolniki s hudo okvaro jeter imajo lahko v primerjavi z bolniki z normalnim delovanjem jeter zvišane koncentracije prostega kobimetiniba v plazmi. Pri zdravljenju z zdravilom Cotellic se lahko pojavijo z jetri povezane laboratorijske nepravilnosti, zato je treba pri bolnikih z okvaro jeter katere koli stopnje zdravilo uporabljati previdno.Varnost in učinkovitost zdravila Cotellic pri otrocih in mladostnikih, mlajših od 18 let, nista ugotovljeni. Način uporabe: Zdravilo Cotellic je za peroralno uporabo. Tablete je treba zaužiti cele, z vodo. Lahko se jemljejo skupaj s hrano ali brez nje. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Zdravilo Cotellic v kombinaciji z vemurafenibom pri bolnikih, katerih bolezen je napredovala med zdravljenjem z zaviralcem BRAF: Podatkov pri teh bolnikih je malo. Ti kažejo, da je učinkovitost kombinacije pri teh bolnikih manjša. Zato je treba razmisliti o drugih možnostih zdravljenja pred uvedbo kombinacije. Zdravilo Cotellic v kombinaciji z vemurafenibom pri bolnikih z zastavki v možganih: Varnost in učinkovitost kombinacije zdravila Cotellic in vemurafeniba pri teh bolnikih ni bila ocenjena. Intrakranialna aktivnost kobimetiniba trenutno ni poznana. Serozna retinopatija: Pri bolnikih, zdravljenih z zaviralci MEK, vključno z zdravilom Cotellic, so opažali serozno retinopatijo. Večina primerov je bila opisana kot hiorioretinopatija ali odstop mrežnice. Večina dogodkov, opaženih v kliničnih preskušanjih, je po prekinitvi ali zmanjšanju odmerka izzvenela ali se izboljšala na nesimptomatsko stopnjo 1. Bolnike je treba na vsakem pregledu oceniti glede simptomov novih motenj vida ali poslabšanja obstoječih motenj vida. V primeru simptomov novih motenj vida ali poslabšanju obstoječih je priporočljiv oftalmološki pregled. Serozno retinopatijo je mogoče obvladati s prekinitvijo zdravljenja, zmanjšanjem odmerka ali prenehanjem zdravljenja. Disfunkcija levega prekata: Pri bolnikih, ki so prejemali zdravilo Cotellic, so poročali o zmanjšanju iztisnega deleža levega prekata (LVEF) v primerjavi z izhodiščem. LVEF je treba izmeriti pred začetkom zdravljenja za določitev izhodiščne vrednosti, nato pa ga kontrolirati po prvem mesecu zdravljenja ter vsaj na 3 mesece oziroma kot je klinično indicirano, do prenehanja zdravljenja. Zmanjšanje LVEF od izhodišča je mogoče obvladati s prekinitvijo zdravljenja, zmanjšanjem odmerka ali prenehanjem zdravljenja. Bolnikov z izhodiščnim LVEF pod spodnjo mejo normalne vrednosti (SMN) za ustanovo ali pod 50 % niso proučevali. Jetrna laboratorijska odstopanja: Jetrna laboratorijska odstopanja se lahko pojavijo tako med uporabo zdravila Cotellic v kombinaciji z vemurafenibom kot med samostojnim zdravljenjem z vemurafenibom. Pri bolnikih, zdravljenih s kombinacijo zdravila Cotellic in vemurafeniba, so opažali jetrna laboratorijska odstopanja, zlasti zvišanje ALT, AST in AF. Jetrna laboratorijska odstopanja je treba kontrolirati z laboratorijskimi preiskavami jeter pred začetkom kombiniranega zdravljenja in vsak mesec med zdravljenjem, lahko pa tudi pogosteje, če je klinično indicirano. Laboratorijska odstopanja stopnje 3 je treba obvladati s prekinitvijo uporabe vemurafeniba ali zmanjšanjem odmerka. Jetrna laboratorijska odstopanja stopnje 4 se obvlada s prekinitvijo zdravljenja, zmanjšanjem odmerka ali prenehanjem zdravljenja z zdravilom Cotellic in z vemurafenibom. Driska: Pri bolnikih, zdravljenih z zdravilom Cotellic, so poročali o primerih driske stopnje ≥ 3 in resni driski. Drisko je treba obvladati z antidiaroiki in podpornim zdravljenjem. V primeru driske stopnje ≥ 3, ki se pojavi kljub podpornemu zdravljenju, je treba obe zdravili, Cotellic in vemurafenib, prekiniti, dokler se driska ne izboljša na stopnjo ≤ 1. Če se driska stopnje ≥ 3 ponovi, je treba odmerek zdravila Cotellic in vemurafeniba zmanjšati. Intoleranca za laktozo: Zdravilo vsebuje laktozo. Bolniki z redko dedno intoleranco za galaktozo, laponsko obliko pomanjkanja laktaze ali malabsorpcijo glukoze/galaktoze se morajo posvetovati z zdravnikom in se z njim pogovoriti, ali zanje koristi zdravljenja odtehtajo tveganje. Podaljšanje intervala QT: Če interval QTc med zdravljenjem preseže 500 ms, prosimo, glejte povzetka glavnih značilnosti vemurafeniba. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Učinki drugih zdravil na kobimetinib: *Zaviralci CYP3A:* Med zdravljenjem s kobimetinibom se izogibajte sočasni uporabi močnih zaviralcev CYP3A. Če se sočasni uporabi močnega zaviralca CYP3A ni mogoče izogniti, je treba bolnike skrbno nadzirati glede varnosti. Previdnost je potrebna, če se kobimetinib uporablja sočasno z zmernimi zaviralci CYP3A. Če se kobimetinib uporablja sočasno z zmernim zaviralcem CYP3A, je treba bolnike skrbno nadzirati glede varnosti. *Induktorji CYP3A:* Treba se je izogibati sočasni uporabi z zmernimi in močnimi induktorji CYP3A. Razmisliti je treba o uporabi drugih zdravil, ki CYP3A inducirajo le malo ali sploh ne. Ker se med sočasno uporabo zmernih do močnih induktorjev CYP3A koncentracija kobimetiniba verjetno bistveno zniža, se lahko njegova učinkovitost pri bolniku poslabša. *Zaviralci P-glikoproteina:* Sočasna uporaba zaviralcev P-gp, npr. ciklosporina in verapamila, lahko zviša koncentracijo kobimetiniba v plazmi. Učinki kobimetiniba na druga zdravila: *Substrati CYP3A in CYP2D6:* Klinična študija medsebojnega delovanja zdravil pri onkološkib bolnikih je pokazala, da se koncentracij midazolama in dekstrometorfana v plazmi v prisotnosti kobimetiniba nista spremenili. *Substrati CYP1A2: In vitro* je kobimetinib potencialen induktor CYP1A2 in lahko zato zmanjša izpostavljenost substratom tega encima, npr. teofilinu. *Substrati BCRP: In vitro* kobimetinib zmerno zavira BCRP. Klinično pomembnega zavrtja BCRP na ravni črevesja ni mogoče izključiti. Druga zdravila proti raku: *vemurafenib:* Pri bolnikih z neoperabilnim ali metastatskim melanomom ni dokazov o klinično pomembnem medsebojnem delovanju med kobimetinibom in vemurafenibom, zato prilagoditve odmerkov niso potrebne. Vpliv kobimetiniba na transportne sisteme zdravil: Študije *in vitro* kažejo, da kobimetinib ni substrat jetrnih privzemnih prenašalcev OATP1B1, OATP1B3 in OCT1, vendar pa jih katerih zavira. Klinični pomen teh izsledkov ni raziskan. Pediatrična populacija: Študije medsebojnega delovanja so izvedli le pri odraslih. *Neželeni učinki:* Neželeni učinki pri bolnikih, zdravljenih z zdravilom Cotellic v kombinaciji z vemurafenibom: *Zelo pogosti:* anemija, serozna retinopatija, zamegljen vid, hipertenzija, krvavitev, driska, navzea, bruhanje, fotosenzibilnost, izpuščaj, makulopapulozen izpuščaj, akneiformni dermatitis, hiperkeratoza, zvišana telesna temperatura, mrzlica, zvišanja CPK, ALT, AST, gama-glutamiltransferaze (GGT) in AF v krvi. *Pogosti:* bazalnocelični karcinom, ploščatocelični karcinom kože, keratoakantom, dehidracija, hipofosfatemija, hiponatriemija, hipergликemija, okvara vida, pnevmonitis, zmanjšan iztisni delež in zvišanje bilirubina v krvi. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** Rp/Spec. **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija. **Verzija: 2.0/16. Informacija pripravljena:** januar 2017.

Skrajšan povzetek glavnih značilnosti zdravila ZELBORAF:

Ime zdravila: Zelboraf 240 mg filmsko obložene tablete. **Kakovostna in količinska sestava:** Ena tableta vsebuje 240 mg vemurafeniba (v obliki precipitata vemurafeniba in hipromeloze acetat sukcinata). **Terapevtske indikacije:** Vemurafenib je indiciran za samostojno zdravljenje odraslih bolnikov z neresektabilnim ali metastatskim melanomom, s pozitivno mutacijo BRAF V600. **Odmerjanje in način uporabe:** Zdravljenje z vemurafenibom mora uvesti in nadzorovati usposobljen zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje raka. Odmerjanje: Priporočeni odmerek vemurafeniba je 960 mg (4 tablete po 240 mg) dvakrat na dan (to ustreza celotnemu dnevnemu odmerku 1920 mg). Vemurafenib lahko vzamemo s hrano ali brez nje, izogibati pa se moramo stalnemu jemanju obeh dnevnih odmerkov na prazen želodec. Zdravljenje z vemurafenibom moramo nadaljevati do napredovanja bolezni ali pojava nesprejemljive toksičnosti. Če bolnik izpusti odmerek, ga lahko vzame do 4 ure pred naslednjim odmerkom za ohranitev sheme dvakrat na dan. Obeh odmerkov pa ne sme vzeti hkrati. Če bolnik po zaužitju vemurafeniba bruha, ne sme vzeti dodatnega odmerka zdravila, ampak mora z zdravljenjem nemudoma nadaljevati. Prilagoditve odmerjanja: Za obvladovanje neželenih učinkov ali ob podaljšanju intervala QTc je potrebno zmanjšanje odmerka, začasna prekinitev in/ali dokončno prenehanje zdravljenja (za podrobnosti o prilagoditvi odmerka, prosimo glejte SmPC zdravila). Zmanjšanje odmerka pod 480 mg dvakrat na dan ni priporočljivo. Če se pri bolniku pojavi ploščatocelični karcinom kože, priporočamo nadaljevanje zdravljenja brez zmanjšanja odmerka vemurafeniba. *Posebne populacije:* Za bolnike, starejše od 65 let, prilagajanje odmerka ni potrebno. O bolnikih z okvaro ledvic ali jeter je na voljo malo podatkov. Bolnike s hudo okvaro ledvic ali z zmerno do hudo okvaro jeter je treba pozorno spremljati. Varnost in učinkovitost vemurafeniba pri otrocih in mladostnikih, mlajših od 18 let, še nista bili dokazani. Podatkov ni na voljo. Način uporabe: Vemurafenib je namenjen peroralni uporabi. Tablete je treba zaužiti cele, z vodo. Ne sme se jih žvečiti ali zdrobiti. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Pred uporabo vemurafeniba je treba z validirano preiskavo potrditi, da ima bolnik tumor s pozitivno mutacijo BRAF V600. Dokazi o učinkovitosti in varnosti vemurafeniba pri bolnikih s tumorji z izraženo redko BRAF V600 mutacijo, ki ni V600E ali V600K, niso prepričljivi. Vemurafeniba se ne sme uporabljati pri bolnikih z malignim melanomom, ki ima divji tip BRAF. *Preobčutljivostne reakcije:* V povezavi z vemurafenibom so bile opisane resne preobčutljivostne reakcije, vključno z anafilaksijo. Hude preobčutljivostne reakcije lahko vključujejo Stevens-Johnsonov sindrom, generaliziran izpuščaj, eritem ali hipotenzijo. Pri bolnikih, pri katerih se pojavijo resne preobčutljivostne reakcije, je treba zdravljenje z vemurafenibom dokončno opustiti. *Kožne reakcije:* Pri bolnikih, ki so prejemali vemurafenib, so v ključnem kliničnem preskušanju poročali o hudih kožnih reakcijah, vključno z redkim Stevens-Johnsonovim sindromom in toksično epidermalno nekrolizo. Po prihodu vemurafeniba na trg so v povezavi z njim poročali o reakciji na zdravilo z eozinofilijo in sistemskimi simptomi. Pri bolnikih, pri katerih se pojavi huda kožna reakcija, je treba zdravljenje z vemurafenibom dokončno opustiti. *Povečanje toksičnosti obsevanja:* Pri bolnikih, ki so se pred, med ali po zdravljenju z vemurafenibom zdravili z obsevanjem, so poročali o primerih vnetnih reakcij na mestu obsevanja (t.i. *radiation recall*) in povečane občutljivosti na obsevanje. Večina primerov je bila po naravi kožnih, a nekaj primerov, ki je vključevalo visceralne organe, je imelo smrtni izid. Pri sočasni ali zaporedni uporabi vemurafeniba in obsevanja je potrebna previdnost. *Podaljšanje intervala QT:* V nekontroliranl, odprti študiji faze II pri predhodno zdravljenih bolnikih z metastatskim melanomom, so opazili podaljšanje intervala QT, odvisnega od izpostavljenosti vemurafenibu. Podaljšanje intervala QT lahko poveča tveganje za ventrikularne aritmije, vključno s t. i. *Torsade de Pointes*. Z vemurafenibom ni priporočljivo zdraviti bolnikov z elektrolitskimi motnjami (vključno z magnezijem), ki jih ni mogoče odpraviti, bolnikov s sindromom dolgega intervala QT in bolnikov, zdravljenih z zdravili, ki podaljšajo interval QT. Pred zdravljenjem z vemurafenibom, en mesec po zdravljenju in po spremembi odmerka je treba pri vseh bolnikih posneti EKG in kontrolirati elektrolite (vključno z magnezijem). Nadaljnje kontrole so priporočljive predvsem pri bolnikih z zmerno do hudo jetrno okvaro, in sicer mesečno prve 3 mesece zdravljenja, potem pa na 3 mesece oziroma pogosteje, če je to klinično indicirano. Zdravljenja z vemurafenibom ni priporočljivo uvesti pri bolnikih, ki imajo interval QTc > 500 ms. *Bolezni oči:* Poročali so o resnih neželenih učinkih na očeh, vključno z uveitisom, iritisom in zaporo mrežnične vene. Bolnikom je treba oči redno kontrolirati glede morebitnih neželenih učinkov na očeh. *Ploščatocelični karcinom kože:* Pri bolnikih, zdravljenih z vemurafenibom, so bili opisani primeri ploščatoceličnega karcinoma kože, vključno s ploščatoceličnim karcinomom, opredeljenim kot keratoakantom ali mešani keratoakantom. Priporočljivo je, da vsi bolniki pred uvedbo zdravljenja opravijo dermatološki pregled in da so med zdravljenjem deležni rednih kontrol. Vsako sumljivo spremembo je treba izrezati, poslati na histopatološko oceno in jo zdraviti v skladu z lokalnimi smernicami. Med zdravljenjem in do šest mesecev po zdravljenju ploščatoceličnega karcinoma mora zdravnik enkrat mesečno pregledati bolnika. Pri bolnikih, ki se jim pojavi ploščatocelični karcinom kože, je priporočljivo nadaljevati zdravljenje brez zmanjšanja odmerka. Nadzor se mora nadaljevati še 6 mesecev po prenehanju zdravljenja z vemurafenibom ali do uvedbe drugega antineoplastičnega zdravljenja. Bolnikom je treba naročiti, naj svojega zdravnika obvestijo o pojavu kakršnih koli sprememb na koži. *Ploščatocelični karcinom, ki se ne nahaja na koži:* Pri bolnikih, ki so prejemali vemurafenib v kliničnih preskušanjih, so poročali o primerih ploščatoceličnega karcinoma, ki se ne nahaja na koži. Bolnikom je treba pred uvedbo zdravljenja in na 3 mesece med zdravljenjem pregledati glavo in vrat (pregled mora obsegati vsaj ogled ustne sluznice in palpacijo bezgavk). Poleg tega morajo bolniki pred zdravljenjem in na 6 mesecev med zdravljenjem opraviti računalniško tomografijo prsnega koša. Pred in po končanem zdravljenju ali kadar je klinično indicirano, je priporočljivo opraviti pregled zadnjika in ginekološki pregled. Po prenehanju zdravljenja z vemurafenibom se mora nadzor glede ploščatoceličnega karcinoma, ki se ne nahaja na koži, nadaljevati še 6 mesecev ali do uvedbe drugega antineoplastičnega zdravljenja. Nenormalne spremembe je treba obravnavati v skladu s klinično prakso. *Novi primarni melanom:* V kliničnih preskušanjih so poročali o novih primarnih melanomih. Bolnike s takšnimi primeri so zdravili z ekscizijo, bolniki pa so nadaljevali z zdravljenjem brez prilagoditve odmerka. Nadzor nad pojavom kožnih lezij je treba izvajati, kot je navedeno zgoraj pri ploščatoceličnem karcinomu kože. *Druge malignosti:* Glede na mehanizem delovanja lahko vemurafenib povzroči napredovanje rakov, povezanih z mutacijo RAS. Pred dajanjem vemurafeniba bolnikom, ki so imeli ali imajo raka, povezanega z mutacijo RAS, skrbno razmislite o koristih in tveganjih. *Pankreatitis:* Pri bolnikih, zdravljenih z zdravilom Zelboraf, so poročali o pankreatitisu. Nepojasnjeno bolečino v trebuhu je treba nemudoma preiskati. Bolnike je treba skrbno spremljati, ko po epizodi pankreatitisa ponovno uvedemo vemurafenib. *Poškodbe jeter:* Med uporabo vemurafeniba so poročali o poškodbah jeter, vključno s primeri hudih poškodb. Pred uvedbo zdravljenja je treba izmeriti jetrne encime (transaminaze in alkalno fosfatazo) ter bilirubin in jih spremljati mesečno med zdravljenjem oz. kot je klinično indicirano. Laboratorijske nepravilnosti je treba obvladati z zmanjšanjem odmerka, prekinitvijo zdravljenja ali prenehanjem zdravljenja (za podrobnosti o prilagoditvi odmerka, prosimo glejte SmPC zdravila). *Ledvična toksičnost:* Med uporabo vemurafeniba so poročali o ledvični toksičnosti v razponu od zvišanega serumskega kreatinina do akutnega intersticijskega nefritisa in akutne tubulne nekroze. Serumski kreatinin je treba izmeriti pred začetkom zdravljenja in ga med zdravljenjem spremljati, kot je klinično indicirano. *Jetrna okvara:* Bolnikom z jetrno okvaro začetnih odmerkov ni treba prilagajati. Bolnike, ki imajo zaradi metastaz v jetrih blago jetrno okvaro in nimajo hiperbilirubinemije, se lahko nadzoruje v skladu s splošnimi priporočili. Podatkov o bolnikih z zmerno do hudo jetrno okvaro je le malo; pri takih bolnikih je izpostavljenost lahko večja. Tako je posebej po prvih tednih zdravljenja potreben skrben nadzor, saj lahko po daljšem obdobju (več tednih) pride do kopičenja. *Ledvična okvara:* Bolnikom z blago ali zmerno *ledvično okvaro začetnih odmerkov ni treba prilagajati. Pri bolnikih z hudo ledvično okvaro je treba vemurafenib uporabljati previdno ter jih pozorno spremljati. Fotosenzibilnost:* Pri bolnikih, ki so v kliničnih študijah prejemali vemurafenib, je bila opisana blaga do huda fotosenzibilnost. Vsem bolnikom je treba naročiti, naj se med jemanjem vemurafeniba ne izpostavljajo soncu. V primeru fotosenzibilnosti stopnje 2 ali več so priporočljive prilagoditve odmerka. *Ženske v rodni dobi* morajo med zdravljenjem in vsaj še 6 mesecev po zdravljenju uporabljati učinkovito kontracepcijsko zaščito. Vemurafenib lahko zmanjša učinkovitost hormonskih kontraceptivov. *Sočasna uporaba ipilimumaba* in vemurafeniba ni priporočljiva. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** *Vpliv vemurafeniba na substrate CYP* Vemurafenib lahko poveča izpostavljenost v plazmi tistim snovem, ki se presnavljajo pretežno s CYP1A2 [npr. agomelatinu, duloksetinu, melatoninu, tizanidinu, teofilinu]; v takem primeru se lahko razmisli o prilagoditvi odmerka, če je klinično indicirano. Vemurafenib lahko zmanjša plazemsko izpostavljenost zdravilom, ki se presnavljajo pretežno s CYP3A4. Tako je lahko učinkovitost kontracepcijskih tablet, ki se presnavljajo s CYP3A4 in se uporabljajo sočasno z vemurafenibom, zmanjšana. Pri substratih CYP3A4, ki imajo ozko terapevtsko okno, se lahko razmisli o prilagoditvi odmerka, če je klinično indicirano. Zaenkrat še ni znano ali lahko vemurafenib pri koncentracijah v plazmi, ki je bila opažena pri bolnikih v stanju dinamičnega ravnovesja, zmanjša plazemske koncentracije sočasno dajanih substratov CYP2B6, kot je bupropion. Kadar se vemurafenib pri bolnikih z melanomom uporabi hkrati z varfarinom (CYP2C9), je potrebna previdnost. Tveganja za klinično pomemben učinek na sočasno uporabljene učinkovine, ki so substrati CYP2C8, ni mogoče izključiti. Zaradi dolge razpolovne dobe vemurafeniba je mogoče, da popolnega inhibitornega učinka vemurafeniba na sočasno dajano zdravilo ne opazimo, dokler ne mine 8 dni zdravljenja z vemurafenibom. Po končanem zdravljenju z vemurafenibom bo morda potreben 8-dnevni premor, da se izognemo interakcijam z nadaljnjim zdravljenjem. *Zdravljenje z obsevanjem:* Pri bolnikih, zdravljenih z vemurafenibom, so poročali o povečanju toksičnosti obsevanja. V večini primerov so bolniki prejeli protokole obsevanja z 2 Gy/dan ali več (hipofrakcionirane protokole). *Medsebojno delovanje vemurafeniba s transportnimi sistemi zdravil:* Ob sočasni uporabi vemurafeniba in substrata P-gp je potrebna previdnost. Pri uporabi zdravil, ki so substrati P-gp in imajo ozko terapevtsko okno (npr. digoksina, dabigatran eteksilata, aliskirena), je treba razmisliti o dodatnem spremljanju koncentracije zdravila. Možnosti, da vemurafenib morda poveča izpostavljenost zdravilom, ki se prenašajo z BCRP, ni mogoče izključiti. *Vplivi sočasno uporabljenih zdravil na vemurafenib:* Vemurafenib je treba uporabljati previdno v kombinaciji z močnimi inhibitorji CYP3A4, glukuronidacije in/ali prenašalnih beljakovin [npr. ritonavirjem, sakvinavirjem, telitromicinom, ketokonazolom, itrakonazolom, vorikonazolom, posakonazolom, nefazodonom, atazanavirjem]. Sočasna uporaba močnih induktorjev P-gp, glukuronidacije in/ali CYP3A4 (npr. rifampicina, rifabutina, karbamazepina, fenitoina ali šentjanževke) lahko vodi v suboptimalno izpostavljenost vemurafenibu in se ji je treba izogibati. Vplivi induktorjev in inhibitorjev P-gp in BCRP na izpostavljenost vemurafenibu niso znani. Ne moremo pa izključiti možnosti, da imajo lahko zdravila, ki vplivajo na P-gp (npr. verapamil, ciklosporin, ritonavir, kinidin, itrakonazol) ali BCRP (npr. ciklosporin, gefitinib), vpliv na farmakokinetiko vemurafeniba. **Neželeni učinki:** Med najpogostejšimi neželenimi učinki (> 30 %), o katerih so poročali v zvezi z vemurafenibom, so artralgija, utrujenost, kožni izpuščaj, fotosenzibilnostna reakcija, navzea, alopecija in srbenje. Zelo pogosto je bil opisan ploščatocelični karcinom kože. Sledijo drugi zelo pogosti in pogosti neželeni učinki. *Zelo pogosti:* seboročna keratoza, kožni papilom, zmanjšanje teka, glavobol, disgevzija, kašelj, driska, bruhanje, zaprtost, aktinična keratoza, makulo-papulozen izpuščaj, papulozen izpuščaj, hiperkeratoza, eritem, suha koža, sončne opekline, mialgija, bolečina v okončini, mišično-skeletne bolečine, bolečine v hrbtu, pireksija, periferni edem, astenija, zvišanje GGT. *Pogosti:* folikulitis, bazalnocelični karcinom, novi primarni melanom, ohromelost sedmega živca, omotica, uveitis, sindrom palmarno-plantarne eritrodisestezije, panikulitis, pilarna keratoza, artritis, zvišanje ALT, alkalne fosfataze, bilirubina in izguba telesne mase, podaljšanje QT in zvišanje kreatinina v krvi. *Posebne populacije:* Pri starejših bolnikih (> 65 let) je možna večja verjetnost neželenih učinkov, vključno s ploščatoceličnim karcinomom kože, zmanjšanjem teka in motnjami srčnega ritma. Med neželene učinke stopnje 3, ki so bili med kliničnimi preskušanji vemurafeniba pri ženskah opisani pogosteje kot pri moških, spadajo kožni izpuščaj, artralgija in fotosenzibilnost. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** Rp/Spec. **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija. **Verzija: 2.0/16. Informacija pripravljena:** januar 2017.

Samo za strokovno javnost.

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

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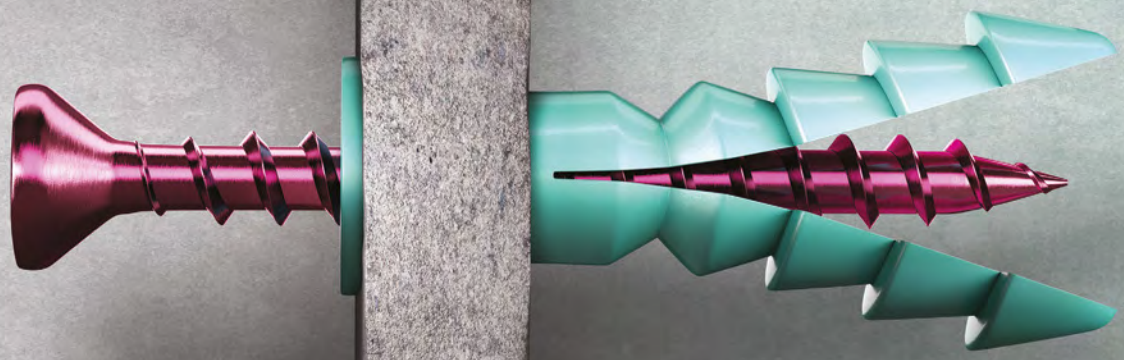

Kombinacija zdravil Cotellic®▼ in Zelboraf® za zdravljenje odraslih bolnikov z neoperabilnim ali metastatskim melanomom, ki ima mutacijo BRAF V600.[1,2]

1 Povzetek glavnih značilnosti zdravila Cotellic. Dostopano januar 2017 na: http://www.ema.europa.eu/docs/sl_SI/document_library/EPAR_-_Product_Information/human/003960/WC500198563.pdf

2 Povzetek glavnih značilnosti zdravila Zelboraf. Dostopano januar 2017 na: http://www.ema.europa.eu/docs/sl_SI/document_library/EPAR_-_Product_Information/human/002409/WC500124317.pdf

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All authors should be listed when their number does not exceed six; when there are seven or more authors, the first six listed are followed by "et al.". The following are some examples of references from articles, books and book chapters:

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XALKORI® – prvi zaviralec ALK, odobren za I. linijo zdravljenja napredovalega, ALK pozitivnega nedrobnoceličnega pljučnega raka[1]

ALK = anaplastična limfomska kinaza

BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

XALKORI 200 mg, 250 mg trde kapsule

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o kateremkoli domnevnem neželenem učinku zdravila. Glejte poglavje 4.8 povzetka glavnih značilnosti zdravila, kako poročati o neželenih učinkih. **Sestava in oblika zdravila:** Ena kapsula vsebuje 200 mg ali 250 mg krizotiniba. **Indikacije:** Prva linija zdravljenja odraslih bolnikov z napredovalim nedrobnoceličnim pljučnim rakom (NSCLC – *Non-Small Cell Lung Cancer)*, ki je ALK (anaplastična limfomska kinaza) pozitiven. Zdravljenje odraslih bolnikov s predhodno zdravljenim, napredovalim NSCLC, ki je ALK pozitiven. Zdravljenje odraslih bolnikov z napredovalim NSCLC, ki je ROS1 pozitiven. **Odmerjanje in način uporabe:** Zdravljenje mora uvesti in nadzorovati zdravnik z izkušnjami z uporabo zdravil za zdravljenje rakavih bolezni. Preverjanje prisotnosti ALK in ROS1: Pri izbiri bolnikov za zdravljenje je treba pred zdravljenjem opraviti točno in validirano preverjanje prisotnosti ALK ali ROS1. Odmerjanje: Priporočeni odmerek je 250 mg dvakrat na dan (500 mg na dan), bolniki pa morajo zdravilo jemati brez prekinitev. Če bolnik pozabi vzeti odmerek, ga mora vzeti takoj, ko se spomni, razen če do naslednjega odmerka manjka manj kot 6 ur. V tem primeru bolnik pozabljenega odmerka ne sme vzeti. *Prilagajanja odmerkov:* Glede na varnost uporabe zdravila pri posameznem bolniku in kako bolnik zdravljenje prenaša, utegne biti potrebna prekinitev in/ali zmanjšanje odmerka zdravila na 200 mg dvakrat na dan; če je potrebno še nadaljnje zmanjšanje, pa znaša odmerek 250 mg enkrat na dan. Za prilagajanje odmerkov pri *hematološki in nehematološki* (povečanje vrednosti AST, ALT, bilirubina; ILD/pnevmonitis; podaljšanje intervala QTc, bradikardija, bolezni oči) *toksičnosti* glejte preglednici 1 in 2 v povzetku glavnih značilnosti zdravila. *Okvara jeter:* Pri blagi in zmerni okvari je zdravljenje treba izvajati previdno, pri hudi okvari se zdravila ne sme uporabljati. *Okvara ledvic:* Pri blagi in zmerni okvari prilagajanje začetnega odmerka ni priporočeno. Pri hudi okvari ledvic (ki ne zahteva peritonealne dialize ali hemodialize) je začetni odmerek 250 mg peroralno enkrat na dan; po vsaj 4 tednih zdravljenja se lahko poveča na 200 mg dvakrat na dan. *Starejši bolniki (U 65 let):* Prilagajanje začetnega odmerka ni potrebno. *Pediatrična populacija:* Varnost in učinkovitost nista bili dokazani. Način uporabe: Kapsule je treba pogoltniti cele, z nekaj vode, s hrano ali brez nje. Ne sme se jih zdrobiti, raztopiti ali odpreti. Izogibati se je treba uživanju grenivk, grenivkinega soka ter uporabi šentjanževke. **Kontraindikacije:** Preobčutljivost na krizotinib ali katerokoli pomožno snov. Huda okvara jeter. **Posebna opozorila in previdnostni ukrepi:** Določanje statusa ALK in ROS1: Pomembno je izbrati dobro validirano in robustno metodologijo, da se izognemo lažno negativnim ali lažno pozitivnim rezultatom. Hepatotoksičnost: V kliničnih študijah so poročali o hepatotoksičnosti, ki jo je povzročilo zdravilo (vključno s primeri s smrtnim izidom). Delovanje jeter, vključno z ALT, AST in skupnim bilirubinom, je treba preveriti enkrat na teden v prvih 2 mesecih zdravljenja, nato pa enkrat na mesec in kot je klinično indicirano. Ponovitve preverjanj morajo biti pogostejše pri povečanjih vrednosti stopnje 2, 3 ali 4. Intersticijska bolezen pljuč (ILD)/pnevmonitis: Lahko se pojavi huda, življenjsko nevarna ali smrtna ILD/pnevmonitis. Bolnike s simptomi ILD/pnevmonitisa, je treba spremljati, zdravljenje pa prekiniti ob sumu na ILD/pnevmonitis. Podaljšanje intervala QT: Opažali so podaljšanje intervala QTc. Pri bolnikih z obstoječo bradikardijo, podaljšanjem intervala QTc v anamnezi ali predispozicijo zanj, pri bolnikih, ki jemljejo antiaritmike ali druga zdravila, ki podaljšujejo interval QT, ter pri bolnikih s pomembno obstoječo srčno boleznijo in/ali motnjami elektrolitov je treba krizotinib uporabljati previdno; potrebno je redno spremljanje EKG, elektrolitov in delovanja ledvic; preiskavi EKG in elektrolitov je treba opraviti čimbližje uporabi prvega odmerka, potem se priporoča redno spremljanje. Če se interval QTc podaljša za 60 ms ali več, je treba zdravljenje s krizotinibom začasno prekiniti in se posvetovati s kardiologom. Bradikardija: Lahko se pojavi simptomatska bradikardija (lahko se razvije več tednov po začetku zdravljenja); izogibati se je treba uporabi krizotiniba v kombinaciji z drugimi zdravili, ki povzročajo bradikardijo; pri simptomatski bradikardiji je treba prilagoditi odmerek. Srčno popuščanje: Poročali so o hudih, življenjsko nevarnih ali smrtnih neželenih učinkih srčnega popuščanja. Bolnike je treba spremljati glede pojavov znakov in simptomov srčnega popuščanja in ob pojavu simptomov zmanjšati odmerjanje ali prekiniti zdravljenje. Nevtropenija in levkopenija: V kliničnih študijah so poročali o nevtropeniji, levkopeniji in febrilni nevtropeniji; spremljati je treba popolno krvno sliko (pogostejše preiskave, če se opazijo abnormalnosti stopnje 3 ali 4 ali če se pojavi povišana telesna temperatura ali okužba). Perforacija v prebavilih: V kliničnih študijah so poročali o perforacijah v prebavilih, v obdobju trženja pa o smrtnih primerih perforacij v prebavilih. Krizotinib je treba pri bolnikih s tveganjem za nastanek perforacije v prebavilih uporabljati previdno; bolniki, pri katerih se razvije perforacija v prebavilih, se morajo prenehati zdraviti s krizotinibom; bolnike je treba poučiti o prvih znakih perforacije in jim svetovati, naj se nemudoma posvetujejo z zdravnikom. Vplivi na ledvice: V kliničnih študijah so opazili zvišanje ravni kreatinina v krvi in zmanjšanje očistka kreatinina. V kliničnih študijah in v obdobju trženja so poročali tudi o odpovedi ledvic, akutni odpovedi ledvic, primerih s smrtnim izidom, primerih, ki so zahtevali hemodializo in hiperkaliemiji stopnje 4. Vplivi na vid: V kliničnih študijah so poročali o izpadu vidnega polja stopnje 4 z izgubo vida. Če se na novo pojavi huda izguba vida, je treba zdravljenje prekiniti in opraviti oftalmološki pregled. če so motnje vida trdovratne ali se poslabšajo, je priporočljiv oftalmološki pregled. Histološka preiskava, ki ne nakazuje adenokarcinoma: Na voljo so le omejeni podatki pri NSCLC, ki je ALK in ROS1 pozitiven in ima histološke značilnosti, ki ne nakazujejo adenokarcinoma, vključno s ploščatoceličnim karcinomom (SCC). **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Zdravila, ki lahko povečajo koncentracije krizotiniba v plazmi (močni zaviralci CYP3A4, npr. atazanavir, indinavir, nelfinavir, ritonavir, sakvinavir, itrakonazol, ketokonazol, vorikonazol, klaritromicin, telitromicin, troleandomicin), tudi grenivke in grenivkin sok. Zdravila, ki lahko zmanjšajo koncentracije krizotiniba v plazmi (močni induktorji CYP3A4, npr. karbamazepin, fenobarbital, fenitoin, rifampicin, šentjanževka). Zmerni induktorji CYP3A4, npr. efavirenz in rifabutin. Zdravila, katerih koncentracije v plazmi lahko krizotinib spremeni (midazolam, alfentanil, cisaprid, ciklosporin, derivati ergot alkaloidov, fentanil, pimozid, kinidin, sirolimus, takrolimus, bupropion, efavirenz, peroralni kontraceptivi, raltegravir, irinotekan, morfin, nalokson, digoksin, dabigatran, kolhicin, pravastatin, metformin, prokainamid). Zdravila, ki podaljšujejo interval QT ali ki lahko povzročijo Torsades de pointes (antiaritmiki skupine IA (kinidin, disopiramid), antiaritmiki skupine III (amiodaron, sotalol, dofetilid, ibutilid), metadon, cisaprid, moksifloksacin, antipsihotiki). Zdravila, ki povzročajo bradikardijo (nedihidropiridinski zaviralci kalcijevih kanalčkov (verapamil, diltiazem), antagonisti adrenergičnih receptorjev beta, klonidin, gvanfacin, digoksin, meflokin, antiholinesteraze, pilokarpin). **Plodnost, nosečnost in dojenje:** Ženske v rodni dobi se morajo izogibati zanositvi. Med zdravljenjem in najmanj 90 dni po njem je treba uporabljati ustrezno kontracepcijo (velja tudi za moške). Zdravilo lahko škoduje plodu in se ga med nosečnostjo ne sme uporabljati, razen če klinično stanje matere ne zahteva takega zdravljenja. Matere naj se med jemanjem zdravila dojenju izogibajo. Zdravilo lahko zmanjša plodnost moških in žensk. **Vpliv na sposobnost vožnje in upravljanja strojev:** Lahko se pojavijo simptomatska bradikardija (npr. sinkopa, omotica, hipotenzija), motnje vida ali utrujenost; potrebna je previdnost. **Neželeni učinki:** Najresnejši neželeni učinki so bili hepatotoksičnost, ILD/pnevmonitis, nevtropenija in podaljšanje intervala QT. Najpogostejši neželeni učinki (U 25 %) so bili motnje vida, navzea, diareja, bruhanje, edem, zaprtje, povečane vrednosti transaminaz, utrujenost, pomanjkanje apetita, omotica in nevropatija. Ostali zelo pogosti (U 1/10 bolnikov) neželeni učinki so: nevtropenija, anemija, levkopenija, disgevzija, bradikardija, bolečina v trebuhu in izpuščaj. **Način in režim izdaje:** Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Izjemoma se lahko uporablja pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. **Imetnik dovoljenja za promet:** Pfizer Limited, Ramsgate Road, Sandwich, Kent CT13 9NJ, Velika Britanija. **Datum zadnje revizije besedila:** 11.11.2016 **Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.**

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Vir: 1. Povzetek glavnih značilnosti zdravila Xalkori, 11.11.2016

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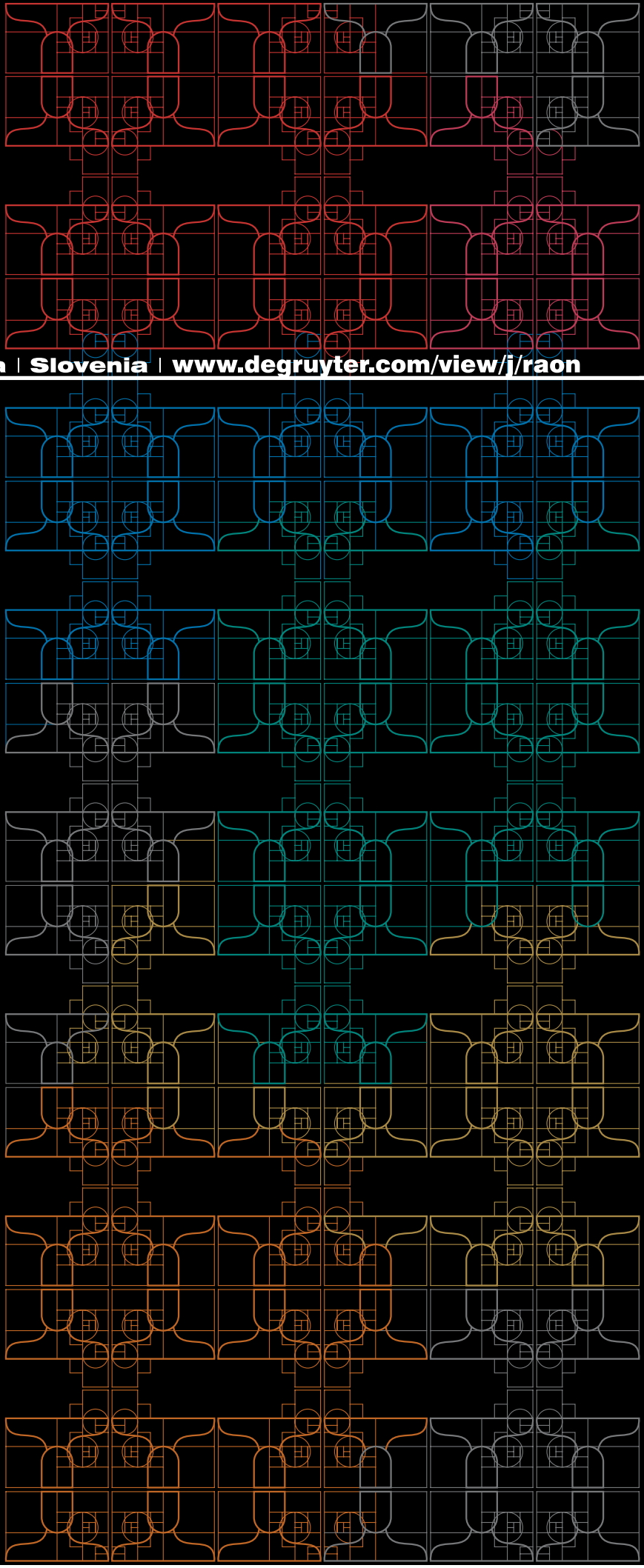

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