RADIOLOGY AND ONCOLOGY

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Halaven®
eribulin

NOVA SMER DO PODALJŠANJA **CELOKUPNEGA PREŽIVETJA**

Eisai

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

- **Halaven** (eribulin): ne-taksanski zaviralec dinamike mikrotubulov, prvo zdravilo iz nove skupine kemoterapevtikov, imenovanih *halihondrini*.
- Zdravilo HALAVEN je indicirano za zdravljenje bolnic z lokalno napredovalim ali metastatskim rakom dojke, ki je napredoval po vsaj enem režimu kemoterapije za napredovalo bolezen. Predhodna zdravljenja morajo vključevati antraciklin in taksan, bodisi kot adjuvantno zdravljenje ali za zdravljenje metastatskega raka dojke, razen če to zdravljenje za bolnice ni bilo primerno.[1]
- Priporočeni odmerek 1,23 mg/m^2, intravensko, v obliki 2- do 5-minutne infuzije, 1. in 8. dan vsakega 21-dnevnega cikla.
- Ena 2 ml viala vsebuje 0,88 mg eribulina.
- Raztopina, pripravljena za uporabo, redčenje ni potrebno.

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

HALAVEN 0,44 mg/ml raztopina za injiciranje (eribulin)
TERAPEVTSKE INDIKACIJE: Zdravljenje lokalno napredovalega ali metastatskega raka dojke, ki je napredoval po vsaj enem režimu kemoterapije za napredovalo bolezen vključno z antraciklinom in taksanom (adjuvantno zdravljenje ali zdravljenje metastatskega raka dojke), razen če to ni bilo primerno. **ODMERJANJE IN NAČIN UPORABE:** Halaven se daje v enotah, specializiranih za dajanje citotoksične kemoterapije, in le pod nadzorom usposobljenega zdravnika z izkušnjami v uporabi citotoksičnih zdravil. Odmerjanje: Priporočeni odmerek eribulina v obliki raztopine je 1,23 mg/m^2 i.v. v obliki 2- do 5-minutne infuzije 1. in 8. dan vsakega 21-dnevnega cikla. Bolnikom je lahko slabo ali bruhajo. Treba je razmisliti o antiemetični profilaksi, vključno s kortikosteroidi. *Preložitev odmerka med zdravljenjem*: Dajanje Halavena je treba preložiti, če se pojavi kaj od naslednjega: absolutno število nevtrofilcev (ANC) < 1 x 10^9/l, trombociti < 75 x 10^9/l ali nehematološki neželeni učinki 3. ali 4. stopnje. *Zmanjšanje odmerka med zdravljenjem*: Za priporočila za zmanjšanje odmerka ob pojavu hematoloških ali nehematoloških neželenih učinkov glejte celoten povzetek glavnih značilnosti zdravila. Okvara jeter zaradi zasevkov: Priporočeni odmerek pri blagi okvari jeter (stopnje A po Child-Pughu) je 0,97 mg/m^2 v obliki 2- do 5-minutne i.v. infuzije 1. in 8. dan 21-dnevnega cikla. Priporočeni odmerek pri zmerni okvari jeter (stopnje B po Child-Pughu) je 0,62 mg/m^2 v obliki 2- do 5-minutne i.v. infuzije 1. in 8. dan 21-dnevnega cikla. Pri hudi okvari jeter (stopnje C po Child-Pughu) se pričakuje, da je treba dati še manjši odmerek eribulina. Okvara jeter zaradi ciroze: Zgornje odmerke se lahko uporabi za blago do zmerno okvaro, vendar se priporoča skrbno nadziranje, saj bo odmerke morda treba ponovno prilagoditi. Okvara ledvic: Pri hudi okvari ledvic (očistek kreatinina < 40 ml/min) bo morda treba odmerek zmanjšati. Priporoča se skrbno nadziranje varnosti. Način uporabe: Odmerek se lahko razredči z do 100 ml 0,9 % raztopine natrijevega klorida (9 mg/ml) za injiciranje. Ne sme se ga redčiti v 5 % infuzijski raztopini glukoze. Pred dajanjem glejte navodila glede redčenja zdravila v celotnem povzetku glavnih značilnosti zdravila ter se prepričajte, da obstaja dober periferni venski dostop ali prehodna centralna linija. Ni znakov, da bi eribulin povzročal mehurje ali dražil. V primeru ekstravazacije mora biti zdravljenje simptomatsko. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov. Dojenje. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Mielosupresija je odvisna od odmerka in se kaže kot nevtropenija. Pred vsakim odmerkom eribulina je treba opraviti pregled celotne krvne slike. Zdravljenje z eribulinom se lahko uvede le pri bolnikih z vrednostmi ANC ≥ 1,5 x 10^9/l in s trombociti > 100 x 10^9/l. Bolnike, pri katerih se pojavijo febrilna nevtropenija, huda nevtropenija ali trombocitopenija, je treba zdraviti v skladu s priporočili v celotnem povzetku glavnih značilnosti zdravila. Hudo nevtropenijo se lahko zdravi z uporabo G-CSF ali enakovrednim zdravilom v skladu s smernicami. Bolnike je treba skrbno nadzirati za znake periferne motorične in senzorične nevropatije. Pri razvoju hude periferne nevrotoksičnosti je treba odmerek prestaviti ali zmanjšati. Če začnemo zdravljenje pri bolnikih s kongestivnim srčnim popuščanjem, z bradiaritmijami ali sočasno z zdravili, za katera je znano, da podaljšujejo interval QT, vključno z antiaritmiki razreda Ia in III, in z elektrolitskimi motnjami, je priporočljivo spremljanje EKG. Pred začetkom zdravljenja s Halavenom je treba popraviti hipokaliemijo in hipomagneziemijo in te elektrolite je treba občasno kontrolirati med zdravljenjem. Eribulina ne smemo dajati bolnikom s prirojenim sindromom dolgega intervala QT. To zdravilo vsebuje majhne količine etanola (alkohola), manj kot 100 mg na odmerek. Eribulin je pri podganah embriotoksičen, fetotoksičen in teratogen. Halavena se ne sme uporabljati med nosečnostjo, razen kadar je to nujno potrebno. Ženske v rodni dobi naj ne zanosijo v času, ko same ali njihov moški partner dobivajo Halaven, in naj med zdravljenjem in še do 3 mesece po njem uporabljajo učinkovito kontracepcijo. Moški naj se pred zdravljenjem posvetujejo o shranjevanju sperme zaradi možnosti nepopravljive neplodnosti. **INTERAKCIJE:** Eribulin se izloča do 70 % prek žolča. Sočasna uporaba učinkovin, ki zavirajo jetrne transportne beljakovine, kot so beljakovine za prenos organskih anionov in beljakovine, odporne na številna zdravila, z eribulinom se ne priporoča (npr. ciklosporin, ritonavir, sakvinavir, lopinavir in nekateri drugi zaviralci proteaze, efavirenz, emtricitabin, verapamil, klaritromicin, kinin, kinidin, dizopiramid itd). Sočasno zdravljenje z indukcijskimi učinkovinami, kot so rifampicin, karbamazepin, fenitoin, šentjanževka, lahko povzroči znižanje koncentracij eribulina v plazmi, zato je ob sočasni uporabi induktorjev potrebna previdnost. Eribulin je blag inhibitor encima CYP3A4. Priporočljiva je previdnost in spremljanje glede neželenih učinkov pri sočasni uporabi snovi, ki imajo ozko terapevtsko okno in se odstranjujejo iz telesa predvsem preko CYP3A4 (npr. alfentanil, ciklosporin, ergotamin, fentanil, pimozid, kinidin, sirolimus, takrolimus). **NEŽELENI UČINKI:** Povzetek varnostnega profila Neželeni učinek, o katerem najpogosteje poročajo v zvezi s Halavenom, je supresija kostnega mozga, ki se kaže kot nevtropenija, levkopenija, anemija, trombocitopenija s pridruženimi okužbami. Poročali so tudi o novem začetku ali poslabšanju že obstoječe periferne nevropatije. Med neželenimi učinki, o katerih poročajo, je toksičnost za prebavila, ki se kaže kot anoreksija, navzea, bruhanje, driska, zaprtost in stomatitis. Med drugimi neželenimi učinki so utrujenost, alopecija, zvečani jetrni encimi, sepsa in mišičnoskeletni bolečinski sindrom. Seznam neželenih učinkov: *Zelo pogosti (≥ 1/10):* nevtropenija (57,0 %) (3./4. stopnje: 49,7 %), levkopenija (29,3 %) (3./4. stopnje: 17,3 %), anemija (20,6 %) (3./4. stopnje: 2,0 %), zmanjšan apetit (21,9 %) (3./4. stopnje: 0,7 %), periferna nevropatija (35,6 %) (3./4. stopnje: 7,6 %), glavobol (17,2 %) (3./4. stopnje: 0,8 %), dispnea (13,9 %) (3./4. stopnje: 3,1 %), kašelj (13,6 %) (3./4. stopnje: 0,6 %), navzea (33,8 %) (3./4. stopnje: 1,1 %), zaprtost (19,6 %) (3./4. stopnje: 0,6 %), driska (17,9 %) (3./4. stopnje: 0,8 %), bruhanje (17,6 %) (3./4. stopnje: 0,9 %), alopecija, artralgija in mialgija (19,4 %) (3./4. stopnje: 1,1 %), bolečina v hrbtu (13,0 %) (3./4. stopnje: 1,5 %), bolečina v udu (10,0 %) (3./4. stopnje: 0,7 %), utrujenost/astenija (47,9 %) (3./4. stopnje: 7,8 %), pireksija (20,4 %) (3./4. stopnje: 0,6 %), zmanjšanje telesne mase (11,3 %) (3./4. stopnje: 0,3 %). *Pogosti (≥ 1/100 do < 1/10):* okužba sečil (8 %) (3./4. stopnje: 0,5 %), pljučnica (1,2 %) (3./4. stopnje: 0,8 %), ustna kandidiaza, ustni herpes, okužba zgornjih dihal, nazofaringitis, rinitis, limfopenija (4,9 %) (3./4. stopnje: 1,4 %), febrilna nevtropenija (4,7 %) (3./4. stopnje: 4,5 %), trombocitopenija (4,3 %) (3./4. stopnje: 0,7 %), hipokaliemija (6,1 %) (3./4. stopnje: 1,7 %), hipomagneziemija (2,9 %) (3./4. stopnje: 0,2 %), dehidracija (2,8 %) (3./4. stopnje: 0,5 %), hiperglikemija, hipofosfatemija, nespečnost, depresija, disgevzija, omotičnost (7,9 %) (3./4. stopnje: 0,5 %), hipoestezija, letargija, nevrotoksičnost, oslinjenje solzenje (6,0 %) (3./4. stopnje: 0,1 %), konjunktivitis, vrtoglavica, tahikardija, vročinski valovi, orofaringealna bolečina, epistaksa, rinoreja, bolečina v trebuhu, stomatitis (9,3 %) (3./4. stopnje: 0,8 %), suha usta, dispepsija (5,9 %) (3./4. stopnje: 0,2 %), gastroezofagealna refluksna bolezen, razjede v ustih, distenzija trebuha, zvišanje alanin-aminotransferaze (7,6 %) (3./4. stopnje: 2,1 %), zvišanje aspartat-aminotransferaze (7,4 %) (3./4. stopnje: 1,5 %), zvišanje gama-glutamiltransferaze (1,8 %) (3./4. stopnje: 0,9 %), hiperbilirubinemija (1,5 %) (3./4. stopnje: 0,3 %), izpuščaj, pruritus (3,9 %) (3./4. stopnje: 0,1 %), bolezni nohtov, nočno potenje, suha koža, eritem, hiperhidroza, bolečina v kosteh (9,6 %) (3./4. stopnje: 1,7 %), mišični spazmi (5,1 %) (3./4. stopnje: 0,1 %), mišično-skeletna bolečina in mišično-skeletna bolečina v prsih, mišična oslabelost, disurija, vnetje sluznice (8,3 %) (3./4. stopnje: 1,1 %), periferni edem, bolečina, mrzlica, bolečina v prsih, gripi podobna bolezen. *Občasni (≥ 1/1.000 do < 1/100):* sepsa (0,5 %) (3./4. stopnje: 0,2 %), nevtropenična sepsa (0,1 %) (3./4. stopnje: 0,1 %), herpes zoster, tinitus, globoka venska tromboza, pljučna embolija, hepatotoksičnost (1,0 %) (3./4. stopnje: 0.6 %), palmarno-plantarna eritrodisestezija, hematurija, proteinurija, odpoved ledvic. *Redki (≥ 1/10.000 do < 1/1.000):* diseminirana intravaskularna koagulacija, intersticijska pljučna bolezen, pankreatitis, angioedem. Za popoln opis neželenih učinkov glejte celoten povzetek glavnih značilnosti zdravila. Vrsta ovojnine in vsebina: viala z 2 ml raztopine. **Režim izdaje: H Imetnik dovoljenja za promet:** Eisai Europe Ltd, European Knowledge Centre, Mosquito Way, Hatfield, Hertfordshire, AL10 9SN, Velika Britanija
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Viri: (1) Povzetek glavnih značilnosti zdravila Halaven, junij 2014; (2) Cortes J *et al. Lancet* 2011; 377: 914–23.

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contents

review

Immunotoxin – a new treatment option in patients with relapsed and refractory Hodgkin lymphoma

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Background. Even though Hodgkin lymphoma is a highly curable disease, some of the patients have either a refractory disease or experience a relapse following a successful primary therapy. Durable responses and remissions in patients with relapsed or refractory disease may be achieved in approximately one-half with salvage chemotherapy followed by high dose chemotherapy (HDT) and autologous hematopoietic cell rescue (SCT). On the other hand, patients who relapse after HDT and autologous SCT or those who have failed at least two prior multi-agent chemotherapy regimens and are not candidates for HDT have limited treatment options.

Conclusions. A new treatment option in this population is an immunotoxin Brentuximab vedotin composed of a CD30 directed antibody linked to the antitubulin agent monomethyl auristatin E. It has demonstrated a substantial effectiveness and an acceptable toxicity. In the pivotal study, the overall response rate was 75% with 34% of complete remissions. The median durations of response were 20.5 and 6.7 months for those with complete remission and all responding patients, respectively. The median overall survival was 40.5 months (3-years overall survival 54%) and the median progression-free survival 9.3 months. The most common non-hematologic toxicities were peripheral sensory neuropathy, nausea, and fatigue while the most common severe side effects were neutropenia, thrombocytopenia, anemia, and peripheral sensory neuropathy.

Key words: Hodgkin lymphoma; relapsed and refractory; new treatment option; treatment effectiveness; toxicity

Introduction

Hodgkin lymphoma (HL) is a highly curable disease. However, some of the patients have either a primary refractory disease or experience a relapse following a successful initial therapy.[1-5] Primary refractory disease refers to those patients who do not achieve a complete remission after initial therapy. The incidence of primary refractory disease varies depending upon the stage of disease at diagnosis and the treatment regimen used and it occurs in approximately 10 to 15% of patients undergoing primary treatment.[4-6] The likelihood of relapse of HL from initial therapy in the era of systemic or combined modality therapy is approximately 10 to 15% for localized HL and 20 to 40% for advanced disease, dependent on prognostic factors. Approximately one-half of these relapses occur in the first 12 months from induction and an additional one-quarter occurs at one to three years thereafter. Late relapses (*i.e.,* more than 3 years following treatment) occur at the rate of a few percent per year, extending up to 12 years post-treatment.[1-3]

Durable responses and remissions in patients with primary refractory disease may be achieved in approximately one-half with second line chemotherapy that incorporates drugs not used in initial treatment followed by high dose chemotherapy and autologous hematopoietic cell rescue (HDT and SCT). The treatment of relapsed disease primarily consists of combination chemotherapy with or without radiotherapy; radiotherapy alone is usu-

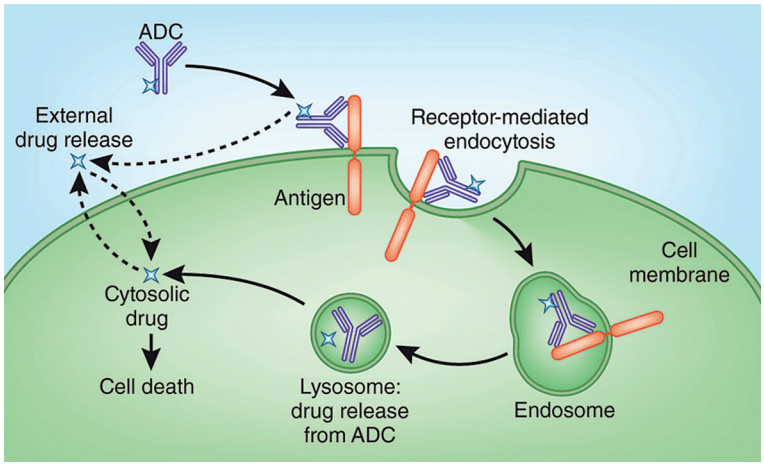

FIGURE 1. Mechanism of action of brentuximab vedotin.

ADC = antibody-drug conjugate

ally not used. Salvage therapy with second or third line regimens can achieve responses in approximately 50% of these patients, although long-term disease-free survival after the treatment of relapse with chemotherapy alone is less common. The choice of therapy is usually based upon prognostic features and patients with a localized, asymptomatic relapse occurring more than 12 months after the initial treatment are usually treated with conventional salvage chemotherapy, often combined with radiation therapy with or without HDT and autologous SCT. The value of HDT is uncertain in this group and may be unnecessarily toxic. High dose chemotherapy and autologous SCT should be considered as the treatment of choice for treatment of early relapses (less than 12 months after treatment) or induction failures, second relapses after conventional treatment for first relapse or generalized systemic relapses even beyond 12 months.[7-12] However, it is of great importance that complete remission is achieved prior to transplantation. This is supported by emerging experience with PET scans obtained at the end of salvage chemotherapy, but prior to high-dose therapy with autologous transplant. In this setting, a negative scan has a markedly positive predictive value with 93% progression-free survival at two years in one series.[13] In comparison, the majority of PET-positive patients relapse despite high-dose therapy.

Radiation therapy is in the relapsed setting indicated for patients with localized residual disease after salvage chemotherapy. In addition, patients with a localized late relapse may achieve long-term remission with chemotherapy followed by involved-field radiation therapy (IF-RT) rather than chemotherapy followed by HDT. The role of IF-RT in the treatment of patients who achieve a complete response to chemotherapy and plan to proceed with HDT is less clear.[6,14,15]

Risk factors for relapse after second line therapy include not only the patient and tumor specific markers (*e.g.*, CD68 expression), but also the initial response to therapy and the duration of this initial response. The German Hodgkin's Lymphoma Study Group (GHSG) identified three adverse risk factors for second relapse following various forms of salvage therapy, which included hematopoietic cell transplantation in one-third of the cases. These adverse risk factors included time to first recurrence of less or equal to 12 months, stage III or IV disease at first relapse and anemia at the time of first relapse.[1]

Patients who relapse following autologous SCT have limited treatment options. A second autologous SCT is an option only in highly selected patients because of their limited bone marrow reserve and due to the fact that the disease that relapses after a first autologous SCT is generally more chemotherapy resistant.[16] Single agent chemotherapy is often used in this setting, but there are no guidelines for the selection of agents. Other options include the use of brentuximab vedotin, bendamustine, rituximab, mTOR inhibitors (*e.g.*, everolimus), immunomodulators (*e.g.*, lenalidomide), histone deacetylase inhibitors, adoptive immunotherapy, local regional irradiation, or allogeneic SCT.[17-26] In addition, the anti PD-1 antibody nivolumab has shown quite impressive responses in patients with HL who relapsed after prior autologous SCT.[27] Allogeneic SCT is usually offered to patients with HL as a salvage therapy following relapse or progression after autologous SCT. Long term remissions can be obtained after allogeneic SCT in a select group of patients. An advantage of an allogeneic SCT graft is the use of tumor-free graft and the transfer of robust immune system from a healthy donor that can mediate the "graft versus lymphoma" effect.

Brentuximab vedotin

Brentuximab vedotin (SGN-35) is an immunotoxin composed of a CD30 directed antibody linked to the antitubulin agent monomethyl auristatin E (MMAE).[17,28] The antibody drug conjugate enables the antibody to selectively deliver a chemotherapy agent to CD30 positive tumor cells. The antibody and MMAE are joined together by a protease-cleav-

able linker that is stable in plasma but degraded by lysosomal enzymes. This conjugate binds to CD30, which is expressed on the surface of HL cells, then gets internalized and traffics to lysosome, where the MMAE is released. The MMAE then disrupts the microtubule network, leading to cell cycle arrest in G2/M phase and apoptosis (Figure 1).

Brentuximab vedotin has been approved by FDA and later EMA for the treatment of patients with relapsed or refractory CD30+ HL after failure of autologous hematopoietic SCT or after failure of at least two prior therapies in patients who are not candidates for HDT and autologous SCT or multiagent chemotherapy.[29] A subset of patients treated with brentuximab vedotin in this setting has been able to proceed with successful reduced intensity conditioning followed by allogeneic SCT.[30] This drug is effective and has been approved also in relapsed or refractory systemic anaplastic large cell lymphoma (sALCL).

Several studies have demonstrated the activity of brentuximab vedotin in patients with HL.[17,31-36] In a phase I trial SG035-0001 of brentuximab vedotin in 45 patients with CD30 positive hematopoietic cancers (42 of whom had HL) relapsed or refractory after a median of three prior therapies, there were 11 complete remissions (CR), six partial remissions (PR), and 19 cases with stable disease.[31] Overall response rate in patients receiving 1.8 mg/kg was 50% and median duration of objective response was 9.7 months with a median progression-free survival of 5.9 months. Side effects were generally mild with the most common being fatigue, pyrexia, diarrhea, nausea, neutropenia, and peripheral neuropathy. The dose limiting toxicity at 2.7 mg/kg was acute renal failure, hyperglycemia, prostatitis, and febrile neutropenia. The maximum tolerated dose was defined as 1.8 mg/kg i.v. every three weeks.

Another phase I trial SG035-0002 was a dose-escalation study (0.4–1.4 mg/kg) of brentuximab vedotin given on days 1, 8, and 15 of 28-day cycles.[37] Forty-four patients with relapsed or refractory CD30+ hematological malignancies were included, 38 of them with relapsed or refractory HL, 68% of all patients were previously treated with HDT. The maximum tolerated dose was defined as 1.2 mg/kg, the most common side effects were peripheral sensory neuropathy (66%), fatigue (52%), and nausea (50%). The overall response rate for all dose groups was 59% (34% CR), and with a median follow-up of 45.1 weeks the median progression-free survival was 28.7 weeks and the median overall survival has not been reached yet.

In a pivotal phase II multicenter trial SG035-0003, 102 patients with relapsed or refractory HL after prior autologous SCT were treated with brentuximab vedotin (1.8 mg/kg every three weeks for up to 16 cycles).[17] The overall response rate was 75% (34% CR) with median times to objective response and CR of 5.7 and 12 weeks, respectively. Median durations of response were 20.5 and 6.7 months for those with CR and all responding patients, respectively. At a median follow-up of 18.5 months, the median progression-free survivals were 21.7 and 5.6 months for patients with CR and all patients, respectively. After responding to brentuximab vedotin, eight patients received an allo-SCT as their first subsequent therapy - five patients with CR and three patients with PR. All eight patients were alive and remained in follow-up at the time of data cut-off. The most common non-hematologic toxicities were peripheral sensory neuropathy (42%), nausea (35%), and fatigue (34%). The most common severe (grade 3/4) side effects were neutropenia (20%), thrombocytopenia (8%), anemia (6%), and peripheral sensory neuropathy (8%).

The data of the SG035-0003 were updated with a median follow-up of 33.3 months post first brentuximab vedotin dose.[38] The updated median overall survival and progression-free survival were estimated at 40.5 months and 9.3 months, respectively. Improved outcomes were observed in patients who achieved a CR, with estimated 3-year overall survival and progression-free survival rates of 73% and 58%, respectively, in this group (and medians not reached). Of the 34 patients who obtained CR, 16 (47%) remained progression-free after a median of 53.3 months of observation, 12 patients remained progression-free without a consolidative allogeneic stem cell transplant. Those patients in remission tended to be younger, predominately females, diagnosed with HL for a shorter period prior to receiving brentuximab vedotin, having a relapsed rather than refractory disease, with a lower ECOG performance score and a smaller disease burden prior to enrollment. They also received more cycles of brentuximab vedotin but with an equal incidence and severity of adverse events relative to other patients.

In another phase II multicenter trial, 25 patients with relapsed HL after prior allogeneic SCT were treated with brentuximab vedotin (1.2 mg/kg or 1.8 mg/kg every three weeks for up to 16 cycles).[32] The overall response rate was 50% (38% CR) with a median time to response of 8.1 weeks. At a median follow-up of 34 weeks, the median progression-free survival was 7.8 months and the median overall

survival has not been reached. The most common toxicities were cough, fatigue, and pyrexia (52% each), nausea and peripheral sensory neuropathy (48% each), and dyspnea (40%). The most common severe (grade 3/4) toxicities were neutropenia (24%) and anemia (20%).

Rothe *et al*.[39] reported effectiveness and safety in 16 patients with HL (14 patients) or sALCL (2 patients) who have not undergone a prior HDT from GHSG enrolled in a named patient program with brentuximab vedotin 1.8 mg/kg every three weeks. Five patients achieved CR and 6 patients PR giving an overall response rate of 69%. Six of the 16 patients proceeded to HDT with autologous transplant in 4 and allogeneic transplant in 2 patients. The 12-month overall survival for all patients was 68% and the 12-month progression-free survival 22%. The toxicity profile was similar as reported in previous studies.

Rothe *et al*.[33] also evaluated brentuximab vedotin in relapsed and refractory HL. Results were pooled from the multicentric study by the GHSG including 45 HL patients (34 patients from the named patient program) and SGN35-007 study (11 patients), 87% of them receiving a previous transplant. The overall response rate was 60%, with 22% of CR and 38% of PR. The median progression-free survival was 8 months and the progression-free survival at 12 months 43% with an overall survival of 83% at 12 months and median overall survival not reached. Grade 3-4 toxicities were of similar frequencies as reported before - there were 13% of neutropenia/sepsis, 7% of thrombocytopenia, and 7% of infections with no grade 3-4 neuropathy reported.

Brentuximab vedotin is usually well tolerated with an acceptable toxicity profile in these usually heavily pretreated patients.[17,31-33,37-43] Infusion reactions are uncommon but severe anaphylaxis has been reported.[29] Peripheral polyneuropathy is one of the most common side effects of brentuximab vedotin treatment with 53% of patients experiencing at least one treatment emerging event. However, in 80% of these patients the neuropathy presented as partially or completely reversible.[17] Concomitant administration of brentuximab vedotin and bleomycin is contraindicated due to unacceptable pulmonary toxicity. Progressive multifocal leukoencephalopathy (PML) and acute pancreatitis are rare but potentially fatal complications of brentuximab vedotin treatment.[42,43] In PML, other possible contributory factors beside brentuximab include prior therapies and underlying disease that may cause immunosuppression.[41]

Brentuximab vedotin has been available since 2012 also in Slovenia. Up till now, we have treated five adult patients with relapsed or refractory HL. All of them were heavily pretreated-in three patients brentuximab vedotin was more than third line treatment (in two of them being the seventh line of treatment), in one patient it was given following the autologous and in one patient following the allogeneic SCT. Patients received from 3 to 16 cycles of brentuximab but no CRs were achieved-there were two PRs and in two patients the disease progressed while the fifth patient is still under treatment awaiting first treatment evaluation. The treatment was very well tolerated in all patients and no dose reductions were needed except in the fifth patient who is receiving brentuximab vedotin at a reduced dosage due to a preexisting neuropathy. Regarding the inferior effectiveness in our patients in comparison with the one reported in the above studies[17,31,38] we conclude that it must be on account of the heavy pretreatment of all our patients suggesting an earlier use of brentuximab vedotin.

Conclusions

Patients with relapsed of refractory HL have limited treatment options - especially those who relapse after HDT and autologous SCT or those who have failed at least two prior multi-agent chemotherapy regimens and are not candidates for HDT. Brentuximab vedotin represents an additional treatment option with substantial effectiveness and acceptable toxicity in this select population.

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research article

[F-18] FDG-PET/CT parameters as predictors of outcome in inoperable NSCLC patients

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Background. We evaluated the prognostic significance of standardized uptake value (SUVmax), metabolic tumour volume (MTV), and total lesion glycolysis (TLG) in [F-18] FDG PET/CT findings in patients with inoperable non-small-cell lung cancer (NSCLC).

Patients and methods. One hundred and three patients (mean age, 65.6 ± 16 years) underwent [F-18] FDG PET/CT before the chemotherapy. The SUVmax value, the MTV (cm^3; 42% threshold) and the TLG (g) were registered. The patients were followed up to 18 months thereafter (range 12-55 months). Failure to respond without progression, progression and/or disease-related death constituted surrogate end-points. The optimal SUVmax, MTV and TLG cut-off to predict the patients' outcome were estimated. PET/CT results were then related to disease outcome (progression free survival; PFS).

Results. The Kaplan-Meier survival analysis for SUVmax showed a significant shorter PFS in patients presenting with lower values as compared to those with higher ($p < 0.05$, log-rank test). MTV and TLG were not suitable for predicting PFS apart from the subset of patients with mediastinal nodal involvement.

Conclusions. Despite the availability of new tools for the quantitative assessment of disease activity on PET/CT, the SUVmax rather than MTV and TLG remains the only predictor for PFS in NSCLC patients. MTV holds a value only when concomitant nodal involvement occurs.

Key words: Non-small-cell lung cancer; [F-18] FDG PET/CT; quantitative assessment; glycolytic activity; survival

Introduction

Lung cancer constitutes the most common cause of cancer death around the world and is the second most common gender unrelated cancer.[1-4] Non–small-cell lung cancer (NSCLC) includes up to 85% of all lung cancer cases.[1] The treatment and prognosis of NSCLC depend mostly upon the stage outlined according to the American Joint Committee on Cancer (AJCC) staging system.[1,5-7] Although NSCLC remains a deadly cancer, to identify prognostic factors represents a clinical challenge since a modulated therapy is still possible. The TNM staging as well as the stage grouping (I–IV) have been largely disputed so far. Other patients' specific factors such as age, pulmonary performance, and co-morbidity might also influence the selection for treatment options.[1,5] In fact, for the early-stage NSCLC, surgical resection is the standard of care, whereas in patients with unresectable, locally advanced tumour, as per stage III NSCLC, the chemotherapy in combination with radiation therapy

represents the best option.[7-9] Systemic chemotherapy is reserved for the stage IV patients.[10-12] Over the paste decade FDG (18F-fluoro-2-deoxy-D-glucose) PET/CT has demonstrated to be a powerful tool for staging and assessment of the treatment response in patients suffering from NSCLC.[13,14] Even if PET/CT is widely used in this setting, only limited data are presently available to describe in concert the role of PET/CT quantitative parameters for the prediction of the disease outcome (with special emphasis to those recently introduced).[15,16] The findings are discordant being alternatively allotted as valuable or worthless.[17-19] At this time, the metabolic tumour volume (MTV) and the total lesion glycolysis (TLG) are measures of the metabolic activity of tumours derived from the [F-18] FDG uptake on PET/CT images. Initial data have recently addressed their value in NSCLC.[20] The MTV and the TLG can be easily calculated in the primary tumour by means of a segmentation technique. The manual or semiautomatic measurement of the pre-treatment MTV has been shown to be better than SUVmax for predicting patients prognosis in different solid neoplasms such as head and neck cancer, with or without metastases.[21,22] Our study was undertaken to investigate the relationship between the functional tumour parameters at staging (SUVmax, MTV, and TLG) and the progression free survival in inoperable patients with NSCLC, presenting with stage IIIB/IV, for whom a chemotherapy (alone or in combination with radiotherapy) was shortly planned.

Patients and methods

Patients

Two hundred and ninety patients suffering from inoperable NSCLC were referred for a staging PET/CT scan prior to the start of chemotherapy between January 2008 and January 2012. Among them 103 (17 women; mean age 65,6 ± 16 years) strictly fulfilled inclusion criteria, which were: age at entry of 18 years or older; negative pregnancy test; stage IIIB and stage IV without distant metastases out of chest (inoperable tumour, CT staged, histologically proven). Patients whose lesions were excised surgically before PET/CT imaging or who had received neo-adjuvant chemo-radiotherapy within the preceding 6 months prior to PET scan were excluded from this study.

Individual data are summarized in Table 1. Our institutional review board provided approval for the procedures included in the study. All patients who underwent PET/CT scan signed an informed consent form in accordance with the Declaration of Helsinki.

Imaging technique

The patients were well-hydrated before receiving [F-18] FDG intravenously (444–555 MBq). Sixty minutes after the tracer injection, PET and CT scans were obtained using a commercial PET/CT scanner (GE Discovery VCT scanner; Waukesha, WI) that combines a PET scanner and a Light Speed VCT sixty-four row MDCT system. MDCT (pitch$_x$ 1.5; 120 mAs; 120 kVp) was performed without the use of an intravenous and/or oral contrast medium. The PET scanning was subsequently performed, acquiring 4 minutes per bed position and six to eight bed positions per patient, depending on patient height. The raw CT data were reconstructed into transverse images with a 3.75-mm section thickness. Sagittal and coronal CT images were generated by reconstruction of the transverse data. Raw PET data were reconstructed with and without attenuation correction into transverse, sagittal, and coronal images. Attenuation correction was based on the CT attenuation coefficients, which were determined by iterative reconstruction. The patients were kept fasting for at least 6 hours prior to the imaging. Blood glucose levels were measured in all patients before [F-18] FDG administration; patients with values above 7.77 mMol/L (140 ml/dl) were excluded from examination.

Imaging evaluation

All images were reviewed by using a PET/CT fusion software (Volumetrix for PET-CT, GE Healthcare, Waukesha, WI, USA). Each PET/CT study was interpreted by two experienced nuclear medicine physicians (G.S., and A.N., each with fifteen years of expertise); one of them also being a board certified radiologist. They were blinded to the patient histories. The examiners first evaluated the CT images alone. The primary lesion size was visually estimated and measured for minimum and maximum diameters by using vendor-provided software. A primary lesion was defined as solitary pulmonary nodule or peripheral nodule with an ill-defined, irregular, and spiculated border as well as identifiable mass or densitometric modifications of lung parenchyma (as per tissue thickening with or without air bronchogram findings and pseudocavitation/focal lucency, ground-glass opacification). Peribronchial/mediastinal lymph

TABLE 1. Overall patient characteristics

Number	103
Age at diagnosis, years, median (range)	68 (48–79)
Gender ratio (M : F)	86 :17
Histological type	
Adenocarcinoma (%)	67 (65)
Squamous cell carcinoma (%)	27 (26)
Broncho-alveolar carcinoma (%)	5 (5)
Adeno-squamous carcinoma (%)	2 (2)
Others (%)	2 (2)
Stage at diagnosis	
IIIB (%)	75 (73)
IV (%)	28 (27)
Chemotherapy (%)	22 (21)
Radiotherapy (%)	21 (20)
Radio-chemotherapy combination (%)	60 (59)
Locoregional/mediastinal lymph node involvement	
Yes (%)	63 (61)
No (%)	40 (39)
Final patient status	
No response/Progression (%)	12 (43)
Death (%)	16 (57)

nodes larger than 1 cm in minimum diameter and with altered shape or hilus, assessed with chest window CT settings, were considered suspicious. The PET studies were evaluated both visually and semi-quantitatively. Then, the maximum standardized uptake values and body weight corrected (SUVmax) as well as the metabolic tumour volume (MTV; cm^3; 42% threshold) and total lesion glycolysis (TLG; g) were determined by using the same vendor-provided software for the primary lesion. The MTV was defined as the volume with SUV over 42% of SUVmax. TLG was calculated as the product of SUVmean and MTV (TLG = SUVmean × MTV). Finally, lymph nodes were evaluated systematically according to the topographic criteria on the PET/CT scan using the SUVmax to determine their metabolic activity, if any.

As previously reported, a conventional SUVmax cut-off value of 2.5 (settled in studies using ROC curve analysis) has been considered to provide excellent specificity and sensitivity for detecting lesions.[15,19,23] Accordingly, a significant uptake was reported to be higher than 2.5.

Follow-up assessment

The patients were categorized into two groups according to SUVmax, MTV and TLG cut-off points determined by receiver-operator-curve (ROC) analysis. The performance status and the status of disease were followed up to 18 months thereafter (range 12–55). The evaluation was carried out by means of clinical and laboratory parameters during scheduled or unscheduled visits, on the basis of diagnostic imaging (*i.e.* CT) results, if any, as well as by phone interview. Failure to respond without disease progression, disease progression after four cycles of chemotherapy and/or disease-related death were defined as surrogate end-points. Disease progression was identified as documentation of a new lesion or enlargement of a previous existing lesion; when there was missing information, the date of unscheduled new, alternative treatment was considered. Clinical parameters and PET/CT results were then correlated to the progression free survival (PFS). PFS was defined as the time from PET/CT until end point occurrence or the time of last censor.

Statistical analysis

Continuous data were expressed as the mean ± 1 SD and median, as appropriate. Comparisons between the mean values were performed with an unpaired Student's t test (two-tailed probability) or Wilcoxon rank-sum test, according to skewness and kurtosis test for normality test. The ROC analysis was performed to estimate the optimal cut-off of SUVmax, MTV and TLG for differentiating patients at high risk of end-points occurrence. Sensitivity and specificity were computed according to the standard method. Both univariate and multivariate regression analysis were used. A multivariate binary logistic regression analysis (enter method) was used to test the chosen (homogeneously available) independent variables such as age, gender, the presence of loco-regional nodal involvement and the lesion SUVmax, MTV and TLG for their association with unsuccessful outcome. The PET/CT results were then correlated to the PFS. The Kaplan-Meier method was used to plot PFS. The predefined cut-off point for SUVmax, MTV and TLG were adopted, and the curves were compared by log-rank testing. Survival analysis was performed by univariate Cox proportional hazard regression analysis. A probability (p) value < 0.05 was considered statistically significant.

Results

Patient characteristics

Patients with NSCLC were evaluated during the study period. Among those enrolled, none had a surgical intervention. All the patients were wild-type (no EGFR mutations, no ALK rearrangements detected) and underwent chemotherapy (from 4 to

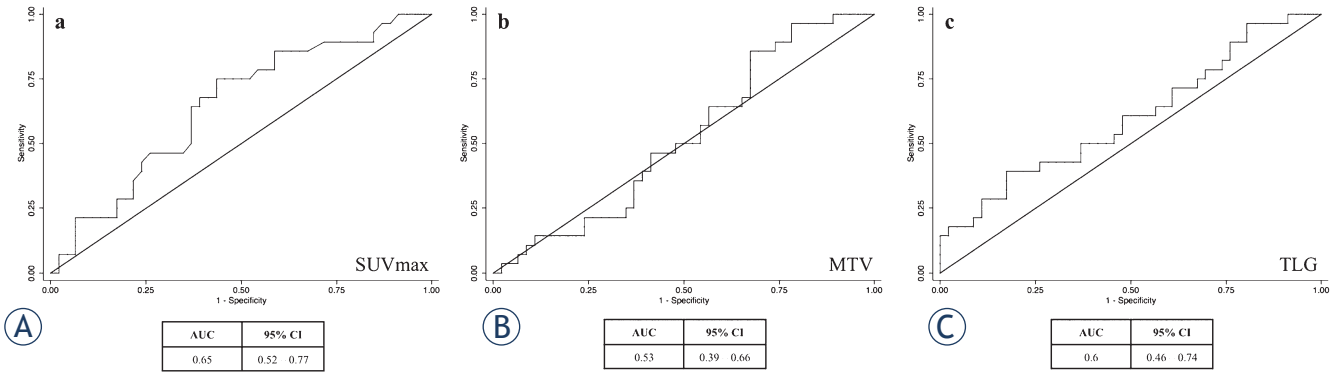


FIGURE 1. ROC curve analysis establishing the cut off value of SUVmax, MTV and TLG for predicting Progression Free Survival. The cut off value of SUVmax **(A)**, MTV **(B)** and TLG **(C)** for stratifying patients was 6.3, 8.4 (cm^3) and 259 (g), respectively.

6 cycles) after the PET/CT study; they were treated with cisplatin-based regimens with or without radiation therapy according to established dose or fractionation schedules up to 60 Gy–70 Gy (in 6–7 weeks). Overall individual patient characteristics are reported in Table 1.

Energetic turnover measurements

The PET/CT was reported as positive in all patients (i.e. significant uptake; visually detectable and SUV higher than 2.5). The median SUVmax value was 7.3 (range 2.7–44), median MTV was 16.5 cm^3 (range 3.7–38.1) and median TLG was 274.5 g (range 72.6–1039.9). According to nodal status, the median SUVmax value was 4.6 (range 2.7–23) and 10.7 (range 2.5–44) in patients without and with nodal involvement, respectively ($p < 0.05$), whereas median MTV was 15.2 (3.7–88) cm^3 and 18.6 (5.1–38.1) cm^3, respectively ($p = 0.07$). TLG values were not significantly different as well.

Prediction and discriminating values of SUVmax, MTV and TLG

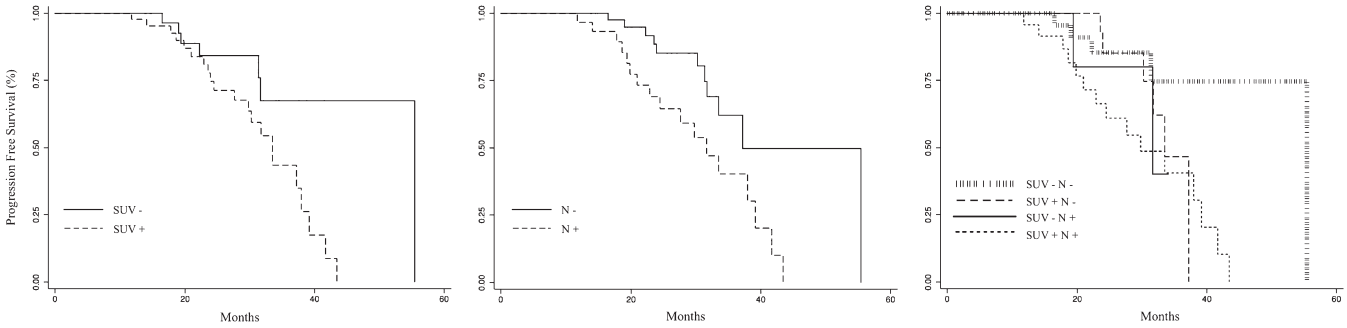
The ROC curve analysis recognizing the cut off value of SUVmax, MTV and TLG is showed in Figure 1. The area under the curve (AUC) for SUVmax was 0.64 and 6.3 was established as the cut off value. The AUC for MTV and TLG was 0.52 and 0.6, respectively whereas MTV and TLG cut off values were 8.4 and 259.0, respectively. The sensitivity of SUVmax cut-off on PET/CT for predicting the outcome was 75% whereas specificity was 57%. The sensitivity and specificity of MTV and TLG cut-off were 85% and 33%, and 61% and 52%, respectively. At univariate logistic regression analysis both the SUVmax ($p < 0.01$; OR 3.9) and presence of nodal involvement ($p < 0.001$; OR 4.3) were predictive of the end-points, whereas MTV, TLG, age and gender were not. At multivariate logistic regression analysis only the presence of loco-regional nodal involvement on PET/CT contributed to the prediction of the end points occurrence ($p < 0.05$; OR 3.2), whereas MTV, TLG, age and gender did not. The SUVmax showed a trend for predicting the outcome ($p= 0.08$; OR 2.6), (Table 2).

Clinical end points and follow up

Overall 28 of 103 patients (27%) reached the end-point, 12 experienced no response/progression (43%) and 16 (57%) died. The mean SUVmax value in patients who reached an end point was higher as compared to those who did not (11.2±4.4 vs 8.4±5.9; $p < 0.05$, Wilcoxon rank-sum test).

The median follow up was 18 months (range 12–55 months). The Kaplan-Meier survival analysis for SUVmax showed a significant difference in PFS ($p < 0.05$, log-rank test). Shorter PFS was observed in patients with lesion SUVmax over 6.3 as compared to those with SUVmax values below this value (median survival: 33 vs 41 months; $p < 0.05$, cox regression) (Figure 2a). Patients with nodal involvement showed a shorter PFS as compared to those who did not (median survival: 30 vs 37 months; $p < 0.05$, cox regression) (Figure 2b). When nodal involvement was aggregated to higher SUVmax the patients showed a shorter PFS as compared to those without nodal involvement and lower SUVmax (Figure 2c).

Noteworthy, in the sub-group with nodal involvement, patients with lower MTV showed better outcome as compared to those with higher MTV value (median MTV 10.8; range 5.1–40 vs 17.6; range 9.7–381; $p < 0.05$). At ROC curve analy-



A

CoxRegression	HR	95% CI	p
SUVmax+	2.62	1.05 – 6.5	0.037

B

CoxRegression	HR	95% CI	p
N+	2.5	1.13 – 5.5	0.023

C

CoxRegression	HR	95% CI	p
SUV- N-	1	Ref.	
SUV+ N-	2.36	.65 – 8.5	0.187
SUV- N+	2.8	.5 – 15.5	0.24
SUV+ N+	4	1.3 – 12.1	0.014

FIGURE 2. Kaplan-Meier survival graphs indicate a significant difference in PFS between the group of patients categorized by SUVmax. **(A)** Kaplan-Meier graph of SUVmax and PFS showing SUVmax above (solid line) and below (dotted line) the cut off of 6.3. **(B)** Kaplan-Meier graph of lymph-nodal involvement and PFS without (N-, solid line) and with (N+, dotted line) nodal concern. **(C)** Survival by combination of SUVmax and nodal positivity. Kaplan-Meier graph of both SUVmax and nodal positivity and Progression Free Survival. SUVmax+ and SUVmax- indicate values of SUVmax above and below the cut off value of 6.3, respectively. N+ and N- indicate the presence and the absence of locoregional lymph-nodal involvement discovered at PET/CT.

TABLE 2. Logistic regression analysis

	Risk of progression					
	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Age	0.95	0.9–1.007	0.093	-	-	-
Gender	2.25	0.75–6.76	0.149	-	-	-
SUVmax*	3.9	1.38–11.0	**0.010**	2.65	0.87–8.06	0.085
MTV*	2.9	0.85–9.88	0.088	-	-	-
TLG*	2.6	0.89–7.81	0.080	-	-	-
Lymph-node	4.37	1.6–11.95	**0.004**	3.2	1.09–9.25	**0.034**

* Dichotomized variables on ROC analysis basis; OR = odds ratio; CI = confidence interval; SUVmax = standardized uptake value; MTV = metabolic tumour volume; TLG = total lesion glycolysis.

sis for this subgroup the AUC was 0.74 and 10.9 was the cut off value. The sensitivity and specificity of MTV cut-off on PET/CT for predicting the outcome were 94% and 54%, respectively. However, in this subgroup the MTV constituted a risk factor when settled as continuous variable (hazard ratio 1.03 per unit increment; $p < 0.05$; 95% CI: 1.005–1.060), but was not able to predict PFS once used as dichotomous variable (hazard ratio 2.14; $p = 0.47$; 95% CI: 0.27–17.3).

Discussion

Current treatment approach based on stage of the disease (operable and inoperable tumours) is not entirely satisfactory in NSCLC, whilst the implementation of more novel therapeutic strategies targeting specific receptors continues to hold great promise.[24] This distressed scenario imposes the re-weighting of the prognostic factors available, especially those allowing the correct understanding of tumour biology and its therapeutic sensitivity.[25] Despite the efficacy of the new therapeutic agents, the necessity arises for directing their strength in accordance to the tumour intrinsic features since metabolic pathways differ from subject to subject, claiming for a further specific treatment approach. Nowadays, [F-18] FDG PET/CT provides a global assessment of NSCLC patients for staging, re-staging and therapy evaluation, having a prognostic value as well.[26] In addition, the PET/CT methodology allows a reproducible semi-quantitative assessment by means of several uptake indices, including the estimate of the whole tumour burden.[20] Among the PET parameters, SUVmax is the most commonly used. It reflects tumour glucose metabolism of the most aggressive cell component, given that previous studies have suggested the association between SUVmax and tumour aggressiveness.[27] Conversely, the metabolic tumour volume as well as the total lesion glycolysis allow to estimate the tumour energetic turn-over throughout the volume of the lesion above a minimum threshold designed to exclude background activity.

Since all the quantitative parameters derived from PET/CT have not yet been comprehensively investigated as prognostic factors in inoperable NSCLC patients, we evaluated, in a similar setting, the predictive impact of SUVmax, MTV, TLG in NSCLC patients, before radio-chemotherapy.

The main finding of the present study is that SUVmax constitutes the only metabolic parameter, among the others, able to predict the progression

free survival in inoperable NSCLC patients having stage IIIB or chest confined stage IV, whereas MTV holds a slightly but predictive value only in case of loco-regional lympho-nodal involvement.

According to previous studies, patients presenting with higher values of SUVmax showed a poor outcome as compared to those having lower, indicating that the high magnitude of glycolytic activity, rather than the extent of metabolic tumour burden, predicts a poor response to subsequent therapy.[28] As a result, lower SUVmax values showed a virtually "protective" significance, whereas the MTV and the TLG did not impact on the outcome of patients. Unfortunately, this dichotomous standpoint does not stratify the grey zone of patients discovered/confirmed to have nodal metabolic load (at PET/CT finding). Such an aspect has not been assessed, if not partially, in previous studies.[29] The patients who showed nodal involvement associated to higher SUVmax had similar PFS to those presenting lower SUVmax values. As expected, the "protective value" of a low SUVmax fails when a lymph nodal involvement occurs; the quote of our patients with nodal involvement had a poor outcome irrespective of SUVmax values. It is well known, however, that, apart from distant metastases, positive mediastinal lymph nodes have the most significant prognostic value for recurrence and death in NSCLC patients. Interestingly, the MTV acquired a prognostic significance in this sub-setting since it was slightly, but significantly associated with the outcome. This finding could be related to the impact of the amount of metabolically active burden when multiple lesions are concomitantly present, which better explains the risk of mediastinal nodal metastases.[30] Some authors have already reported similar results but in more compromised, surgically treated patients whose nodal involvement was confirmed by histology and, not by PET/CT.[29] Additionally, they described the incremental risk of developing nodal involvement due to MTV whereas in our setting it was related to PFS.

From a patho-physiological point of view, this finding is not surprising since SUVmax represents an index of cell glucose utilization and substantially reflects the first step of aerobic glycolysis, including cell uptake, whilst MTV better depicts the comprehensive metabolic tumour burden.

Considering the reasonable natural history of the NSCLC, it could be envisaged that the persistent metabolism of glucose to lactate even in aerobic conditions is an adaptation to intermittent hypoxia in initial lesions, those supposed to be not yet metastatic (see SUVmax significance). However, once upregulation of glycolysis has taken place, cell populations acquire acid resistant phenotypes and powerful growth advantage. Such a condition is presumably thought to favour further, continuous hypoxia moving tumour and non-tumour cells automatically toward a type of anaerobic respiration. As a consequence, SUVmax depicting the sole hottest pixel within a tumour, might no longer indicate the whole tumour burden. On the other hand, the MTV, which embodies a volumetric representation of the metabolic charge, out of the more active pixel, seems to acquire a prognostic significance when tumour has dimensionally progressed and metastasized giving an incremental risk of event per unit increase.

From a methodological point of view, it is conceivable that the lack of actually standardized criteria for determining MTV and TLG implies that these volumetric measurements might not replicate the absolute values produced elsewhere and continue to hold some intrinsic limits. However, several studies are being performed accounting for and validating their value.

The prognostic impact of F-18 FDG PET/CT quantification parameters in non-surgical NSCLC patients remains to be completely established. In view of that, our results demonstrate, as a rule, that SUVmax is a better predictive indicator of progression free survival than new volumetric parameters even if the MTV purchases a prognostic power in case of concomitant nodal metabolic concern. These findings endorse the idea that metabolic quantification parameters may be implemented in daily practice redirecting the concept that size (morphologically assessed) and histology constitute the only key-points for determining the biological aggressiveness of lung cancer.

This study included NSCLC patients that strictly fulfilled inclusion criteria (non-operable tumour) and it was performed in a single centre, which gives reason for the relatively small samples size.

Conclusions

The quantitative assessment by F18-FDG PET/CT quantification parameters may be helpful to manage inoperable NSCLC patients before chemotherapy. In this setting, the magnitude of glycolytic activity rather than the tumour burden extent seems to be generally predictive of response to subsequent therapy apart from a loco-regional metastatic concern.

Acknowledgments

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research article

Optimal scan time for evaluation of parathyroid adenoma with [^{18}F]-fluorocholine PET/CT

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Background. Parathyroid adenomas, the most common cause of primary hyperparathyroidism, are benign tumours which autonomously produce and secrete parathyroid hormone. [^{18}F]-fluorocholine (FCH), PET marker of cellular proliferation, was recently demonstrated to accumulate in lesions representing enlarged parathyroid tissue; however, the optimal time to perform FCH PET/CT after FCH administration is not known. The aim of this study was to determine the optimal scan time of FCH PET/CT in patients with primary hyperparathyroidism.

Patients and methods. 43 patients with primary hyperparathyroidism were enrolled in this study. A triple-phase PET/CT imaging was performed five minutes, one and two hours after the administration of FCH. Regions of interest (ROI) were placed in lesions representing enlarged parathyroid tissue and thyroid tissue. Standardized uptake value (SUV_{mean}), retention index and lesion contrast for parathyroid and thyroid tissue were calculated.

Results. Accumulation of FCH was higher in lesions representing enlarged parathyroid tissue in comparison to the thyroid tissue with significantly higher SUV_{mean} in the second and in the third phase ($p < 0.0001$). Average retention index decreased significantly between the first and the second phase and increased significantly between the second and the third phase in lesions representing enlarged parathyroid tissue and decreased significantly over all three phases in thyroid tissue ($p< 0.0001$). The lesion contrast of lesions representing enlarged parathyroid tissue and thyroid tissue was significantly better in the second and the third phase compared to the first phase ($p < 0.05$).

Conclusions. According to the results the optimal scan time of FCH PET/CT for localization of lesions representing enlarged parathyroid tissue is one hour after administration of the FCH.

Key words: [^{18}F]-fluorocholine PET/CT; lesions representing enlarged parathyroid tissue; triple-phase; standardized uptake value; retention index; lesion contrast

Introduction

Primary hyperparathyroidism is an endocrine disorder that develops as a result of autonomous production and secretion of parathyroid hormone (PTH) from parathyroid glands. The most common cause of primary hyperparathyroidism is solitary adenoma (80–85%). Multi-glandular disease (multiple adenomas and parathyroid hyperplasia) is much rarer (15–20%).[1] Parathyroid adenomas are benign monoclonal tumours that arise from neoplastic proliferation of a single abnormal cell. They are composed of main cells, oxyphil cells or a combination of both. In normally functioning parathyroid glands only a small number of cells are in the growth phase while in primary hyperparathy-

roidism the number of cells in the growth phase increases. The highest degree of proliferation is in parathyroid adenomas, followed by parathyroid gland hyperplasia.[2,3]

Symptomatic primary hyperparathyroidism is routinely treated with parathyroidectomy with a cure rate greater than 95% and a complication rate below 4%.[4] Traditional surgical approach was a bilateral neck exploration with identification of all four parathyroid glands.[5,6] Because most primary hyperparathyroidism cases can be attributed to a single adenoma[7], a minimally invasive parathyroidectomy with selective exploration and excision of only abnormally functioning parathyroid glands can be performed. The first minimally invasive parathyroidectomy was performed in 1996.[8] Since then, the minimally invasive parathyroidectomy became a mainstay treatment for single adenoma with primary hyperparathyroidism, providing decreased cost and patient discomfort[9,10], and similar cure rates as a classic bilateral neck exploration.[11] A prerequisite for successful minimally invasive parathyroidectomy is accurate preoperative localization of lesions representing enlarged parathyroid tissue (LREPT). [^{99m}Tc]-sestaMIBI (MIBI) single-photon emission computed tomography/computed tomography (SPECT/CT)[12] is a current gold standard for preoperative localization of LREPT with the sensitivity in the identification of a single adenoma of 80–90%.[13-15] The ultrasonography that is often used in the preoperative or intraoperative setting as an adjunct for patients with negative MIBI scans, have the accuracy in identifying single adenomas of 70–80%.[16,17] Both techniques have significantly lower accuracy for the detection of multi-glandular disease. Therefore, better imaging technique for preoperative localization of enlarged parathyroid glands is needed for wider acceptance of a minimally invasive parathyroidectomy.

[^{11}C]-choline and [^{18}F]-fluorocholine (FCH), the PET markers of cellular proliferation, was incidentally demonstrated to accumulate in LREPT.[18] Therefore; FCH was proposed for the preoperative localization in patients with a primary hyperparathyroidism.[19] In a pilot study, our group was the first to demonstrate the effectiveness of FCH in the preoperative localization of LREPT in patients with a primary hyperparathyroidism. In a group of 24 patients the performance of FCH PET/CT was superior to standard MIBI SPECT/CT particularly in patients with multiglandular disease.[20]

There are very limited data in the literature on the tissue kinetics of FCH and additionaly they are all from studies in prostate cancer patients. The time course of FCH accumulation and release from LREPT and adjacent thyroid tissue has not yet been described. The aim of present study was to determine the optimal scan time, i.e. time between radiopharmaceutical administration and FCH PET/CT imaging in patients with a primary hyperparathyroidism. At the optimal scan time the highest values of lesion contrast should be expected, but also the radiopharmaceutical properties, such as administered activity and isotope half-life as well as particular department logistics, especially radiopharmaceutical availability and scanner availability should be considered.

Patients and methods

From May 2012 to May 2014, FCHPET/CT triple-phase point imaging was performed in addition to conventional MIBI SPECT/CT and neck ultrasound in 43 patients with a biochemically proven primary hyperparathyroidism (8 male and 35 female, mean age 59.6 ± 11 years; range 36–77 years). All patients had increased levels of preoperative calcium (mean 2.8 mmol/l; range 2.6–4.1 mmol/l; normal range 2.1–2.6 mmol/l) and increased preoperative iPTH levels (mean 311.5 ng/l; range 70.6–2022 ng/l; normal range 10–65 ng/l). Patients with known history of malignant and/or inflammatory disease of the area of head and neck (other than autoimmune thyroid disease) were excluded from the study. All patients underwent surgery and had a histopathological examination of the removed parathyroid tissue. National medical ethics committee approved the study and informed consent was obtained from all patients.

After the administration of 100 MBq FCH (range: 96.8–104.5 MBq; mean 99.6 ± 2.2 MBq) the PET/CT imaging was performed at three time points: 5 minutes (first phase), one hour (second phase) and two hours (third phase). An integrated PET/CT scanner (Biograph mCT, Siemens) was used. At all three time points of the imaging process the neck and upper mediastinum were scanned in a single bed position with a scan time of 4 minutes, the time-of-flight information capture being enabled. The protocol included a low dose (120 kV; 25 mA) non-enhanced CT scan of the neck and upper mediastinum for the attenuation correction, followed by 3D PET acquisition in the same anatomical area. The images were reconstructed with iterative reconstruction with 2 iterations and 21 subsets, utilizing the scanner-specific point spread function. Data sets were reconstructed into standard 200×200×109 matrix

size using a 4×4×2 mm^3 voxel size. A 3D Gaussian post-reconstruction filtration with 4 mm full-width at half maximum was applied. All the images were acquired at the Department of Nuclear Medicine, University Medical Centre Ljubljana.

All FCH PET/CT images were masked and interpreted by two experienced observers on OASIS SEGAMI processing software. The image findings were scored for 5 different locations of LREPT: upper right/left, lower right/left and ectopic. Focally increased uptake outside the normal FCH biodistribution was estimate as positive for LREPT.

Scans of all three phases of FCH PET/CT were shown simultaneously on the monitor in the sagittal, transverse and coronary plane. Accumulated activity of FCH in LREPT was (semi)quantitatively valuated by placing a circular region of interest (ROI) adjusted to the metabolic volume of the gland. Maximum and mean standardized uptake values corrected for body weight (SUV_{max} and SUV_{mean}) were obtained and SUV_{mean} was used for analysis. In the thyroid gland, the circular ROI was positioned in an area in the lateral lobe without any thyroid pathology on metabolic and anatomical (low dose CT) images. ROIs were copied and transferred to scans of all three time points, with automatic placement of the ROI in the appropriate location through automatic linking feature and manual correction if needed. Placement of ROIs was repeated four times to evaluate potential measurement error/dispersion.

For patients positive in all three phases, SUV_{mean} values were used to calculate the retention index (RI) and the lesion contrast (LC) – a quantitative measure obtained to evaluate the differential dynamics of tracer uptake in LREPT and thyroid tissue in order to determine optimal FCH PET/CT scan time. RI determines the percentage variation of the standard uptake values in LREPT and thyroid tissue and was compared between first-second and second-third phase.[26] RI was calculated specifically for LREPT and thyroid gland. RI was calculated as:

$$RI_{t,p} = \frac{{}^{t,p}SUV_{mean}^{late} - {}^{t,p}SUV_{mean}^{early}}{{}^{t,p}SUV_{mean}^{early}} \cdot 100\,\% \qquad [1]$$

where the $RI_{t,p}$ is retention index of LREPT (p) or thyroid tissue (t), and SUV_{mean} is standardized uptake values in LREPT (p) or thyroid tissue (t) for corresponding phase.[21]

LC refers to the difference in the visual intensity of LREPT and thyroid gland in the image that corresponds to different levels of radiopharmaceutical accumulation in these tissues. LC is important for the visual assessment of FCH PET/CT images and its higher value assists in the identification of abnormalities, because the radiotracer accumulation in LREPT is higher in most cases, but may also be lower in comparison to surrounding tissue; in such cases, the lesion contrast is negative. LC was calculated as:

$$LC = \frac{{}^{P}SUV_{mean} - {}^{T}SUV_{mean}}{{}^{T}SUV_{mean}} \cdot 100\,\% \qquad [2]$$

where the LC is lesion contrast, ${}^{P}SUV_{mean}$ is mean SUV in LREPT and ${}^{T}SUV_{mean}$ is mean SUV in the thyroid tissue. LC was calculated for all three phases using the SUV_{mean} of LREPT and thyroid tissue for corresponding phase.[22]

FCH PET/CT results were compared with histopathological results, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated. SUV_{mean}, RI and LC values are shown as average ± standard deviation (range). Group means were compared by two-tailed Student's *t*-test for paired

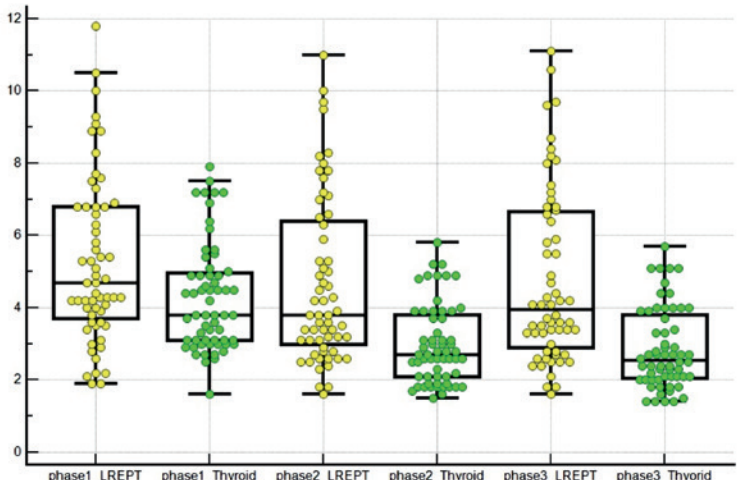

FIGURE 1. Different SUV_{mean} in all three phases of the described kinetics of FCH in LREPT and thyroid tissue.

TABLE 1. Comparison of binary classification test between all triple-phase FCH PET/CT

	5 min FCH PET/CT	1 h FCH PET/CT	2 h FCH PET/CT	Together PET/CT
Sensitivity	90.5%	93.6%	93.6%	95.3%
Specificity	98.2%	98.2%	98.2%	98.2%
PPV	96.6%	96.7%	96.7%	96.8%
NPV	94.7%	96.4%	96.4%	97.3%
Accuracy	94.1%	96.5%	96.5%	97.0%

TABLE 2. Comparison of average SUV_{mean} LREPT and thyroid tissue

Tissue	Average SUV_{mean}		
	5 min	1 h	2 h
LREPT	5.29 ± 2.29 (1.9% to 11.8%)	4.69 ± 2.31 (1.6% to 11.0%)	4.77 ± 2.39 (1.6% to 11.5%)
Thyroid tissue	4.48 ± 1.55 (2.5% to 7.9%)	3.15 ± 1.11 (1.8% to 5.8%)	3.04 ± 1.13 (1.8% to 5.7%)
p	0.03	<0.0001	<0.0001

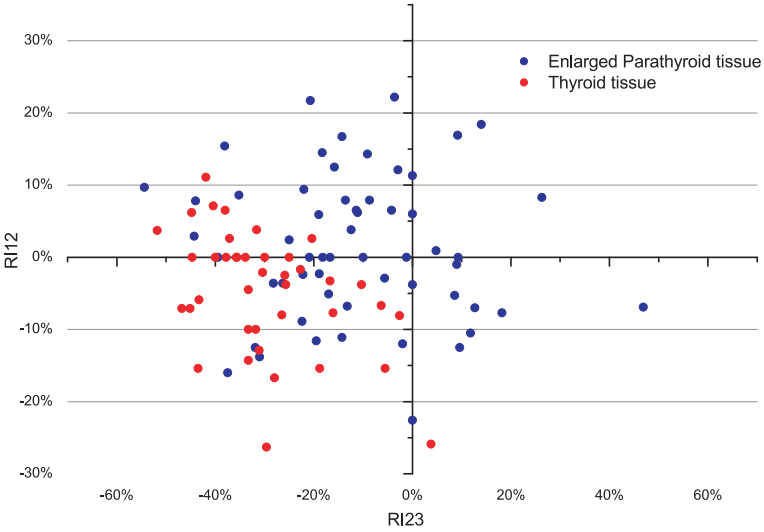

FIGURE 2. Retention index (RI) between the second and the third phase (RI23) versus RI between the first and the second phase (RI12) for all LREPT **(A)**, and for thyroid tissue **(B)**. Positive (negative) values of both RI12 and RI23 represent SUV_{mean} increase (decrease) through different phases; whereas positive (negative) RI12 and negative (positive) RI23 represent an increase (decrease) of SUV_{mean} after the first phase, followed by a decrease (increase) after the second phase.

or unpaired data, as appropriate. A *p*-value < 0.05 was considered statistically significant. Statistical analysis was performed with the use of the SPSS software (version 16).

Results

Sixty lesions of enlarged parathyroid tissue were localized by the FCH PET/CT scanning 43 patients. A primary hyperparathyroidism resolved and serum calcium normalized in 40/43 patients after surgery in which 60 parathyroid glands were removed (1.4 parathyroid gland/patient). According to histopathological diagnosis there were 34 solitary adenomas, one double adenoma, one cancer and hyperplasia in 7 patients (2/7 patients had a combination of primary and secondary hyperparathyroidism). Sensitivity, PPV, NPV and accuracy of the FCH PET/CT were higher in the second and third phase compared to the first phase (Table 1).

Triple-phase PET/CT images showed a different distribution of FCH in the LREPT in comparison to the thyroid tissue (Figure 1, Table 2). On average, SUV_{mean} in LREPT was highest in the first phase, and then decreased significantly in the second phase ($p < 0.0001$), and increased non-significantly in the third phase ($p = 0.2$). Average SUV_{mean} in thyroid tissue was also highest in the first phase, then decreased significantly in the second ($p < 0.0001$) and the third phase ($p = 0.009$). The difference of the average SUV_{mean} between the LREPT and background thyroid tissues was significant in the second and the third phase. Figure 2 shows RI between the first and the second phase and between the second and the third phase. Average RI decreased significantly in LREPT between the first and the second phase, and increased significantly between the second and the third phase (both at $p < 0.0001$) (Table 3).

Observed LC of LREPT and thyroid tissue was 31.5% ± 60.8% in the first phase, 70.4% ± 95.5% in the second phase and 75.6% ± 121.5% in the third phase (Table 4).The difference in LC was statistically significant between the first and the second phase ($p = 0.012$) and between the first and the third phase ($p = 0.015$). The positive LC had values of up to 600% and the maximum negative LC had a value of 52%. Distribution of number of lesions along the ranges of LC values in all three phases is presented in Figure 3a. Additionally, in Figure 3b, the number of both – the positive and the negative - contrast lesions having the absolute LC greater than the selected value is presented.

Discussion

MIBI SPECT/CT is the current gold standard for preoperative localization of LREPT in patients with a primary hyperparathyroidism with sensitivity in the identification of a single gland disease of 80-90%.[13-15] However, in the case of multiglandular disease the diagnostic performance of MIBI SPECT/CT is significantly lower.[23] Therefore, better imaging techniques for preoperative localization of LREPT are being searched. Among different radiopharmaceuticals tested for PET scan in preoperative diagnosis of a primary hyperparathyroidism, [^{11}C]-methionine is the most common one. However, the results of reported studies were not convincing enough to replace MIBI SPECT/CT.[24-26] The first report of FCH accumulation in

parathyroid adenomas and hyperplasia was based on incidental findings of Quak and Mapelli in patients with prostate cancer using FCH and [^{11}C]-choline.[18-19] It was our group that published the first study of comparison between FCH PET/CT and MIBI SPECT/CT and concluded that the FCH PET/CT is an accurate, efficient imaging modality for localization of hyper functioning parathyroid tissue, particularly in patients with multi glandular disease, where by its diagnostic performance is superior to the standard MIBI SPECT/CT.[20] Better spatial resolution and LC are most probably responsible for higher sensitivity of FCH PET/CT.

The aim of our present study was to determine the optimal FCH uptake period that maximizes tumor-to-normal-tissue activity ratio. In order to determine the optimal scan time, SUV_{mean}, RI and LC in LREPT and thyroid tissue were measured at three time points - 5 minutes (first phase), one hour (second phase) and two hours (third phase) after the administration of FCH (Figure 4).

Average accumulation of FCH was higher in LREPT in comparison to thyroid tissue in all three phases. However, the statistically significant difference in tracer uptake between LREPT and thyroid tissue, as assessed by SUV_{mean}, was only found in the second and the third phase; in these two phases, LC was also significantly higher in comparison to the first phase. Additionally, in comparison to LREPT a higher SUV_{mean} in the thyroid tissue was found in more than a third (18/57; LREPT positive in all three phases) of lesions (8 solitary adenomas and 10 multi-glandular diseases). An underlying thyroid disease might be an explanation for a higher accumulation of FCH in the thyroid tissue. Three of these patients indeed had autoimmune thyroiditis; unfortunately, we did not have clinical data on thyroid disease status in the rest of these patients, but there was no known history of thyroid disease.

The highest SUV_{mean} value in LREPT was achieved shortly after the FCH administration and decreased gradually between the first and the second phase in the majority of lesions. Surprisingly, in approximately half of these lesions a slight increase of SUV_{mean} value in LREPT was observed between the second and the third phase. There is very limited data in the literature on the kinetics of FCH – all these studies include prostate cancer patients. Giussani *et al.*[27] have developed a model of FCH kinetics based on biodistribution measurements that describes recirculation of radiopharmaceutical from major organs of early uptake (liver, spleen, kidneys) back into the blood pool, which

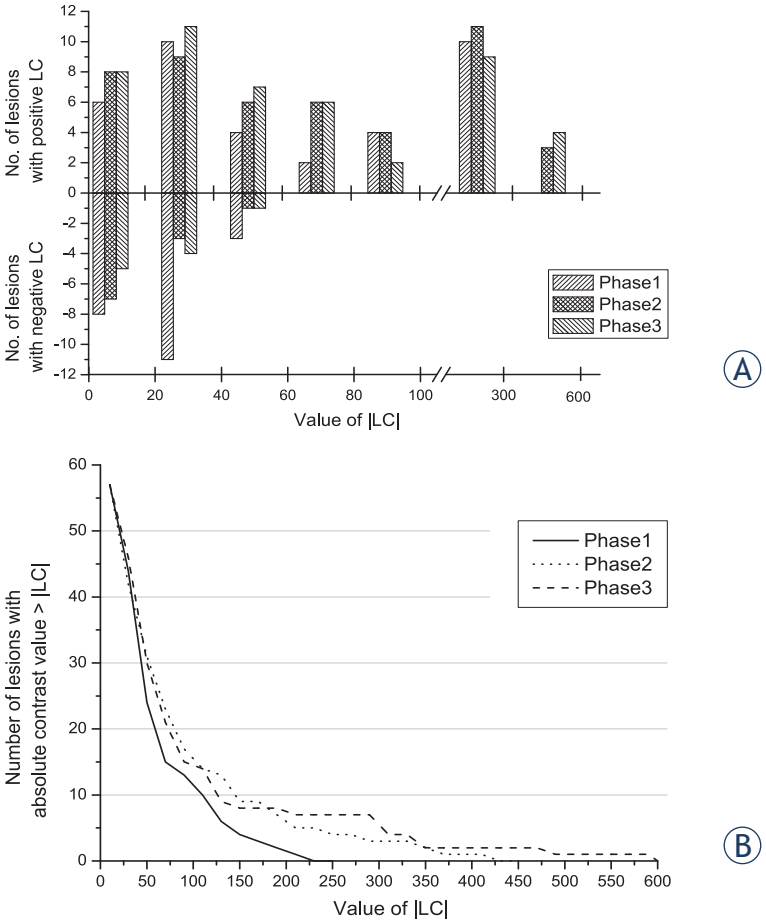

FIGURE 3. (A) The number of lesions in ranges of lesion contrast (LC) values for all three phases; for both positive and negative LC. **(B)** The number of both positive and negative lesions having absolute LC value equal or greater to the value on horizontal axis.

TABLE 3. Comparison of average RI in LREPT and thyroid tissue

Tissue	Average RI	
	between first and second phase	between second and third phase
LREPT	-11.1% ± 18.5% (-54.4% to 26.3%)	1,7% ± 10.2% (-22.8% to 22.2%)
Thyroid tissue	-29.8% ± 12.8% (-51.7% to 3.8%)	-4.5% ± 8.4% (-26.3% to 1.1%)
p	<0.0001	0.001

TABLE 4. Lesion contrast calculation and temporal comparison

	5 min	1 h	2 h
LC	31,1% ± 60.8% (-52% to 217.7%)	70.4% ± 95.5% (-44.8% to 410.5%)	75.6% ± 121.5% (-40.3% to 592.8%)

	Between first and second phase	Between second and third phase	Between first and third phase
Lesion contrast comparison *(p)*	0.012	0.8	0.015

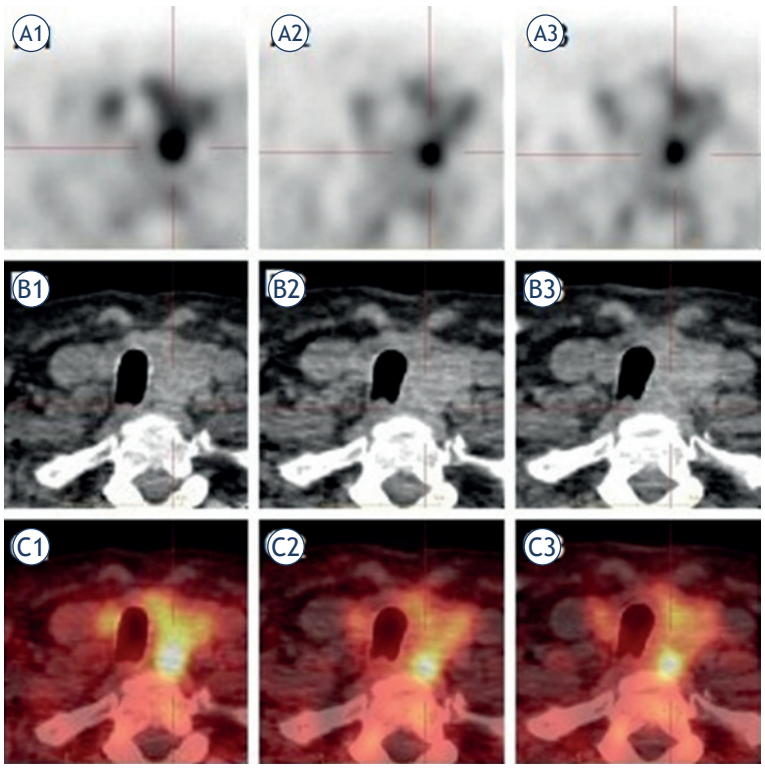

FIGURE 4. A secluded lower left LREPT. FCH PET/CT was performed in triple-phase after administration of 100 MBq of FCH. The LREPT is well delineated according to the thyroid tissue on PET axial image in the first **(A1)**, the second **(A2)** and the third phase **(A3)**. SUV_{mean} in the LREPT and the thyroid tissue was in the first phase 6.3 and 3.6, in the second phase 7.1 and 3, and in the third phase 6.6 and 2.9. The CT axial image of the LREPT and the thyroid tissue in all three phases **(B1, B2, B3)**, and the FCH PET/CT axial fusion image in all three phases **(C1, C2, C3)**.

may provide an explanation for late SUV increase in the parathyroid tissue. Tavola *et al.*[28] concluded in their study that the simple linear model cannot adequately describe the kinetics of FCH, due to non-linear kinetics, which is associated with the release of FCH from the organs back into the blood. The non-linear kinetic model caused a slight overestimation of the activity in the liver and kidneys, most probably due to a physiological activity.

In addition to a higher accumulation of FCH in most LREPT in all three phases, in comparison to the thyroid tissue, there was also a slower (efflux) release of FCH from the LREPT, reflected by the highest LC between LREPT and thyroid tissue in the third phase. However, the difference in LC between the second and the third phase is not statistically significant, allowing PET/CT investigations to be performed from one to two hours after the administration of the radiopharmaceutical. Since the daily radiopharmaceutical dose was delivered to the department in the mornings, and its activity diminished relatively fast due to the 110 minutes half-life of $18F$[29], earlier scanning times were prefered. Therefore we are suggestting the optimal scan time of one hour with 4 minutes acquisition time. Such scanning protocol did allow us to image up to 12 patients with a primary hyperparathyroidism, each with small activities (100 MBq) administration of the radiopharmaceutical.

Despite the generally higher LC between LREPT and thyroid tissue in the second and the third phase, there was a single patient in our study with the uptake only in the first phase. In this patient's case, an intense accumulation was present in the bone marrow in the second and the third phase, while almost no activity could be perceived in LREPT and thyroid tissue. A possible explanation for this unusual situation could be *polycythemia rubra vera*, which the patient was treated for. In order to avoid negative test results, early imaging might also be recommended in patients with potentially extensive hypermetabolic tissues, such as haematological and other malignancies.

Due to poor contrast between LREPT and thyroid gland in the first phase, three lesions were not localized, while in the second and the third phase the LC improved due to rapid wash out of FCH from the thyroid tissue and all three lesions were correctly localized. In one patient with hyperplasia two lesions were correctly localized as double adenomas, while two were false positive – histological results showed they were lymph nodes.

Conclusions

Preoperative localization of parathyroid glands in patients with a primary hyperparathyroidism is possible with FCH PET/CT imaging. Optimal imaging time is one hour after the administration of FCH. Due to rare comorbidities, lesion uptake may be present exclusively in the early phase (immediately after administration) therefore if logistically possible, early phase imaging is recommended as well.

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research article

Endobronchial ultrasound elastography strain ratio for mediastinal lymph node diagnosis

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Background. Ultrasound elastography is an imaging procedure that can assess the biomechanical characteristics of different tissues. The aim of this study was to define the diagnostic value of the endobronchial ultrasound (EBUS) elastography strain ratio of mediastinal lymph nodes in patients with a suspicion of lung cancer. The diagnostic values of the strain ratios were compared with the EBUS brightness mode (B-mode) features of selected mediastinal lymph nodes and with their cytological diagnoses.

Patients and methods. This prospective, single-centre study enrolled patients with an indication for biopsy and mediastinal staging after a non-invasive diagnostic workup of a lung tumour. EBUS with standard B-mode evaluation and elastography with strain ratio measurement were performed before endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA).

Results. Thirty-three patients with 80 suspicious mediastinal lymph nodes were included. Malignant infiltration was confirmed in 34 (42.5%) lymph nodes. The area under the receiver operating characteristic curve for the strain ratio was 0.87 ($p < 0.0001$). At a strain ratio ≥ 8, the accuracy for malignancy prediction was 86.25% (sensitivity 88.24%, specificity 84.78%, positive predictive value [PPV] 81.08%, negative predictive value [NPV] 90.70%). The strain ratio is more accurate than conventional B-mode EBUS modalities for differentiating between malignant and benign lymph nodes.

Conclusions. EBUS-guided elastography with strain ratio assessment can distinguish malignant from benign mediastinal lymph nodes with greater accuracy than conventional EBUS modalities. This new method may reduce the number of mediastinal EBUS-TBNAs and thus reduce the invasiveness and expense of mediastinal staging in patients with non-small lung cancer (NSCLC).

Key words: cancer staging; elastography; endobronchial ultrasound; lung cancer; needle biopsy

Introduction

Mediastinal lymph node staging is essential for optimal treatment decisions in patients with non-small cell lung cancer (NSCLC) who do not have distant metastases.[1,2] Current NSCLC guidelines recommend endosonographically guided needle biopsy of mediastinal lymph nodes as a reliable first-choice method, reducing the need for more invasive surgical staging.[1] A combination of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) can define the mediastinal lymph node stage with a sensitivity of 91% and a negative predictive value (NPV) of 96%.[1] Mediastinal lymph nodes can also be non-invasively characterised by conventional brightness mode (B-mode) EBUS.[2,3] Power/colour Doppler-mode image analysis of vascular patterns of lymph nodes can be helpful in predicting metastatic infiltration during the EBUS-TBNA procedure.[4]

Ultrasound elastography is an imaging procedure that can assess the biomechanical character-

istics of different tissues and their deformation under compression.[5] Malignant tissues are generally stiffer than native healthy tissues and can therefore be distinguished on the basis of decreased elasticity. Elastographic image analysis is based on qualitative pattern analysis and/or on semi-quantitative histogram analysis. New EBUS software supports visualisation of the tissue elasticity modulus by colour-coding tissue deformability: the hardest tissues are shown in blue, intermediate tissues in green and soft tissues in red. Moreover, it is possible to quantify the strain in two operator-selected areas. Comparing these different areas of tissue allows a numeric representation of the strain ratio between the two areas.

Ultrasound elastography has previously been applied as an external procedure (for example, in the diagnosis of thyroid, breast and prostate tumours) and as an endoscopic procedure (for example, in the diagnosis of pancreatic tumours, and nodal involvement of rectal and oesophageal cancer).[6-13] A meta-analysis of EUS elastography trials on the differentiation of benign and malignant lymph nodes reported a sensitivity of 88%, a specificity of 85% and an area under the receiver operating characteristic (ROC) curve of 0.95.[14] However, only one of the included trials evaluated the strain ratio as a diagnostic standard.[15] A recent preliminary report on endobronchial ultrasound elastography suggests that this method may improve diagnostic yield.[16]

The aim of this pilot study was to define for the first time the diagnostic value of the EBUS elastography strain ratio for mediastinal lymph node staging in patients who were suspected to have lung cancer. Diagnostic values for the strain ratios were compared with EBUS B-mode features of selected mediastinal lymph nodes and with cytological diagnosis.

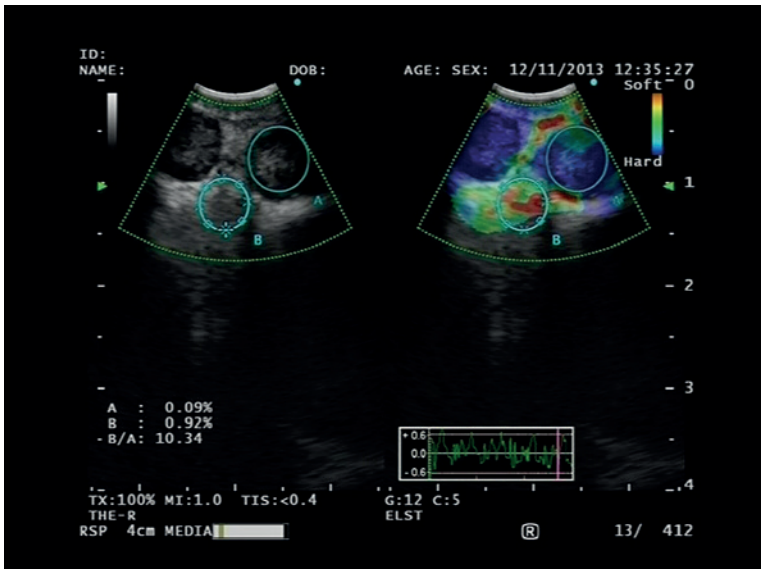

FIGURE 1. Endobronchial ultrasound (EBUS) elastography image of subcarinal lymph nodes. The image on the left-hand side displays greyscale ultrasound features. The image on the right-hand side is a superimposed elastographic image with a colour-coded scale (the hardest tissues are shown in blue and the softest in red). Symbol B/A represents the strain ratio, calculated between selected areas of the lymph node and the surrounding tissue.

Patients and methods

Patients

This prospective single-centre study was conducted between August and December 2013. Evaluations were performed on consenting consecutive patients who were at least 18 years old and been referred for bronchoscopy with a suspicion of lung cancer according to a chest CT scan. Eligible patients had enlarged but discrete N2/N3 lymph nodes, a centrally located tumour with normal-sized mediastinal lymph nodes, or enlarged N1 lymph nodes with normal-sized mediastinal lymph nodes. Exclusion criteria were metastatic disease, severe co-morbidity that disqualified surgical treatment, mediastinal tumour infiltration, and small peripheral lung tumours with normal-sized mediastinal lymph nodes.

Written informed consent was obtained from each patient prior to bronchoscopy. The study was approved by the National Medical Ethics Committee and was registered at ClinicalTrials.gov under the clinical trial number NCT02009319.

Instruments and procedure

All bronchoscopy procedures were performed under deep sedation that was carried out by an anaesthesiologist. EBUS procedures were performed with two BF-UC180F linear ultrasound bronchoscopes (Olympus Tokyo, Japan). Real-time EBUS B-mode and elastography with strain ratio measurements were performed using a pre-production model of the Endoscopic Ultrasound Center EU-Y0008 (Olympus Tokyo, Japan), which was later commercialised under the name EU-ME2 Premier Plus after minor modifications.

Mediastinal lymph nodes were initially evaluated by B-mode EBUS as in Fujiwara *et al*. for anatomical location, size, shape, border distinction, echogenicity, central hilar structure and coagula-

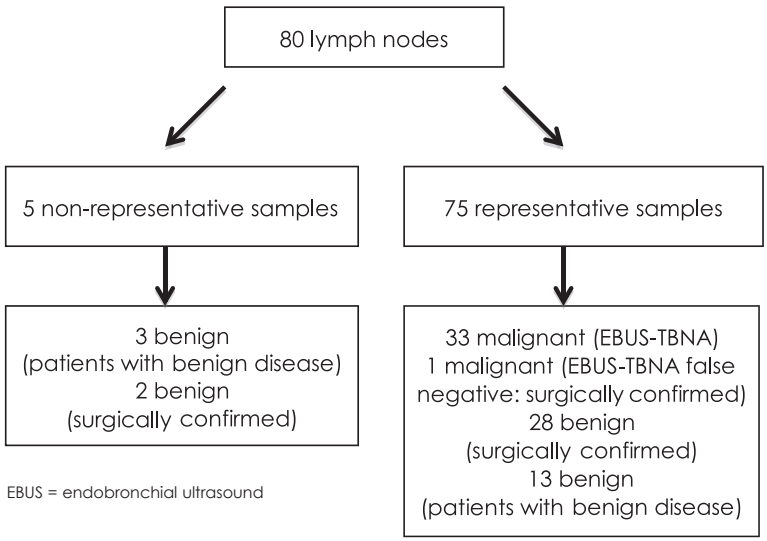

FIGURE 2. Flow diagram of lymph node confirmation.

tion necrosis.[3] A size of greater than 10 mm, round shape, distinct margin, heterogeneous echogenicity, absence of central echogenic hilum and coagulation necrosis were considered to be signs of malignant infiltration of the lymph node.

The region of interest (ROI) for the elastographic evaluation was selected using a trackball, avoiding large vessels because structures with very low elasticity might induce artefacts in the evaluation of stiffness distribution.[13] The elastography pattern as the result of tissue compression was produced by vascular pulsations and respiratory movement and not by direct bronchoscope pressure on the bronchial wall. After obtaining an artefact-free image, the "freeze" function was used, and the largest possible area of the lymph node was outlined to determine the strain. As a reference, an area of normal-appearing soft tissue from the surrounding mediastinum was selected to determine the strain ratio by EBUS processor unit (Figure 1). We selected tissue between the lymph node and bronchial cartilage or lateral to the lymph node. The strain ratio was measured twice for each selected lymph node. Whenever possible, the EBUS bronchoscope was inserted into the oesophagus to determine the strain ratio of the same lymph nodes to assess the influence of tracheal cartilage on the transtracheal measurements.

After non-invasive evaluation, EBUS-TBNA was performed using a 22-gauge needle. The pathologist who performed the cytological analysis was blinded to the strain ratio and other EBUS B-mode features.

Definitive diagnosis

EBUS-TBNA was performed at least twice per lymph node. Malignancy, where present, was accepted as a definitive diagnosis. Patients with a benign outcome from EBUS-TBNA (*i.e.*, lymphocytes) and proven cancer were sent for surgical treatment with lymph node dissection and histological examination. In cases where malignant disease was excluded, those patients were followed up meticulously until a benign outcome for the course of the disease was also confirmed.

Data analysis

Data are presented as frequencies, ranges, means ± SD and percentages. The strain ratio for each lymph node was measured twice, and the mean of both measurements was accepted for further analysis. Sensitivity, specificity, accuracy, positive predictive values (PPVs), and NPVs were calculated. ROC analysis was performed to show the specificity/sensitivity for different strain ratio cut-off values. The area under the ROC curve was calculated. The optimal cut-off value for the strain ratio was selected at the point with the highest sensitivity and specificity. A paired *t*-test was used to analyse tracheal and oesophageal measurements of the strain ratio. The analysis was performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, California, USA; www.graphpad.com).

Results

Patients and lymph nodes

EBUS elastography was performed on 80 lymph nodes at different lymph node stations in 33 patients (25 male and 8 female) with an average age of 67.5 (± 8.2) years. Twenty-seven patients had a final diagnosis of lung cancer (14 adenocarcinoma, 8 squamous cell carcinoma, 3 small cell carcinoma and 2 non-small cell carcinoma); six patients had benign diagnoses at the end of the study (2 post-tuberculotic changes, 2 pneumonia/lung abscesses, one sarcoidosis and 1 infectious mononucleosis). Cytological specimens were adequate from 75 (93.7%) lymph nodes and non-representative from five (6.3%) lymph nodes. Malignant infiltration was confirmed in 34 (42.5%) lymph nodes, primarily by EBUS-TBNA (sensitivity 97.1%, NPV 97.6%; Figure 2).

The average size of the evaluated lymph nodes was 11.1 (± 4.8) mm, with a range of 4–26 mm.

Thirty-three (41.3%) lymph nodes were larger than 10 mm, and forty-seven (58.7%) were 10 mm or smaller at the shortest diameter.

EBUS elastography strain ratio

The mean strain ratio for malignant lymph nodes was 18.96 ± 18.32 and 6.27 ± 7.30 for benign lymph nodes (Figure 3). The ROC area under the curve for the strain ratio was 0.87 (95% confidence interval [CI] 0.78–0.96, $p < 0.0001$; Figure 4). The optimal cut-off point for distinguishing malignant and benign lymph nodes, determined by a ROC sensitivity/specificity decision plot was at the strain ratio of 8 (Figure 5). Strain ratio values of 8 or higher represented the probability of malignant involvement with an accuracy of 86.25% (sensitivity 88.24%, specificity 84.78%, PPV 81.08%, NPV 90.70%; Table 1). There was no correlation between lymph node size and strain ratio when benign and malignant lymph nodes were evaluated separately.

Fifteen lymph nodes at stations 7 and 4L were evaluated through tracheal and oesophageal approaches of an EBUS bronchoscope inserted consecutively (Figure 6). The method of strain ratio measurement was statistically insignificant ($p = 0.26$), although pairs of strain ratio measurements correlated significantly ($p < 0.0001$).

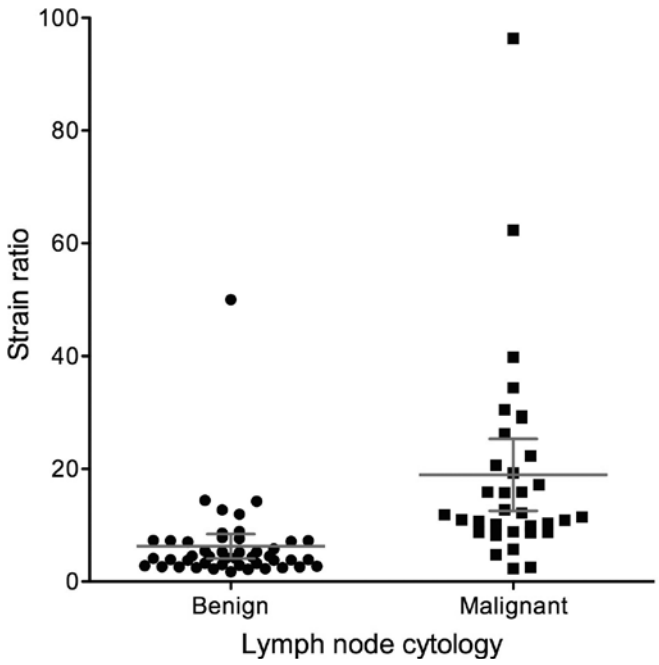

FIGURE 3. Plot of elastography strain ratio for benign and malignant lymph nodes at a cut-off of 8. Bars represent mean with 95% confidence interval (CI) of the mean.

Elastography strain ratio vs. EBUS B-mode features

The ability to differentiate between malignant and benign lymph nodes based on the elastography strain ratio was compared with the EBUS B-mode features of the same lymph nodes; the data are shown in Table 1. Elastography strain-ratio assessment was more accurate than any other lymph node characteristic assessed using EBUS B-mode criteria.

Subgroup analysis

Subsequently, a group of patients with malignant disease (lung cancer) was analysed separately. Again, the optimal cut-off point for distinguishing malignant and benign lymph nodes was at the strain ratio of 8. The ROC area under the curve for the strain ratio was 0.91 (95% CI 0.83–0.98, $p < 0.0001$). Strain ratio values higher than 8 represented a probability of malignant involvement with an accuracy of 89.39% (sensitivity 88.24%, specificity 90.61%, PPV 90.91%, NPV 87.88%).

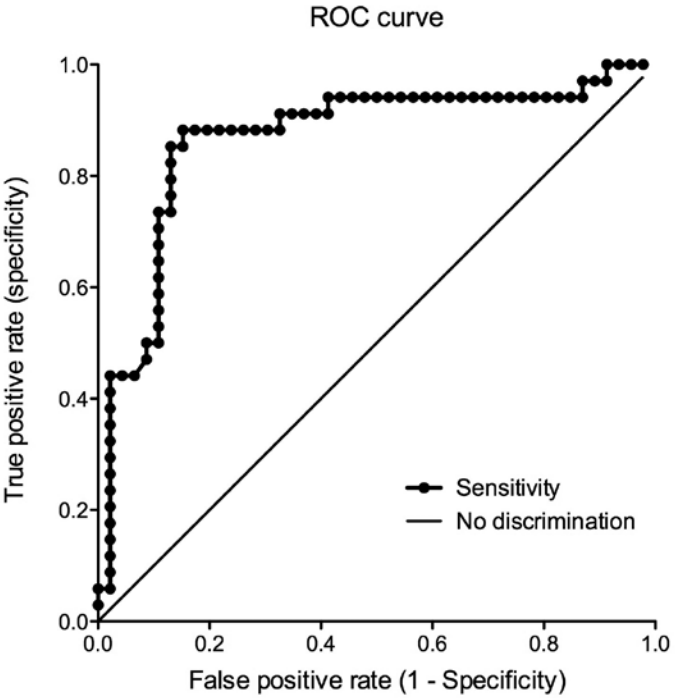

FIGURE 4. Receiver operating characteristic (ROC) curve for elastography strain ratio. Area under the ROC curve was 0.87 ($p < 0.0001$).

TABLE 1. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each endobronchial ultrasound image category for metastatic lymph nodes in comparison to strain ratio (%)

	Size (cm)	Shape	Margin	Echogenicity	CHS absent	CNS present	Strain ratio
Sensitivity	67.65	76.47	79.41	67.65	88.24	41.18	88.24
Specificity	78.26	78.26	56.52	84.78	69.57	91.30	84.78
PPV	69.70	72.22	57.44	76.67	68.18	77.78	81.08
NPV	76.60	81.82	78.79	78.00	88.89	67.74	90.70
Accuracy	73.75	77.50	66.25	77.50	77.50	70.00	86.25

CHS = central hilar structure; CNS = coagulation necrosis sign

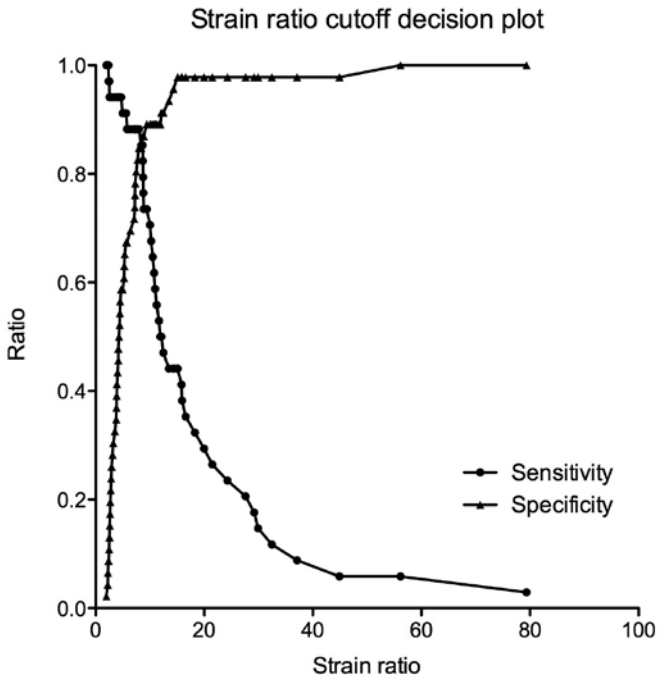

FIGURE 5. Sensitivity and specificity decision plot to determine the optimal cut-off for strain ratio. Curves cross at the strain ratio value 8.

Eleven (28.21%) of 39 normal-sized lymph nodes sampled in patients with malignant disease were metastatic. The statistical data for the strain ratios in the group of normal-sized lymph nodes were as follows: acc. 89.74%, sensitivity 90.91%, specificity 89.29%, PPV 76.92%, and NPV 96.15%.

Discussion

The strain ratio determined by endobronchial ultrasound elastography seems to be a promising new method for differentiating between malignant and benign lymph nodes in patients with lung cancer. This study confirmed the feasibility of elastography and strain ratio analysis using an EBUS bronchoscope in mediastinal lymph nodes. Moreover, the initial results showed a higher accuracy in the differentiation of malignant and benign lymph nodes than the conventional non-invasive EBUS modalities evaluated in this study.

The most accurate methods currently available for mediastinal staging in NSCLC patients are FNA under ultrasonic guidance and mediastinoscopy.[1] These methods require tissue sampling and disrupt the integrity of mediastinal structures, carrying the risk of complications such as bleeding, infection and pneumothorax.[17] Although rapid on-site evaluation (ROSE) can reduce the number of punctures, many patients are nonetheless exposed to numerous sampling procedures at several different lymph node stations and to subsequent mediastinoscopy.[18,19] Less invasive techniques for accurate mediastinal lymph node diagnosis are therefore desirable for optimal mediastinal staging.

Cervical mediastinoscopy, which was the golden standard for mediastinal staging, seems to have limited utility in the patients with T1 and T2 clinically staged N0 by positron emission tomography - computed tomography (PET-CT).[20] However, in case of CT-enlarged or PET-positive mediastinal lymph nodes, tissue confirmation is indicated.[2] Endosonography (EBUS/EUS) with fine needle aspiration is still the first choice since it is minimally invasive and has a high sensitivity to rule in mediastinal nodal disease. If negative, surgical staging with nodal dissection or biopsy is indicated. Video-assisted mediastinoscopy is preferred over mediastinoscopy.[21]

The elastography strain ratio as a semi-quantitative method has been evaluated as part of an endoscopic procedure in gastroenterology, and favourable results have been reported for mediastinal lymph node analysis in patients with oesophageal cancer.[15] The conclusions from the staging of upper

gastrointestinal cancer have been less promising.[22] Elastography without a strain ratio determination has been shown to be feasible during an EBUS procedure on a small group of patients.[16]

Our preliminary results describe for the first time the value of the strain ratio for the mediastinal staging of patients with lung cancer. A strain ratio cut-off point 8 had the highest ratio between sensitivity and specificity (sensitivity 88.24%, specificity 84.78%). However, the sensitivity and NPV of the strain ratio were still lower than for EBUS-TBNA.

In addition to 34 cytologically confirmed malignant lymph nodes, there were seven cases with false-positive elastography strain ratios. Three of these lymph nodes with prominent strain-ratio values contained substantial calcium deposits and were found in patients with post-tuberculotic lymph node abnormalities.

In four cases, the elastography strain ratio gave a false-negative result. Two lymph nodes were small and only partially overgrown with malignant tissue, which was identified upon subsequent histological examination. One of the lymph nodes was also a false negative on EBUS-TBNA. The other two lymph nodes were larger, heterogeneous and necrotic on conventional EBUS. Possible explanations for the false-negative results may lie in the highly vascularised or necrotic structure of particular nodes that appeared soft under elastographic evaluation.

In the subgroup of normal-sized mediastinal lymph nodes in patients with lung cancer, we found that 28.21% of the lymph nodes had malignant infiltration. The interesting feature was the high NPV (96.15%) of the strain ratio for this subgroup of lymph nodes, which was comparable to the EBUS-TBNA NPV. The potential role of the strain ratio may be in excluding lymph nodes with benign features from further invasive sampling. That would result in reduced invasiveness and reduced costs for mediastinal staging.

In general, EBUS elastography cannot currently replace the more accurate EBUS-FNA for mediastinal lymph node diagnosis. However, it may be useful as a supplemental method to reduce the number of punctures.[14] This method can help the operator to select stiffer sections of lymph nodes for FNA to avoid necrosis or blood vessels, thus improving the quality of the samples. It could also help in choosing the most suspicious lymph node for sampling in regions where multiple lymph nodes are present. Some punctures could possibly even be avoided in groups of normal-sized lymph nodes that have a low strain ratio.

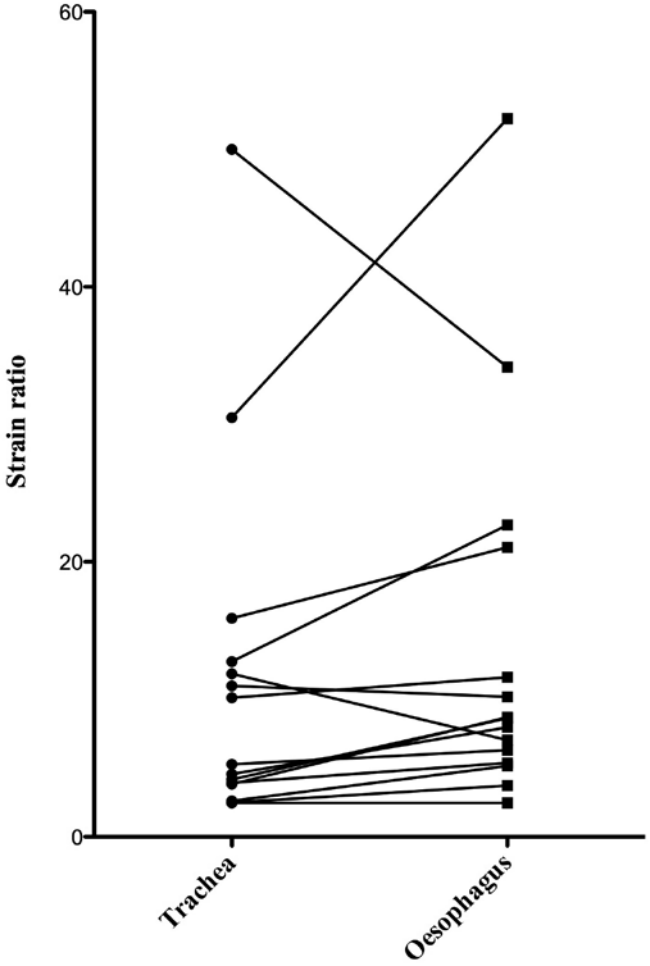

FIGURE 6. Strain ratios of the same mediastinal lymph nodes evaluated through tracheal and oesophageal approaches.

Several technical challenges were encountered during the study. Elastography relies on the movement and compression deformation of observed tissues. Obtaining a good and consistent recording was more problematic in the right paratracheal region, particularly in certain patients with more mediastinal fat tissue. Our impression was that heart and vascular pulsations were less pronounced on the right side of the trachea, and thus less tissue displacement could impede strain calculation.

In most cases, tracheobronchial cartilage did not interfere with image acquisition, and the strain ratios of the same lymph nodes measured through the trachea and through the oesophagus showed a strong correlation. However, in several patients, calcified cartilage cast intense acoustic shadows and narrowed the field of vision.

The third challenge was a scarceness of tissue in the mediastinum and the presence of large vessels. Although we tried to avoid the inclusion of large

vessels in the ROI, this was not entirely possible in all cases.

The study design had several limitations. The first limitation was that this was a single-centre, single-operator scheme, and thus no inter-observer variations were taken into account. The gold standard for the determination of malignant involvement is a combination of several methods: a positive EBUS-TBNA result (assuming that there were no false-positive results), lymph node dissection during surgery (with station evaluation rather than direct node-to-node comparison) and a final benign diagnosis in the remaining patients. Because the strain ratio was determined from "frozen" EBUS images, selection bias might be created during static image selection, especially during the selection of the reference area in the strain ratio evaluation.

In conclusion, this study shows that EBUS-guided elastography with strain ratio assessment can distinguish malignant and benign mediastinal lymph nodes with greater accuracy than conventional EBUS modalities. The high NPV for normal-sized mediastinal lymph nodes in lung cancer patients is comparable to the NPV obtained with EBUS-TBNA. This new method may potentially reduce the number of mediastinal EBUS-TBNAs and thus reduce the invasiveness and expense of mediastinal staging in NSCLC patients. Further multicentre trials are needed to confirm these preliminary results.

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research article

Analysis of risk factors for perifocal oedema after endovascular embolization of unruptured intracranial arterial aneurysms

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Background. Endovascular embolization is a treatment of choice for the management of unruptured intracranial aneurysms, but sometimes is complicated with perianeurysmal oedema. The aim of our study was to establish incidence and outcomes of perianeurysmal oedema after endovascular coiling of unruptured intracranial aneurysms, and to reveal possible risk factors for development of this potentially serious complication.

Methods. In total 119 adult patients with endovascular embolization of unruptured intracranial aneurysm (performed at Department for Interventional Neuroradiology, Clinical Center, Kragujevac, Serbia) were included in our study. The embolizations were made by electrolite-detachable platinum coils: pure platinum, hydrophilic and combination of platinum and hydrophilic coils. Primary outcome variable was perianeurysmal oedema visualized by magnetic resonance imaging (MRI) 7, 30 and 90 days after the embolization.

Results. The perianurysmal oedema appeared in 47.6% of patients treated with hydrophilic coils, in 21.6% of patients treated with platinum coils, and in 53.8% of those treated with mixed type of the coils. The multivariate logistic regression showed that variables associated with occurrence of perianeurysmal oedema are volume of the aneurysm, hypertension, diabetes and smoking habit. Hypertension is the most important independent predictor of the perianeurysmal oedema, followed by smoking and diabetes.

Conclusions. The results of our study suggest that older patients with larger unruptured intracranial aneurysms, who suffer from diabetes mellitus and hypertension, and have the smoking habit, are under much higher risk of having perianeurysmal oedema after endovascular coiling.

Key words: intracranial aneurysms; endovascular embolization; perianeurysmal oedema; hypertension

Introduction

During the past decade method of endovascular embolization became a treatment of choice for the management of unruptured intracranial aneurysms, which are increasingly being diagnosed in the era of modern imaging methods. Endovascular embolization has been validated as a minimally invasive and effective treatment that can prevent rupture of an aneurysm and intracranial haemorrhage, with shortening of hospital stay and faster patient recovery compared to the surgical treatment. Further step in development of this therapeutic procedure was design of bioactive coils, which are very effective, but sometimes followed by perianeurysmal oedema. There are a few pub-

lished case reports linking these coils with development of the perianeurysmal edema[1-7], but we are still far away from complete understanding of this phenomenon. Almost identical post embolization reactions could be seen after use of bare platinum coils[8,9], and it is not clear whether the per aneurysmal oedema is an adverse reaction to the coils itself, or just to specific type(s) of the coils.

The perianeurysmal oedema could clinically present itself with headache, lethargy, confusion, meningismus, seizures, disorders of visual function and/or cranial nerve(s) palsy.[1,2,4,5,7] Majority of the reported perianeurysmal oedema cases appeared after coiling large aneurysms[6], but oedema was also recorded after embolization of small aneurysms.[9] From the cases described so far, we could conclude that the clinical appearance, the time frame of oedema occurrence and the final patient outcome are highly variable.

Perianeurysmal oedema is relatively rare complication that follows embolization of intracranial aneurysms. Most of the patients with this complication are clinically inconspicuous, asymptomatic or with nonspecific symptoms, which leads to underestimation of the incidence and importance of this complication.[5] We are currently unaware of any risk factors that could predetermine appearance or severity of the perianeurysmal oedema after endovascular coiling.[10-13]

The aim of our study was to establish incidence and outcomes of perianeurysmal oedema after endovascular coiling of unruptured intracranial aneurysms, and to reveal possible risk factors for development of this potentially serious complication.

Material and methods

Study design

Our study was designed as case series, with an embedded case-control study. The case series consisted of patients who underwent endovascular embolization of unruptured intracranial aneurysm; those of them who developed perianeurysmal oedema were then considered to be the „cases", and the rest of them were classified as „controls". The cases and controls were not matched.

The study was approved by the Clinical Center Kragujevac Medical Ethics Committee (No. 01/8642) and was carried out according to the Declaration of Helsinki as well as we have followed the relevant guidelines in this investigation. All patients signed informed consent.

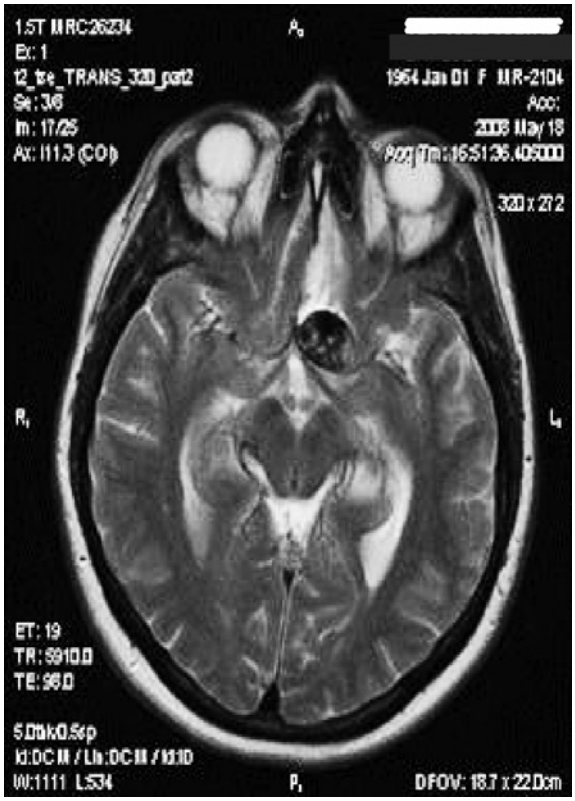

FIGURE 1. T2W axial MR tomogram of a patient showing perifocal oedema in frontal region after embolization of the left ophtalmic artery aneurysm.

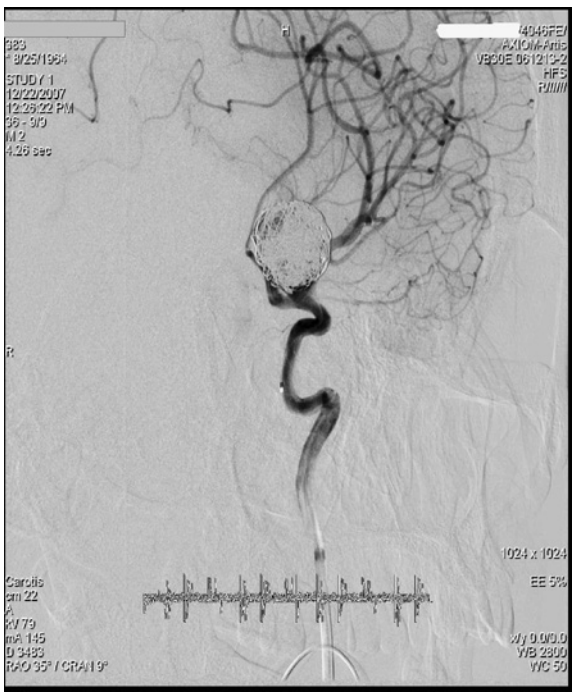

FIGURE 2. Digital subtraction angiography (DSA) of a patient after embolization of the unruptured left ophtalmic artery aneurysm, 13 x 10mm in diameter.

Study population

In total 119 patients (of both sex and older than 18 years) with endovascular embolization of unruptured intracranial aneurysm were included in our study. The embolizations were performed by experienced interventional neuroradiologists (more than 200 performed embolizations each) at Department for Interventional Neuroradiology, Clinical Center, Kragujevac, Serbia, from January 2008 to December 2012. The embolizations were made by etachable platinum coils: pure platinum, hydrophilic and combination of platinum and hydrophilic coils. In order to prevent extrusion of the coils from aneurysms with broad neck, self-expanding stents were used.

Study variables

Primary outcome variable was perianeurysmal oedema visualized by magnetic resonance imaging (MRI) imaging 7, 30 and 90 days after the embolization, using standard T1 weighted (W), T2W and fluid attenuated inversion recovery (FLAIR) sequences (Figures 1–4). There are predictor variables that were followed in the study: age of the patients, sex, arterial blood pressure, serum cholesterol level, size and location of the aneurysm, type of the coils, patient medication and smoking habits.

Statistical analysis

The study data were primarily described using medians, means and standard deviations for continuous variables, and percentages and odds for categorical variables. Normality of the data distribution was checked by Kolgomorov-Smirnof test. The differences in values of predictor variables among the study groups (patients with and without perianeurysmal oedema) were tested for significance by Student's T-test (for continuous variables) or by Chi-square test (for categorical variables). Univariate and multivariate logistic regression analyses were used to study the relationship between occurrence of perianeurysmal oedema and the predictor variables. The differences among the study groups were considered to be significant if probability of null hypothesis was less than 0.05.

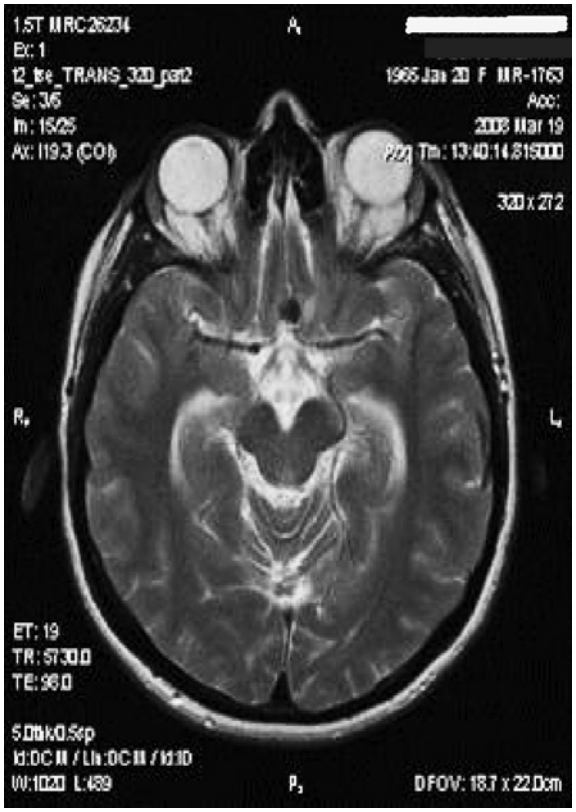

FIGURE 3. T2W axial MR tomogram of a patient showing minimal perifocal oedema in left frontal region after embolization of the anterior communicating artery aneurysm 5 x 5 mm in diameter.

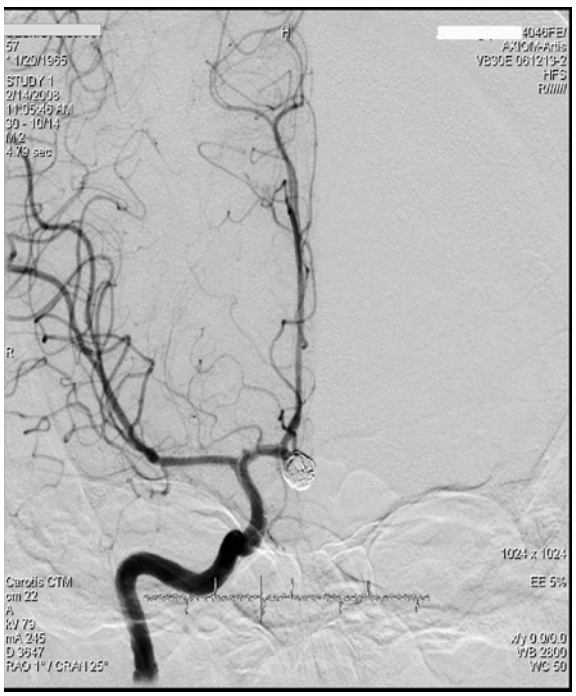

FIGURE 4. Digital subtraction angiography (DSA) of a patient after endovascular embolization of the unruptured anterior communicating artery aneurysm, 5 x 5 mm in diameter.

TABLE 1. The patients' characteristics

	Without perianeurysmal oedema; n = 74	With perianeurysmal oedema; n = 45	p
Age	46.30 ± 8.26*	56.87 ± 9.33*	***0.0005
Gender (F)	26 (35.1)	20 (44.4)	0.414
Hypertension	43 (58.1)	44 (97.8)	***0.0005
Diabetes	8 (10.8)	20 (44.4)	***0.0005
Smoking	39 (52.7)	34 (75.6)	0.022
Hypercholesterolemia	19 (25.7)	30 (66.7)	***0.0005
Corticosteroids before the embolization	29 (39.2)	13 (28.9)	0.346
Volume of an aneurysm	75 (33 – 154)**	518 (215 – 898)**	***0.0005
The coil type:			***0.006
- hydrophilic	22 (52.4%)	20 (47.6%)	
- platinum	40 (78.4%)	11 (21.6%)	
- mixed	12 (46.2%)	14 (53.8%)	

* = mean ± SD, ** = median (25-th percentile – 75-percentile); *** = significant difference categorical variables shown as n (%);

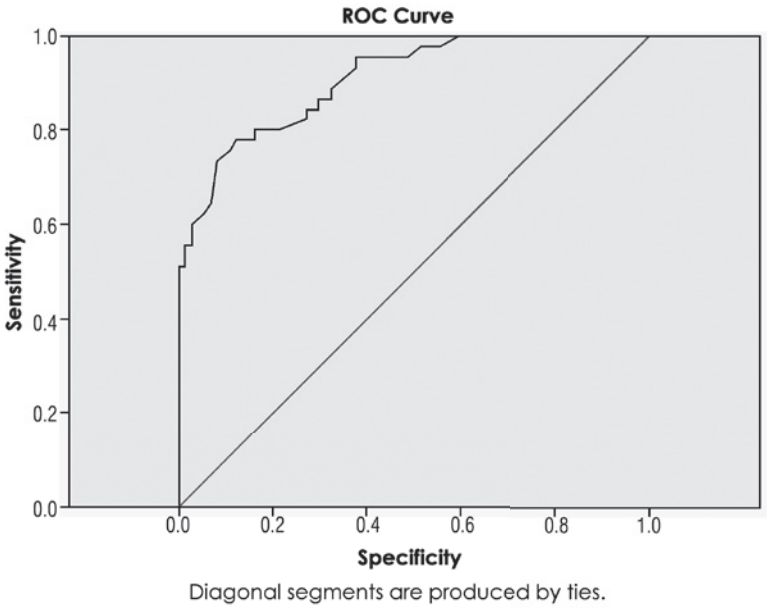

FIGURE 5. The receiver-operator curve (ROC) for volume of the intracranial aneurysms and occurrence of the perianeurysmal oedema after endovascular embolization.

Results

The perianeurysmal oedema was observed by NMR imaging in 45 of 119 patients (37.8%). However, only 8 patients (6.7%) developed symptomatic oedema, with transient headache and malaise. The symptoms withdrew spontaneously in the next 48 hours. The characteristics of the patients with and without the perianeurysmal oedema are shown in Table 1. Location of the aneurysms was similar in the both groups (p = 0.268), but type of the coils determined occurrence rate of the perianeurysmal oedema: it appeared in 47.6% of patients treated with hydrophilic coils, in 21.6% of patients treated with platinum coils, and in 53.8% of those treated with mixed type of the coils. However, the only significant difference in rate of perianurismal oedema was between the patients treated with platinum coils and the patients treated with mixed type of coils. All of the patients with symptomatic oedema were treated with mixed type coils.

From the Table 1 one could see that the higher the volume of an aneurysm, the perianeurysmal oedema is more frequent. If a Receiver-Operator Curve (ROC) is constructed for volume of the aneurysms and occurrence of the oedema after endovascular embolization (Figure 5), it turns out that growth of volume of aneurysm for 1 mm^3 increases risk of perianeurysmal oedema for 1.3% (AUROC = 0.908, p = 0.0005). If a cut-off value of 174 mm^3 is taken into account, the volume of an aneurysm could predict occurrence of perianeurysmal oedema with sensitivity of 80.08%, and specificity of 83.8% (positive predictive value is 75.0%, and negative predictive value is 87.3%).

The results of univariate and multivariate binary logistic regression for occurrence of perianurysmal oedema as dependent variable are shown in Table 2. After adjustment for other followed variables, the multivariate regression showed that variables associated with occurrence of peri-

TABLE 2. Binary logistic regression for occurrence of perianeurysmal oedema as dependent variable

	Univariate analysis		Multivariate analysis	
	p	Odds ratio (CI)	p	Odds ratio (CI)
Age	0.0005	1.146 (1.085 – 1.211)		
Hypertension	0.001	31.721 (4.144 – 242.784)	0.022	30.599 (1.624 – 576.504)
Smoking	0.015	2.774 (1.223 – 6.291)	0.026	5.391 (1.226 – 23.710)
Diabetes	0.0005	6.600 (2.577 – 16.901)	0.039	5.336 (1.091 – 26.099)
Hyperholesterolemia	0.0005	5.789 (2.575 – 13.015)		
Volume of the aneurysm	0.0005	1.010 (1.006 – 1.015)	0.0005	1.013 (1.006 – 1.019)

aneurysmal oedema are volume of the aneurysm, hypertension, diabetes and smoking habit. Hypertension is the most important independent predictor of the perianeurysmal oedema (30-fold increased risk), followed by smoking and diabetes (about 5-fold increased risk with each of the variables).

Discussion

Most of previous reports on perianeurysmal oedema after endovascular coiling described patients with developed clinical picture.[4,5,9] However, perianeurysmal oedema occurs also in asymptomatic and patients with mild non-specific symptoms, so frequency and importance of this phenomenon could be underestimated. According to scarce published data, the oedema could be found in 9% of asymptomatic patients[3], but our data showed much higher rate of 37.8%. Considering recently found link between occurrence of perianeurysmal oedema after endovascular embolization and recurrence of anurysm[14], it becomes very important to identify risk factors for the oedema and to modify them with an aim to prevent its occurrence.

Mechanism of perianeurysmal oedema development remains unclear for the time being, but it seems that inflammation plays very important role. Several mechanisms have been proposed to explain perianeurysmal oedema. It may represent a normal healing response after coiling and is probably related by the inflammatory changes. This inflammatory response can be exaggerated in some cases, leading to peryaneurysmal oedema. In other cases, oedema is observed several months after aneurysms treatment and is generally associated with massive recurrence. Oedema can develop also immediately after treatment due to thrombus formation and secondary expansion of aneurysmal sac. It was described around both coiled and uncoiled aneurysms, but the uncoiled ones were invariably thrombosed. If the aneurysm was not thrombosed completely, continuous water-hammering effect against the residual lumen of the aneurysm (aneurysm pulsing) could cause haemorrhage in the aneurysmal wall, triggering inflammation.[15] This happens mostly in larger aneurysms with wide neck, which explains why in our study the aneurysms with larger volume were more frequently followed with perifocal oedema after embolization. Moreover, larger aneurysms have bigger endothelial surface, which when damaged by coils and blood jet produces larger quantity of autacoids that can initiate process of inflammation.[1] As a confirmation of these hypotheses, both release of cytokines to cerebrospinal liquor[13] and inflammation of meninges[12] were found in patients with perianeurysmal oedema after endovascular coiling. However, although clinical improvement of perianeurysmal oedema was demonstrated in some studies[12,13], others did not confirm such beneficial effect.[15] In our study pre-embolization administration of corticosteroids did not affect the occurrence of perianeurysmal oedema.

Older patients have higher risk for perianeurysmal oedema after embolization[6,8,9,10]; the patients with oedema in our study were on average 10 years older than the patients without oedema. Hypertension, diabetes mellitus and smoking are also risk factors for perianeurysmal oedema, according to our and some other studies.[6] This is not surprising, since all these factors adversely affect microcirculation[16,17] leading to increased permeability of capillaries and propensity for inflammation and oedema formation.

Perianeurysmal oedema could be observed after endovascular embolization with any type of coils.[1-11] However, certain studies hypothesized that bioactive coils increase the risk for the oedema up to nine-fold.[8,10,12] In our study bare platinum coils did have the smallest rate of perianeurysmal oedema, but the difference was not significant in comparison to the hydrophilic coils. This issue requires further research before any kind of recommendation about choice of coil type could be made.

According to published data the incidence of perianeurysmal oedema is much lower, from a few percentages to 14, 3%.[18,19] However all published cases were symptomatic, presented commonly with headache, malaise or cranial neuropathies. Perianeurysmal oedema is actually much more frequent, but mostly remains asymptomatic (as shown in our study), and is rarely followed by serious complications.

The results of our study suggest that older patients with larger unruptured intracranial aneurysms, who suffer from diabetes mellitus and hypertension, and have the smoking habit, are under much higher risk of having perianeurysmal oedema after endovascular coiling. Such patients should be more strictly controlled by MRI imaging after the embolization, in order to reveal the oedema earlier, and adjust the care for the patient accordingly.

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research article

Estimation of cell response in fractionation radiotherapy using different methods derived from linear quadratic model

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Background. The aim of this study was to use various theoretical methods derived from the Linear Quadratic (LQ) model to calculate the effects of number of subfractions, time intervals between subfractions, dose per subfraction, and overall fraction time on the cells' survival. Comparison of the results with experimental outcomes of melanoma and breast adenocarcinoma cells was also performed. Finally, the best matched method with experimental outcomes is introduced as the most accurate method in predicting the cell response.

Materials and methods. The most widely used theoretical methods in the literature, presented by Keall *et al.*, Brenner, and Mu *et al.*, were used to calculate the cells' survival following radiotherapy with different treatment schemes. The overall treatment times were ranged from 15 to 240 minutes. To investigate the effects of number of subfractions and dose per subfraction, the cells' survival after different treatment delivery scenarios were calculated through fixed overall treatment times of 30, 60 and 240 minutes. The experimental tests were done for dose of 4 Gy. The results were compared with those of the theoretical outcomes.

Results. The most affective parameter on the cells' survival was the overall treatment time. However, the number of subfractions per fractions was another effecting parameter in the theoretical models. This parameter showed no significant effect on the cells' survival in experimental schemes. The variations in number of subfractions per each fraction showed different results on the cells' survival, calculated by Keall *et al*. and Brenner methods ($P<0.05$).

Conclusions. Mu *et al*. method can predict the cells' survival following fractionation radiotherapy more accurately than the other models. Using Mu *et al*. method, as an accurate and simple method to predict the cell response after fractionation radiotherapy, is suggested for clinical applications.

Key words: fractionation radiotherapy; survival; dose per fraction; number of fractions; linear quadratic model

Introduction

Radiotherapy is one of the main procedures of cancer treatment. The goal of radiotherapy is to deliver as much dose to the tumor site while keeping the dose to the surrounding normal tissues as low as possible.[1, 2] In radiotherapy, in addition to the conventional techniques used in clinical practice, some state of the art specialized techniques such as Intensity Modulated Radiation Therapy (IMRT),

Respiratory-Gated, stereotactic, and Image Guide Radiotherapy (IGRT) have also been developed.[3-7] These modern techniques optimize the radiotherapy dose distribution since they include more segments in the radiation field which are usually shaped using more complicated equipment.[3-7] These techniques enhance tumor local control and have lower radiation-induced toxicities in normal organs around the tumor compared to conventional techniques. Moreover, they vary in the dose delivery due to using more subfractions per each treatment fraction, different treatment times between subfractions, and the prolonged treatment time of one fraction.[4,8-15]

The radiobiological efficiency of these techniques might be different from conventional one mainly due to the repair of sublethal damages.[8-16] However, the rate and the mechanism of repair is a complicated function of different parameters such as dose per fraction, dose rate, repairs half time, and state and nature of the organs of interest (*i.e.* α/β ratio of the organ).[8-16]

To predict the results of different radiation delivery procedures on the cells' survival, the basic theoretical model is the incomplete repair model of Thames[17] generalized to multiple fractions by Nilsson *et al*.[18] that is a developed form of Linear Quadratic (LQ) model. Some studies have investigated the effects of prolonged time of radiation delivery on the survival of some cell lines and compared the results with theoretical methods derived from the LQ model.[8-15]

Although these theoretical methods are all derived from the basic LQ model, however, the rate of agreement between their results in researches and experiments was significantly different for diverse dose schedules.[8,9,19-21]

Therefore, more investigations are needed in order to evaluate the effect of various treatment factors on the cells' survival. In addition, it seems beneficial to compare the results of these methods theoretically and experimentally in order to find the best method that can be used to predict the cells' survival after different fractionation radiotherapy schemes.

The aim of this study was to compare various theoretical methods widely used in the literature[8,9,19-21] to estimate the effects of number of subfractions, time intervals between subfractions, dose per subfraction, and overall fraction time on the F10B16 skin melanoma and 4T1 breast adenocarcinoma cells' survival. Comparison of the results with experimental outcomes of melanoma and breast adenocarcinoma cells was also performed. Moreover, in this work, the best matched method with experimental outcomes is introduced as the most accurate one in predicting the cell response in fractionation radiotherapy.

Materials and methods

Theoretical methods

Three methods of calculation derived from LQ model, presented by Keall *et al*., Brenner, and Mu *et al*.[8,9,19-21], were compared to investigate the effect of different dose schemes (dose per subfraction, time intervals between subfractions, total treatment time of each fraction) on the survival of F10B16 skin melanoma and 4T1 breast adenocarcinoma cells. The basic idea of these methods is based on the completed LQ model as:

$$S = \exp(-\alpha D - G\beta D^2) \quad [1]$$

Which is a developed form of the basic LQ model:

$$S = \exp(-\alpha D - \beta D^2) \quad [2]$$

Where α and β are cell parameters, D is the total dose delivered to the cells, S is the survival fraction of cells, and G parameter is defined in intermittent radiotherapy to investigate the effect of subfractions. The G parameter has been formulated differently by various investigators.[8,9,19-21]

The first method (method I) was presented by Keall *et al*.[9] They have experimentally and theoretically investigated the temporal effects of respiratory-gated and IMRT treatment delivery for dose of 2 Gy and in the total treatment times of 1.67 min (in conformal radiotherapy) to 15 min (in gated IMRT) on the cells' survival. Keall *et al*. have used a simplified form of G to predict the cells' survival and have compared the outcomes with experimental results.[9] They have assumed negligible cell proliferation and unchanging radiosensitivity.[9] According to Keall *et al*. study, the G parameter is calculated as[9]:

$$G = \frac{1}{n}\left[2\left(\frac{\mu\tau-1+\exp(-\mu\tau)}{(\mu\tau)^2}\right) + 2\left(\frac{\cosh(\mu\tau)-1}{(\mu\tau)^2}\right) \times \left(\frac{2}{n}\left(\frac{\emptyset}{1-\emptyset}\right)\left(n - \frac{1-\emptyset^n}{1-\emptyset}\right)\right)\right] \quad [3]$$

Where

$$\emptyset = \exp\left(-\mu(\tau + \Delta t)\right) \quad [4]$$

In this method, μ is the rate constant for repair of sublethal damages, n is the number of subfrac-

tions, τ is the time of exposure and Δt is the time between subfractions. This method assumes a constant value for both exposure time (t) and the time between exposures (Δt).[9] Keall *et al*. results showed no significant difference between the experimental observations and theoretical calculations.[9] Moreover, this method indicated a good agreement with experimental results for the total dose of 2 Gy.[9]

The second method (method II) was utilized by Brenner.[20] This method was also proposed in some review papers.[19,21] Brenner has simplified the LQ model and experimentally and theoretically investigated the temporal effects of fractionation treatment delivery on *in vitro* survival.[20]

In Brenner method, the G factor accounts for fraction protection and acts on the quadratic component as follow[20]:

$$G = \frac{2}{\tau^2}\int_0^{\tau}\int_0^{t} e^{-\mu(t-t')}dt'\,dt = \frac{2}{(\mu\tau)^2}(\mu\tau + \exp(-\mu\tau) - 1) \qquad [5]$$

In this method, the used parameters are the same as Keall *et al*. method.[9] As this formula (equation 5) shows, the effects of time intervals between subfractions are ignored, however, Brenner has confirmed that there was a good agreement between the outcomes of this formula and the experimental results.[20] Therefore, it has been proposed that, this formula can be used to calculate the cell response after prolonged treatment delivery.[20] In addition, this method can be employed to calculate the protraction effects in a single fractionation delivered at a constant rate, splitting dose, multi-fraction irradiation protocols and continuous low dose rates radiotherapy such as brachytherapy.[20]

The third theoretical method (method III) was reported by Mu *et al*.[8] In Mu *et al*. study, the G parameter is defined as below[8]:

$$G = \frac{2}{n^2}\left[\frac{\exp(-\mu\,\Delta t)}{1-\exp(-\mu\,\Delta t)}\right]\left[n - \frac{1-(\exp(-\mu\,\Delta t))^n}{1-\exp(-\mu\,\Delta t)}\right] + \frac{1}{n} \qquad [6]$$

All the used parameters in this method are explained above. In this method, it is assumed that there is no recovery during actual irradiations but rather during the time between subfactions.[8]

Cell culture and assay

The cells were cultured in plastic flasks at 37°C in a humidified atmosphere of 50 mL/L CO2 and 95% air with the RPMI1640 medium containing 10-15% fetal calf serum (FCS or FBS) with 100 U/mL penicillin and 100 μg/mL streptomycin.

TABLE 1. The F10B16 and 4T1 cell parameters as input data for the used models

Symbols (unit)	Definitions	F10B16	4T1
α (Gy^{-1})	Linear parameter of LQ model	0.0956	0.0424
β (Gy^{-2})	Quadratic parameter of LQ model	0.0177	0.0399
$T_{1/2}$ (hour)	Half time of sublethal repair	0.524±0.035	0.344±0.015

Due to tree shaped structure of these cell lines, complexity of counting their colonies, and significant number of samples used in this study, an automated and faster assay method was used. Therefore, instead of the clonogenic assay, the multi 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT) assay was used. This method was offered in other similar researches[22-24] and all the details of experimental procedure are published in papers by our team for these two cell lines (F10B16 melanoma and 4T1 breast adenocarcinoma) of interest.[25,26]

Theoretical schemes

In this paper, α and β parameters were calculated using the basic LQ model (equation 2). Hence, the cell survival fractions (S) following doses of 2, 4, 6, 8 and 10 Gy were experimentally determined for both melanoma and breast adenocarcinoma cells and then inserted in the basic formula of the LQ model. Using the S and D parameters and inserting them in the mentioned formula, the survival curves of cell lines were drawn and α and β parameters were derived using the MATLAB software (Version 7.11, R2010b, MathWorks, USA).[8,25]

In order to determine the time constant for repair of sublethal damage ($T_{1/2}$), the cells were exposed in two fractions with different time intervals between the fractions. Then, the surviving fraction was plotted against the time between fractions and finally the half value of sublethal damage repair was investigated.[8,25]

All the cell's parameters for both cell lines of interest, used in this study, are illustrated in Table 1.

Different treatment schemes were designed in order to investigate the effects of the most important radiobiological parameters including; the number of subfractions, time intervals between subfractions, subfraction doses, and overall treatment time, in complex radiotherapy practices.

To investigate the effect of total treatment time, the survival fraction (SF) were calculated for dose of 2, 4 and 6 Gy in two subfractions of 1, 2 and 3 Gy, respectively. The overall treatment times were

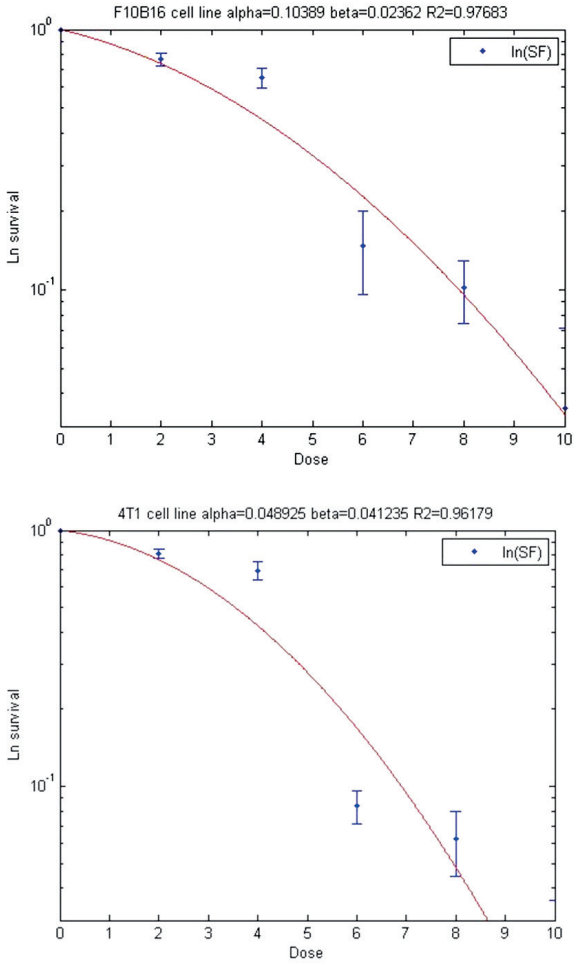


FIGURE 1. The survival curves of the **(A)** melanoma F10B16 and **(B)** breast adenocarcinoma 4T1 cell lines (R^2 is a statistical measure of how close the data are to the fitted regression curve. It is also known as the coefficient of determination).

ranged from 15 to 240 minutes. Although, total treatment time in complicated radiotherapy is about 1 hour and longer treatment time is not practical, however, we followed the investigations for up to 4 hours to determine comprehensive results and investigate the ability of the developed models to predict the cells' survival.

To investigate the effects of increasing the number of subfractions and dose per subfraction, the survival was calculated for total dose of 2, 4 and 6 Gy in 4 and 8 subfractions as follow: 4 fractions of 0.5 Gy and 8 fractions of 0.25 Gy (both for a total dose of 2 Gy), 4 fractions of 1 Gy and 8 fractions of 0.5 Gy (both for a total dose of 4 Gy) and 4 fractions of 1.25 Gy and 8 fractions of 0.75 Gy (both for a total dose of 6 Gy). They all delivered through fixed overall treatment times of 30, 60 and 240 minutes.

It should be noted that the theoretical methods presented by Keall *et al*., Brenner, and Mu *et al*. can be used in predicting survival in fractionation radiotherapy and some of them have flaw in predicting survival when the dose is delivered continuously in one fraction.[8,9] Therefore, in this work, the basic LQ model (equation 2) was used to predict the cell survival following continuous dose delivery.

Experimental schemes

The cells were picked out from the flasks when they reached to linear phase of exponential grow in the day before irradiation and were put in 96 well plates with density of 1000 cells in each well.[22-25] There were 7 samples for each experiment and, to avoid the variability inherent to the assay used, all tests were performed for 3 independent experiments. A Co-60 source with a dose rate of 0.81 Gy/min was used for irradiation. The ionizing radiation was delivered in a 25×25 cm^2 field size. All irradiations were performed at a distance of 20 cm between the radiation sources and plate.

To measure the absorbed dose rate of the Cobalt-60 beam, a Farmer-type ionization chamber with a standard ^{60}Co buildup cap, and positioned in air using a customized stand, was used. "For traceability to international standards, the ionization chamber was calibrated in comparison with the response of the Secondary Standard Dosimetry Laboratory (SSDL, Karaj Complex, Atomic Energy Organization of Iran) reference and working standard ionization chambers in the ^{60}Co gamma ray beam of a teletherapy unit. All of the SSDL ionization chambers used for calibrations are themselves calibrated at the International Atomic Energy Agency (IAEA) dosimetry laboratory".[27]

To design the experimental tests, firstly, continuous radiation with doses of 2, 4 and 6 Gy, similar to conventional radiotherapy techniques, were delivered to the cells. Next, to investigate the effect of overall treatment time on the cells' survival, the same as the theoretical schedules, 6 groups from both of the studied cell lines were exposed to 2, 4 and 6 Gy in two subfractions with dose of 1, 2 and 3 Gy, respectively. In this step, the overall treatment time was 15 to 240 minutes. Then, to simulate the effects of the number of subfractions as well as dose per subfraction, 4 and 8 subfractions with dose of 1 and 0.5 Gy, respectively (total dose of 4 Gy), were delivered to the cells at overall treatment times of 30, 60 and 240 minute. After that, the results were compared with those of continuous radiation.

It should be noted that, although the conventional treatment dose used in clinical situation is approximately 2 Gy per fraction[8,9], however, the

effect of this low level of dose on the cell culture environment was negligible for the two cell lines of interest (Figure 1), and consequently the dose of 4 Gy was used in this experiment.

Statistical analysis

Statistical analysis was performed using the SPSS software version 14 (SPSS, Inc., Chicago, IL, USA). To assess the effects of different irradiation protocols, the analysis of variance (ANOVA) was used. A significant level of 0.05 was considered to the tests.

Results

Figure 1 illustrates the survival curves for the two cells of interest as well as the calculated α and β parameters. Figures 2, gives the survival of the cells in continuous radiation with dose of 2, 4 and 6 Gy and also in fractionation delivery in two subfractions of 1, 2 and 3 Gy, during the overall treatment time of 15 to 240 minute. Figures 3 to 5, show the predicted survival using the theoretical methods of Keall *et al.*, Brenner, and Mu *et al.*, as well as the experimental results. For a total dose of 2 Gy and all irradiation times in both two cell lines of interest (4T1 and F10B16), there was no significant difference ($P<0.05$) between the calculated survival by the three used methods (Figure 3).

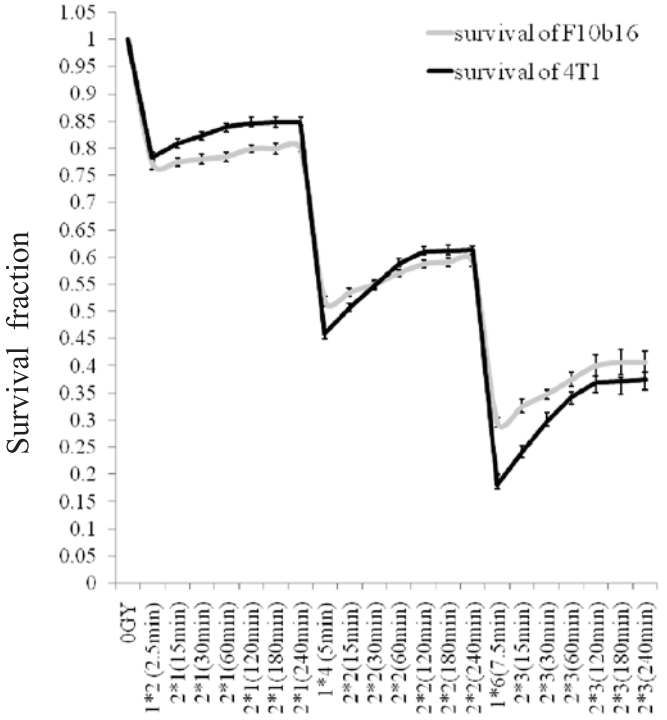
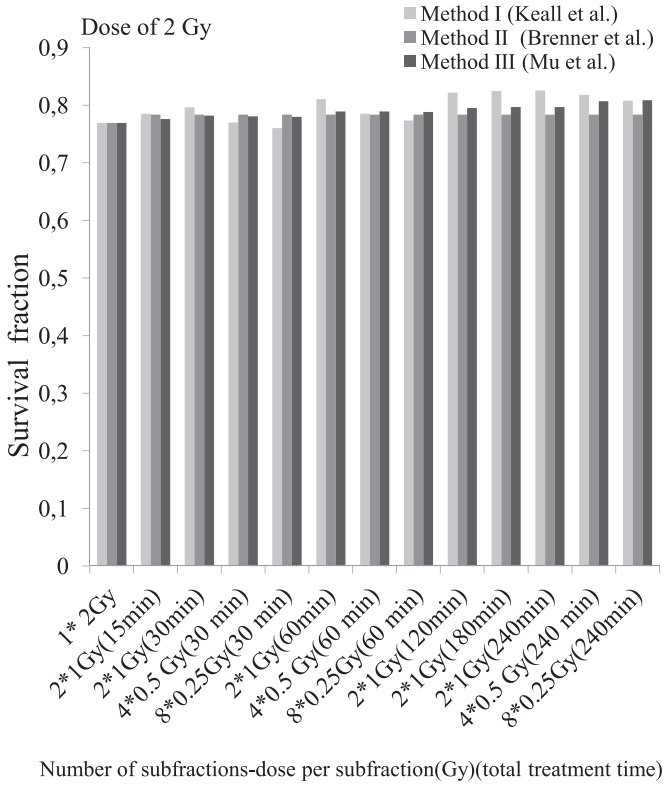


FIGURE 2. The survival fraction of F10B16 and 4T1 cells in dose levels of 2, 4 and 6 Gy delivered continuously and in two subfractions of 1, 2 and 3 Gy, respectively, for overall treatment time of 15 to 240 minutes.

For a dose of 4 Gy, there was no significant difference ($P<0.05$) between survivals calculated by three methods in total treatment time of up to 60

TABLE 2. Experimental and calculated survival using method III for total dose of 4 Gy

		Survival fractions of F10B16 and 4T1 cells			
		Experimental calculations		Theoretical calculations	
Number of subfractions×dose (Gy)	Total treatment time (min)	F10b16	4T1	F10b16	4T1
0×0	0	1	1	1	1
1×4	5	0.518±0.019	0.459±0.017	0.513±0.038	0.445±0.012
2×2	15	0.535±0.027	0.506±0.018	0.534±0.036	0.505±0.048
2×2	30	0.549±0.017	0.547±0.018	0.550±0.023	0.545±0.019
2×2	60	0.570±0.016	0.588±0.017	0.569±0.029	0.587±0.042
2×2	120	0.586±0.016	0.609±0.018	0.585±0.019	0.609±0.027
2×2	180	0.590±0.018	0.612±0.019	0.590±0.054	0.612±0.038
2×2	240	0.591±0.029	0.613±0.016	0.591±0.024	0.613±0.029
4×1	30	0.546±0.026	0.546±0.017	0.542±0.038	0.529±0.035
8×0.5	30	0.549±0.015	0.547±0.026	0.540±0.034	0.523±0.043
4×1	60	0.570±0.017	0.585±0.017	0.567±0.047	0.595±0.047
8×0.5	60	0.570±0.016	0.583±0.008	0.564±0.028	0.589±0.038
4×1	240	0.598±0.018	0.653±0.027	0.622±0.024	0.706±0.038
8×0.5	240	0.607±0.008	0.674±0.008	0.627±0.023	0.732±0.042



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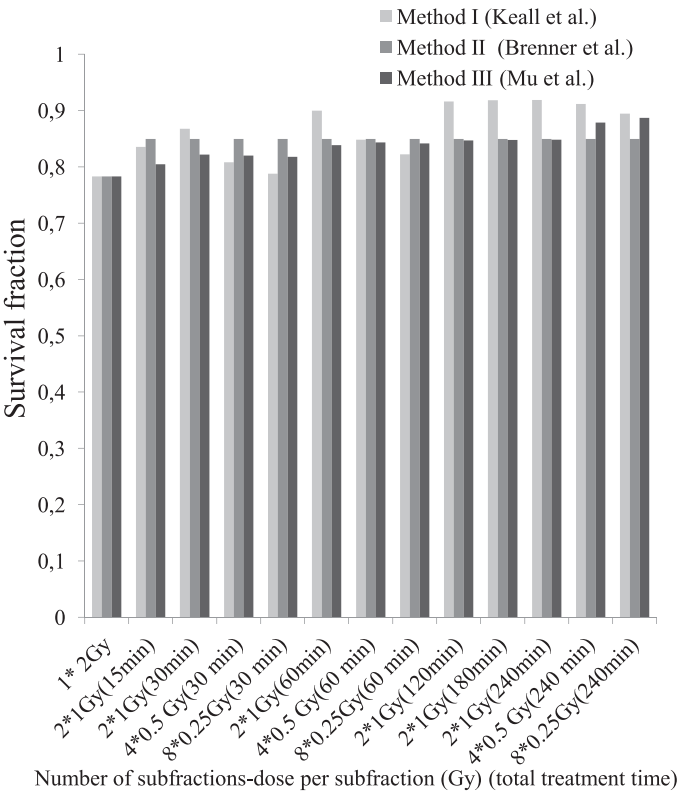


B

FIGURE 3. The survival fraction predicted by the used methods for F10B16 melanoma **(A)** and 4T1 breast adenocarcinoma **(B)** cell lines in different fraction numbers, dose per fractions, and total treatment times, for dose of 2 Gy.

minute (Figure 4). After 60 minute, for the F1B16 cells, there was a significant difference between method I and the other two methods. While in the treatment time less than 240 minute there was no significant difference between methods I and III ($P<0.05$). For the 4T1 cells, there were significant differences ($P<0.05$) in calculated survival between method I and the two other methods. Considering total treatment time, these variations increased considerably after 60 minute. Small differences observed between methods II and III in groups with 4 or 8 subfractions (Figure 4).

For a total dose of 6 Gy, the results of calculations for the F10B16 cells were the same as dose of 4 Gy (Figure 5). For the 4T1 cells, a significant difference between the used methods observed, especially between the method I and the other two methods (Figure 5).

These results showed that, when the total treatment time increased, the survival of both two cell lines increased significantly according to all three methods.

Increasing the number of subfractions showed different results according to the used methods.

According to the method I, increasing the number of subfractions in a fixed total treatment time reduced the survival in all three doses of 2, 4 and 6 Gy. The predicted survival according to the method II did not show any significant difference ($P<0.05$) due to the variations in number of subfractions.

The calculated survival by the method III for F10B16 melanoma cell line, showed a significant decrease by increasing the number of subfractions from 2 to 4 and 4 to 8, for total dose of 2, 4 and 6 Gy and both treatment times of 30 and 60 minute. While, for the 240 minute treatment time, increasing the number of subfractions increased the survival of the cells (Figures 3 to 5).

For 4T1 cell line, increasing the number of subfractions decreased the survival of the cells in 30 minute treatment time. For the total treatment time of 60 minute, increasing the number of subfractions from 2 to 4 fractions enhanced the survival of the cells, whereas, increasing the subfractions to 8 declined the cells survival. For the 240 minute treatment time, increasing the number of subfractions raised the cells survival (Figures 3 to 5).

Considering the three used methods, differences between the exposed groups to 2 Gy was not significant for the F10B16 cells and was negligible for 4T1 cells. Therefore, to investigate the effects of number of subfractions and dose per subfraction in experimental investigations, a total dose of 4 Gy was used. The results of this experiment were

assessed in 2, 4 and 8 fractions during treatment times of 30, 60 and 240 min, for both F10B16 and 4T1 cell lines.

Comparisons between the experimental results with those calculated by the three used methods showed that experimental results were in a good agreement with method III. The results of experimental investigations and the calculated survival by the method III are shown in Table 2.

The results showed that, in a fixed overall treatment time, there was no statistical significant difference ($P<0.05$) between the irradiated groups in different subfractions. Considering the overall treatment time, there was an agreement between experimental results and those predicted by the method III for the irradiated cells in total treatment time of 1 h, as opposed to the 4 h irradiated group.

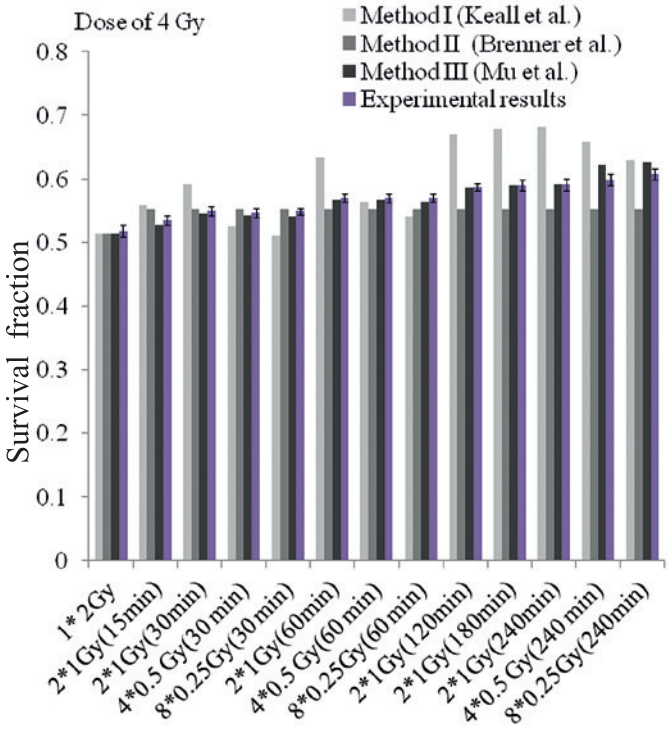
Discussion

Recently, some researchers have shown the effect of prolonged dose delivery time on the cell survivals.[8-15] In this regard, several models have been offered to predict the effects of variations in the treatment procedures on the cells survival.[8,9,20] One of these models is the developed LQ model by Thames and Dale.[17] However, different theoretical methods have been derived from this model in some researches in order to predict the survival of cells after prolonged dose delivery schemes.[8,9,10,19] As stated earlier, these researches have just investigated the effect of total treatment time and have not considered the effects of number of subfractions, dose per subfarction and the time intervals between subfractions in detail.

Therefore, more investigations were needed in order to determine the effect of different treatment factors on cells' survival. In addition, it seems useful to compare the results of these methods theoretically and experimentally in order to find the best method for clinical application in fractionation radiotherapy.

In this study, three calculation methods derived from the basic LQ model proposed in different researches[8,9,20] were used to evaluate the effects of different parameters such as total treatment time, number of subfractions, and subfractions interval on survival of cell lines with constant α, β and μ parameters. Then, the results were compared with experimental outcomes of F10B16 skin melanoma and 4T1 breast adenocarcinoma cells.

Comparison between the results of the used three methods with those of experimental results



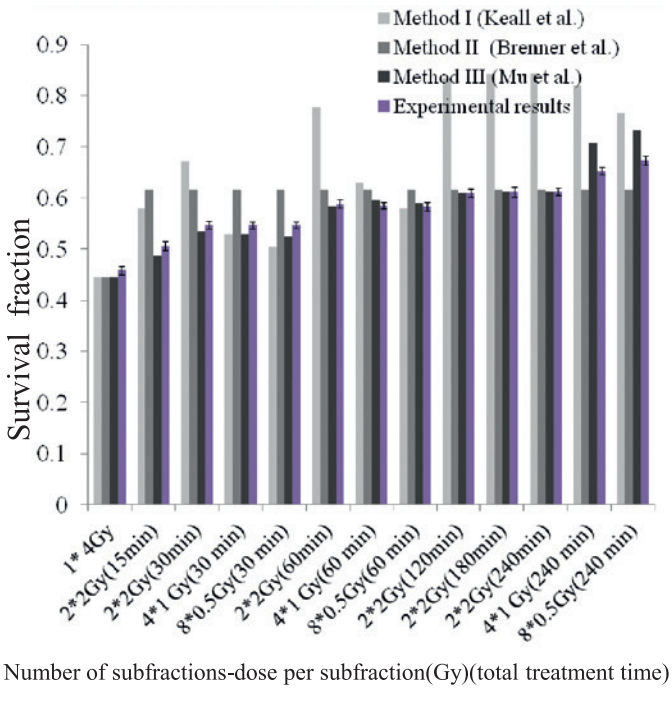
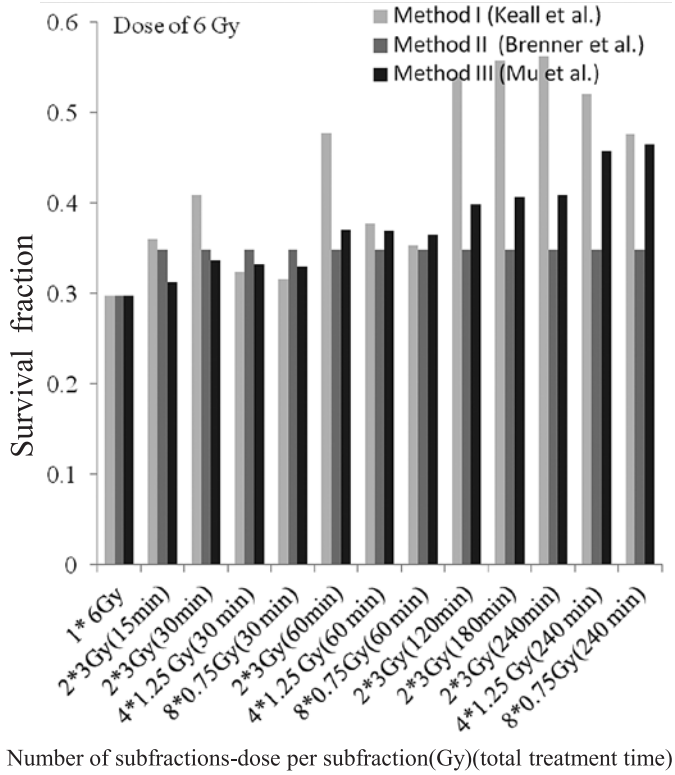
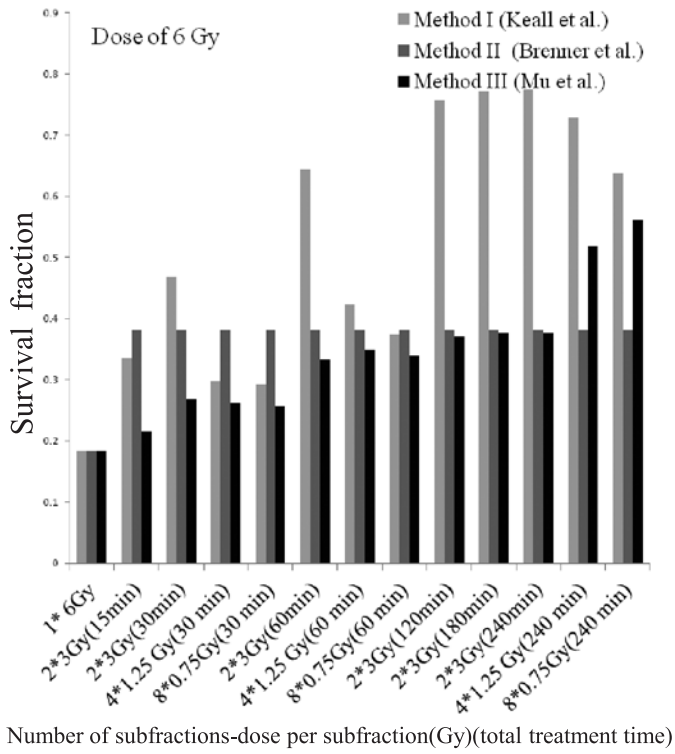


FIGURE 4. The survival fraction predicted by the used methods as well as the experimental results for F10B16 melanoma **(A)** and 4T1 breast adenocarcinoma **(B)** cell lines in different fraction numbers, dose per fractions, and total treatment times, for dose of 4 Gy.



A



B

FIGURE 5. The survival fraction predicted by the used methods for F10B16 melanoma **(A)** and 4T1 breast adenocarcinoma **(B)** cell lines in different fraction numbers, dose per fractions, and total treatment times, for dose of 6 Gy.

showed that method III (Mu *et al*. model) was in a better agreement with experimental outcomes. Mu *et al*. proposed a method to calculate the effect of prolonged treatment time on the Chinese hamster fibroblasts (V79-379-A) cells' survival for total treatment dose of 2 and 8 Gy. They have shown that, there is a good agreement between experimental and theoretical results for the total dose of 2 Gy and treatment time below 1 hour. While in our study, different mathematical methods presented by Keall *et al*., Brenner and Mu *et al*., were used to calculate the cells' survival after different treatment schemes such as 2, 4, and 6 Gy continuous dose in two subfractions with dose of 1, 2, and 3 Gy, respectively. In this work, to investigate the effects of the number of subfractions and dose per subfraction, the cells' survival after total doses of 2 Gy (4 subfractions of 0. 5 Gy and 8 subfractions of 0.25 Gy), 4 Gy (4 subfractions of 1 Gy and 8 subfractions of 0.5 Gy), 6 Gy (4 subfractions of 1.25 Gy and 8 subfractions of 0.75 Gy) were calculated through fixed overall treatment times of 30, 60 and 240 minutes.

Considering the method III investigations in predicting the F10B16 cells survival (T1/2 = 30 minute), it is expected that increasing the number of subfractions reduced the survival, in total treatment times of 30 and 60 minute. The reason was due to the repair of sublethal damages. For all defined subfractions (2, 4 and 8), the intervals between subfractions was lower than T1/2, therefore, after the first irradiation there was not enough time for the cells to repair their sublethal damages, hence the survival reduced. This effect was found for 4T1 cells in total treatment time of 30 minute. However, for the 60 minute treatment time, considering the T1/2 of about 20 minute (significantly lower than F10B16) the time between 4 subfractions was higher than the repair time, and therefore, after irradiation in the first subfraction the damages were repaired before starting the next exposure, consequently the survival increased. However, for the 8 subfractions in 60 minute treatment time, the results were the same as before. These explanations can justify the behavior of the used both two cell lines in 240 minute treatment time, too. Therefore, the survival of cells increased for this total treatment time.

Experimental results showed that increasing the total treatment time, similarly occurred in new complicated methods such as IMRT, increased the cell survival in both cell lines and all three total dose of 2, 4 and 6 Gy in up to 2 hour treatment time. However, the extent of this effect was not considerable for F10B16 cells with shorter T1/2, and was negligible for the dose of 2 Gy for this cell line.

Moreover, the results of this research confirmed that a cell with lower α/β ratio is considered to have a greater ability to undergo sublethal damage repair. The rate of sublethal damage repair may be represented by T1/2; therefore, cells with a shorter T1/2 have more repairs. In addition, the survival of 4T1 cell line with lower α/β and T1/2 was dramatically different than the F10B16, when the time interval between subfractions increased.

In total treatment time of 4 hours, both theoretical and experimental results showed an increase in survival with fractionated irradiation.

Some studies have been performed to investigate the ability of LQ model in predicting the survival in low dose levels (< 1 Gy).[21,28,29] Cherubini *et al*. and Jones *et al*. explained that in low doses (less than 1 Gy) the LQ model cannot predict the cell survival accurately.[28,21] While, Smith *et al*. claimed that the LQ model calculates the survival precisely in such low doses.[29] Brenner has shown that in total dose of 2 to 15 Gy, the LQ model can accurately predict the survival in *in-vitro* and *in-vivo* conditions.[20] In this study, in line with Brenner, the results suggest that, in fractionation radiotherapy, the developed LQ model can potentially reach close agreement with reality in total treatment dose of 2 to 4 Gy.

Compared with other studies, using small subfractions of 0.25–0.5 Gy, Marples *et al*.[30], and Mu *et al*.[8] investigated the phenomenon of hypersensitivity to low doses per fraction. Marples *et al.* showed that, this would lead to a more effective cell killing than predicated by the LQ model.[30] While Mu *et al*. study showed that there was no evidence for such effect since it should have resulted in lower survival than expected and not higher.[8] They explained that this effect is perhaps because of the effective dose rate in each fraction which is too high to avoid activating a possible repair.[8] However, in our study which lower dose rate was used, cell killing reached close agreement to the amount predicted by the LQ model that is in an agreement with the Marples *et al*. result. The factors that influence the dose rate are radical recombination and sublethal damage repair.[30] It should be noted that, at the dose levels and dose rates encountered in radiotherapy, the effect of radical recombination on cell killing is negligible.[31] Ling *et al*.[32] and Michaels *et al*.[33] have compared the survival of CHO cells at dose rates of 0.6 Gy/min from a Co-60 unit, and their results showed that the obtained survival curves were exactly the same with up to 15 Gy/min dose rates. Hence, based on the results of our study and comparisons with other works, an idea to reduce the effect of fractionation or prolonged treatment time is using higher dose rates or more treatment dose in one fraction.

In other work by Keall *et al*.[9], they have shown that both respiratory gating and IMRT delivery will decrease survival compared with continuous delivery of the same dose in the same overall time. Therefore, for a given treatment time, delivery method is another factor affecting the cell survival.

Conclusions

According to presented experimental and theoretical results, in treatment of tumors in radiotherapy by new complicated methods, this should be noted that exceeding the treatment time will increase the survival of tumor cells and may decrease tumor control. Increasing the number of subfractions in a course of treatment could reduce the cell survivals if the fractions time interval be lower than the repair time of sublethal damages. Although, this parameter has a negligible effect on the survival of the cell lines of interest in our experimental study, this factor can be considered in compensating the increase in cell survival due to the time prolongation.

It seems appropriate to use the method proposed by Mu *et al*. to predict the cell response following fractionation radiotherapy, especially in new fractionation radiotherapy procedures with more number of subfractions and with prolonged total treatment times. This method can simply and accurately determine the cell survival after each radiotherapy assessment and can be used to calculate the compensating dose for these treatment schedules. Although the effect of fractionation dose delivery is negligible for one session (with dose of 2 Gy), and it seems that there is no need to compensate these effect, but it can be important for a radiotherapy period (30 or 35 session with 2 Gy in each fraction) because of the cumulative effect of dose.

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research article

Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) in breast cancer - correlation with traditional prognostic factors

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Background. Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) play a key role in tumour invasion and metastasis. High levels of both proteolytic enzymes are associated with poor prognosis in breast cancer patients. The purpose of this study was to evaluate the correlation between traditional prognostic factors and uPA and PAI-1 expression in primary tumour of breast cancer patients.

Patients and methods. 606 primary breast cancer patients were enrolled in the prospective study in the Department of gynaecological oncology and breast oncology at the University Medical Centre Maribor between the years 2004 and 2010. We evaluated the traditional prognostic factors (age, menopausal status, tumour size, pathohistological type, histologic grade, lymph node status, lymphovascular invasion and hormone receptor status), together with uPA and PAI-1. We used Spearman's rank correlation, Mann Whitney U test and χ^2 test for statistical analysis.

Results. Our findings indicate a positive correlation between uPA and tumour size ($p < 0.001$), grade ($p < 0.001$), histological type ($p < 0.001$), lymphovascular invasion ($p = 0.01$) and a negative correlation between uPA and hormone receptor status ($p < 0.001$). They also indicate a positive correlation between PAI-1 and tumour size ($p = 0.004$), grade ($p < 0.001$), pathohistological type ($p < 0.001$) and negative correlation between PAI-1 and hormone receptor status ($p = 0.002$).

Conclusions. Our study showed a relationship between uPA and PAI-1 and traditional prognostic factors. Their role as prognostic and predictive factors remains to be further evaluated.

Key words: urokinase plasminogen activator; plasminogen activator inhibitor; breast cancer; prognostic factor

Introduction

Urokinase plasminogen activator system (uPAS) consists of urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA), urokinase plasminogen activator receptor (uPAR), and plasminogen activator inhibitor type-1 (PAI-1) and type-2 (PAI-2). Proteolytic enzyme uPA converts the proenzyme plasminogen into proteolytically active form (plasmin), which takes part in physiological and pathophysiological processes on the basal membrane and inside the extracellular matrix, which are important for tumour growth and its metastases.[1] Plasminogen activator inhibitor type-1,

which functions as a natural inhibitor of uPA, is the most important factor among fibrinolytic inhibitors for the development of vascular diseases and cancer. uPA and PAI-1 do not only have proteolytic characteristics, but also have impact on the fundamental cellular processes, such as chemotaxis, migration, invasion, adhesion, proliferation and angiogenesis.[2-6] uPA and PAI-1, as part of the fibrinolytic system, are the first factors with a confirmed clinical role in breast cancer (level of evidence I).[7,8] According to the conclusions of the meta-analysis by Look *et al*.[7], uPA and PAI-1 are in addition to axillary lymph node involvement the most important independent prognostic factors. uPA and PAI-1 are supposed to be useful in deciding upon adjuvant systemic therapy in women with low-risk primary breast cancer.[7] Use of PAI-1 for therapeutic purposes has shown promising results on tumour models; however, the results are yet to be confirmed.[9] The increase of PAI-1 could represent a response to the increased proteolytic activity caused by uPA inside the tumour. It is also possible that PAI-1 has a direct effect on the development of the disease.[10]

Prognostic and predictive factors are clinically important for planning the treatment of breast cancer, which improves disease-free survival, overall survival and quality of life. Prognostic factors predict the course of the disease independently of treatment and are connected with disease-free survival and overall survival. Tumour size, axillary lymph node involvement, pathohistological tumour type, malignancy grade and lymphovascular invasion are prognostic factors in the case of breast cancer. To assess patients with a high risk of recurrence, traditional prognostic factors do not suffice. Therefore, numerous studies are being conducted to discover better factors. uPA in PAI-1 are related to the course of breast cancer as statistically important independent prognostic factors.[11-15] Numerous studies have shown that patients with low concentrations of uPA and PAI-1 have better survival than patients with high concentrations.[16-17] The prognostic roles of DNA ploidy and S-phase fraction are not clearly defined yet.[18]

Predictive factors are biological markers by means of which it is possible to predict response to a certain type of treatment. Status of the hormone receptors, which predicts the response to hormonal therapy, and human epidermal growth factor receptor 2 (HER2) expression, which predicts the response to anti-HER2 therapy in patients with HER2-positive breast cancer, were confirmed to be reliable predictive factors in breast cancer. High level of evidence supports the predictive significance of uPA and PAI-1, which are the subject of many studies.[19] Protein over-expression and/or amplification of the HER2 gene are found in around 20% of all breast cancer patients. Pre-clinical studies show that HER2 accelerates cellular adhesion and migration and therefore plays a key role in tumour cell invasion.[20-23] Certain clinical studies indicate that in some cancer types HER2 stimulates the invasion of tumour cells with the effect on the accelerated release of proteolytic enzyme uPA and its inhibitor (PAI-1)[24-27], whereas other studies did not confirm this assumption.[28,29] The international coordinated guidelines, adopted at the conference in St. Gallen in 2007, require knowledge of factors such as tumour size, malignancy grade, age, axillary node involvement, status of hormone receptors and HER2 expression as the basis for choosing adjuvant therapy.[30]

Despite excellent evidence about the prognostic value of uPA and PAI-1, determination of these markers is not yet routinely used for planning adjuvant treatment. It is not completely clear if routine determination of uPA and PAI-1 would add important new information as opposed to simply confirming what can already be deduced from the traditional prognostic factors.

The aim of this study was to evaluate the correlation between uPA and PAI-1 and traditional prognostic factors in primary breast cancer. Statistically significant correlation between uPA and PAI-1 and traditional prognostic factors was expected. HER2 expression and its correlation with the traditional prognostic factors were also included.

Patients and methods

Patients

Six hundred and six patients with primary breast cancer, treated at the Department of Gynaecologic Oncology and Breast Oncology of the Division of Gynaecology and Perinatology, University Medical Centre Maribor, between the years 2004 and 2010 were included in this prospective study. The study was conducted in accordance with good clinical practice and all applicable regulatory requirements, including Declaration of Helsinki. The study was approved by the institutional review board and registered at Slovenian Research Agency under the clinical trial number P3-0321. All patients had pathohistologically confirmed invasive breast cancer. None of the patients had clinically or radiologically registered metastatic disease at the beginning of primary treatment. The characteristics of patients and tumours are present-

ed in Table 1. Traditional prognostic factors such as menopausal status, pathological tumour size, pathohistological tumour type, malignancy grade, axillary lymph node involvement and lymphovascular invasion were assessed by means of clinical examination and pathohistological examination of tumour tissue. Tumours were classified according to the UICC-WHO criteria and malignancy grade according to Scarff-Bloom-Richardson (SBR) classification, modified by Elston.[31] Lymphovascular invasion was evaluated as positive if tumour cell emboli were present in the vascular space lined by endothelium. Hormone receptors were evaluated by means of immunohistochemical staining of paraffin-embedded tumour tissue sections. Tumours in which at least 1% of tumour cells expressed oestrogen (ER) and/or progesterone (PR) receptors were marked as hormone receptor positive.

The study group was a cohort of women with breast cancer primarily treated at our institution during a period of seven years. During this time, recommendations for determination of some histological parameters have changed. Progesterone receptors and HER2 were not routinely determined in all patients throughout the study period and for some early cases of HER2 determination in situ hybridization was not performed in cases that were immunohistochemically marked as 2+. Besides, tumour grade was not reported for lobular histological subtypes in the past. Unfortunately, all this has lead to a high rate of missing data in these fields.

All patients were radically locally treated with a modified radical mastectomy or conservative operation (tumorectomy, quadrantectomy) and postoperative radiation. They further received adjuvant systemic therapy (chemotherapy and/or hormone therapy). Most patients with positive axillary lymph nodes and patients with a high risk and negative lymph nodes received adjuvant chemotherapy. All patients with positive hormone receptors received adjuvant hormone therapy.

TABLE 1. Characteristics of primary breast cancer patients and tumours (n = 606)

Characteristics	Number of patients	Percentage (%)
Age		
< 50 years	136	22
≥ 50 years	470	78
Menopausal status		
Premenopausal	162	27
Postmenopausal	444	73
Pathological tumour size		
< 2 cm	282	46
≥ 2 cm	319	53
Unknown	5	1
Pathohistological classification of tumours		
Invasive ductal	496	82
Invasive lobular	45	7
Other invasive	61	10
Unknown	4	1
Malignancy grade		
G1	126	21
G2	212	35
G3	235	39
Unknown	33	5
Axillary lymph node involvement		
Negative	333	55
Positive	243	40
Unknown	30	5
Oestrogen receptors		
Negative	119	20
Positive	478	79
Unknown	9	1
Progesterone receptors		
Negative	219	36
Positive	337	56
Unknown	50	8
Hormone receptors		
Negative	101	17
Positive	492	81
Unknown	13	2
Lymphovascular invasion		
Yes	103	17
No	481	79
Unknown	22	4
HER2		
Negative	127	21
Positive	373	62
Unknown	106	17

G = grade; HER2 = human epidermal growth factor receptor 2

Laboratory measurements of uPA, PAI-1 and HER2

After histological examination of tissue sections, the tumour tissue obtained by surgery was stored for further analysis in liquid nitrogen. Samples of frozen tumour tissue were then pulverized with a Micro-dismembrator, dissolved in buffer (pH 5.5) composed of 0.02 M Tris-HCl, 0.125 M NaCl and 2% Triton X-100 and after 3 hours of stirring at 4°C centrifuged at 100,000 x g for 30 minutes. Protein content was measured with the bicinchoninic acid (BCA) method (Pierce, Rockford, IL). Antigens uPA and PAI-1 were quantified with standardized immunometric method using ELISA sets (American Diagnostica, Greenwich, CT, USA). Values of uPA and PAI-1 were expressed in ng/mg of proteins.

Based on the assesed intensity of membrane reaction due to the overexpression of HER oncoprotein, the tumour tissue was categorized into one out of three groups: negative (0, 1+), equivocal (2+) and positive (3+). The immunohistochemically HER2 3+ result indicates positive HER2 status of the tumours. In all cases of the equivocal HER2 2+ results, the tumour tissue was retested with the fluorescence in situ hybridization (FISH) method

TABLE 2. Levels of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) in tumour tissue of primary breast cancer patients

	Range* (min–max)	Median value* (25 / 75 percentile)	Limit values*	Number of patients	Percentage (%)
uPA	0–24.95	2.34 (1.08 / 4.20)	< 3 ≥ 3	319 223	59 41
PAI-1	0–170.92	10.6 (6.93 / 18.27)	< 14 ≥ 14	347 195	64 36

* ng / mg of proteins

using PathVysion™ HER-2 PROBE KIT, in order to determine the amplification of HER2 proto-oncogene. All breast cancer patients with HER2 positive tumour status were treated with the monoclonal antibody trastuzumab.

Statistical analysis

Spearman Rank Correlation was used to test the relation between continuous variables. We used the non-parametric Mann-Whitney U test to compare continuous and categorical variables and chi-square test for the comparison of categorical variables. Continuous variables uPA and PAI-1 were converted into binary variables to divide the patients into those with high risk and those with low risk by using limit values of 3 ng uPA / mg of proteins and 14 ng PAI-1 / mg of proteins.[32] Statistical analysis was performed by means of the SPSS 17.0 program. The value of $p < 0.05$ was considered statistically significant. uPA and PAI-1 values are shown in Table 2.

Results

606 patients with primary breast cancer were included in the prospective study. Mean age of patients was 60.1 ± 12.7 years. The youngest patient was 22 years old, and the oldest 95 years old.

Correlation between uPA and PAI-1 values

A strongly positive statistically significant correlation was established between uPA and PAI-1 values ($r_s = 0.576$, $p < 0.001$). The relation between uPA and PAI-1 values is shown in Figure 1.

Correlation between uPA and PAI-1 and traditional prognostic factors

Statistically significant correlation between uPA and PAI-1 and most of the traditional prognostic factors was established. They were related with tumour size, pathohistological tumour type, malignancy grade, status of oestrogen (ER) and hormone receptors. The relation between uPA and malignancy grade is shown in Figure 2.

No statistically significant correlation between uPA and PAI-1 and age, menopausal status, progesterone receptors (PR) and axillary lymph node involvement was found. Interestingly, only uPA's correlation with lymphovascular invasion was sta-

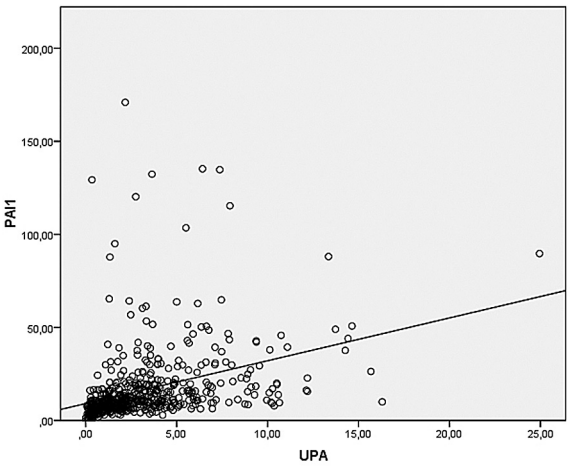

FIGURE 1. Correlation between urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) values.

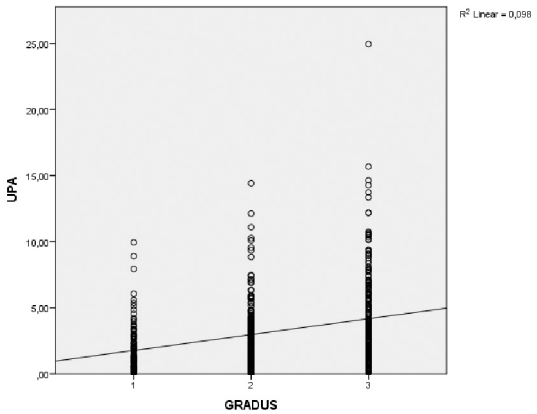

FIGURE 2. Correlation between urokinase plasminogen activator (uPA) and malignancy grade. Similar correlations were found for uPA and tumour size, pathohistological tumour type and lymphovascular invasion. Negative correlation was found between uPA and hormone and oestrogen receptor status. Analogous correlations between and plasminogen activator inhibitor type-1 (PAI-1) and all these factors except lymphovascular invasion were also determined.

tistically significant. The correlation between uPA, PAI-1 and traditional prognostic factors is shown in Table 3.

Correlation between tumour HER2 positive status and traditional prognostic factors

HER2 tumour status was statistically related to all the traditional prognostic factors, except to axillary lymph node status (Table 3).

Patients with a high risk due to high uPA and PAI-1 values and tumour HER2 positive status

High uPA levels were present in 223 patients (41%) and high PAI-1 levels in 195 (36%) patients, as shown in Table 2. High levels of one or both proteolytic enzymes, uPA and PAI-1, were identified in 288 (53%) patients. HER2 overexpression was identified in 127 (25%) patients.

Among 183 patients with high uPA, 50 patients (27%) had HER2-positive tumours. In the group of 263 patients with low uPA, 60 patients (23%) had HER2-positive tumours. Out of 157 patients with high PAI-1, 46 patients (29%) had HER2-positive tumours. Among 289 patients with low PAI-1, 64 patients (22%) had HER2-positive tumours. In the group of 238 patients with high values of one or both proteolytic enzymes, uPA and PAI-1, 65 patients (27%) had HER2-positive tumours and out of the 214 patients with low values of uPA and PAI-1 47 patients (22%) had HER2-positive tumours.

TABLE 3. Correlations between urokinase plasminogen activator (uPA), plasminogen activator inhibitor type-1 (PAI-1), human epidermal growth factor receptor 2 (HER2) and traditional prognostic factors

Variables	Number of patients*	uPA	PAI-1	HER2
Age				
< 50 years	136	p = 0.700	p = 0.402	**p = 0.017**
≥ 50 years	470			
Menopausal status				
Premenopausal	162	p = 0.896	p = 0.218	**p = 0.008**
Postmenopausal	444			
Pathological tumour size				
< 2cm	282	**p < 0.001**	**p = 0.004**	**p = 0.005**
≥ 2cm	319			
Pathological tumour type				
Ductal invasive	541	**p < 0.001**	**p < 0.001**	**p = 0.021**
Other invasive	61			
Malignancy grade				
G1 + G2	338	**p < 0.001**	**p < 0.001**	**p < 0.001**
G3	235			
Axillary lymph node involvement				
Negative	333	p = 0.052	p = 0.171	p = 0.385
Positive	243			
Oestrogen receptors				
Negative	119	**p < 0.001**	**p < 0.001**	**p < 0.001**
Positive	478			
Progesterone receptors				
Negative	219	p = 0.162	p = 0.960	**p = 0.003**
Positive	337			
Hormone receptors				
Negative	101	**p < 0.001**	**p = 0.002**	**p < 0.001**
Positive	492			
Lymphovascular invasion (LVI)				
Yes	103	**p = 0.010**	p = 0.292	**p = 0.006**
No	481			

* Due to missing values the number of patients is not always 606; G = grade

Discussion

Prognostic and predictive factors play a key role in the proper treatment of breast cancer patients. Knowledge about these factors enables the patients with aggressive malignant tumours the possibility of adjuvant systemic therapy along with better survival and spares the patients with less aggressive malignant tumours unnecessary systemic treatment with numerous side effects while having the same chance of recovery. Establishing prognostic factors that are independent of treatment became very challenging in modern medicine, as most patients with breast cancer receive adjuvant systemic therapy after the primary treatment. It would be unethical to discontinue the adjuvant systemic therapy for research purposes regarding the characteristics of the disease.

The purpose of the study was to evaluate the relation between uPA and PAI-1 and traditional prognostic factors in primary breast cancer. HER2 overexpression as a prognostic and predictive factor and its correlation with traditional prognostic factors was also included in the study.

The sample size (606 patients) in our study is comparable with the reports by other authors.[33-39] The study provides exact data on the characteristics of patients with primary breast cancer and their tumours. More than a third of patients (78%) were aged 50 or more and 73% of patients were postmenopausal. We determined that HER2 overexpression is more frequently present in younger and premenopausal patients. The same correlation was not established with uPA and PAI-1. Similarly, Look *et al*.[7] found no significant relationship between uPA and age or menopausal status. However, they reported a correlation between PAI-1 and age and higher PAI-1 in postmenopausal women. They nevertheless considered these relationships not to be clinically meaningful.

Axillary lymph node involvement, tumour size, pathohistological tumour type, malignancy grade and lymphovascular invasion are the most important prognostic factors of the clinical course of breast cancer. In numerous studies these tumour characteristics were proved as independent prognostic factors since the disease recurred more often and affected the survival in patients with affected axillary lymph nodes, larger tumours, invasive ductal carcinoma, higher malignancy grade and lymphovascular invasion.

Numerous previous studies have shown that high levels of uPA and PAI-1 in primary tumour tissue negatively affect the outcome of breast cancer. uPA enables the development of metastases through proteolytic degradation of the extracellular matrix. Furthermore, PAI-1 also has an important role in invasion and metastasis, because it does not act only as inhibitor of uPA in plasminogen activator system but also affects most basic cell processes, such as adhesion, migration, invasion, proliferation and apoptosis of normal and malignant cells. Eljuga *et al.*[17] even showed that PAI-1 determined immunohistochemically in tumour cells as opposed to the less available ELISA testing may carry important prognostic information in node-negative breast cancer patients.

A strongly positive correlation between both proteolytic enzymes, uPA and PAI-1, was established, which is in line with the findings of other studies.[7,28,29,34,36] Despite this correlation, it is clinically important to determine both factors. Establishing both values allows differentiation between groups with high risk (with a high level of one or both factors) and making a decision on the proper individual adjuvant therapy.[40]

Size of the primary tumour is a known prognostic factor for the course of breast cancer. Patients with primary tumours equal to or larger than 2 cm, with a 53% share in our study, had more often high uPA and PAI-1 levels. A similar share of patients with large tumours (56%) was reported by Look *et al.*[7] In larger tumours HER2 overexpression was also more frequently present.

Axillary lymph node involvement is related to tumour size, as larger tumours more often develop regional lymph node metastases. Interestingly though, high uPA and PAI-1 values in our patients were not related to axillary lymph node involvement. The reason may lie in the role of uPA and PAI-1 as independent prognostic and predictive factors in patients with primary breast cancer without axillary lymph node involvement. uPA was proved to be a stronger prognostic factor than tumour size, axillary lymph node involvement and oestrogen receptor status. Furthermore, it was also proved to be the strongest predictive factor of disease-free survival and overall survival in patients with primary breast cancer and no axillary lymph node involvement in numerous studies.[7,8,11,15,41,42] In our study, 154 patients (40%) had a high uPA level and no axillary lymph node involvement. High PAI-1 levels were present in 136 patients (36%). High levels of both, uPA and PAI-1, were present in 88 patients (21%) with no axillary lymph node involvement. Individually or both, the uPA and PAI-1 levels were high in 202 patients (54%). De Cremoux *et al.*[42] established high one or both levels of uPA and PAI-1 in 56% of patients with no axillary lymph node involvement, which is comparable to our results. Neither was axillary lymph node involvement related to the HER2 overexpression.

Malignancy grade of primary tumour is a confirmed prognostic factor in breast cancer. We have proved that patients with high levels of uPA and PAI-1 and HER2 overexpression more frequently have high-grade tumours. Poorly differentiated tumours (G2 and G3) were present in 74% of patients. Evaluating the malignancy grade by means of studying the histological structure and cytological characteristics of malignant cells is a subjective method. Sotiriou *et al.*[43] found that it was necessary in patients with grade 2 tumours (that is 30–60% of all tumours) to determine the genomic grade index of the tumour. By doing so, the patients would be divided into those with a high and low risk for recurrence. G2 tumours were present in 35% of patients in our study.

We found that high levels of uPA and PAI-1 and HER2 overexpression are more often present in invasive ductal carcinoma than in lobular and other cancer types, which is in line with their different clinical outcomes. Invasive ductal type of breast cancer was present in 82% of our patients. Descotes *et al.*[39] discovered 84% of invasive ductal breast cancer type.

Oestrogen and progesterone receptor status is an important predictive factor of the response to hormone therapy. Patients with positive ER and PR in the tumour have better survival than patients with positive only one type of hormone receptors. Patients with hormone receptor negative tumours have the worst survival rate. ER are mostly predictive factors of the response to hormone therapy, whereas PR are prognostic factors of the disease course, therefore it is important to measure both in the primary breast cancer tissue. In our study, high levels of uPA and PAI-1 had a negative correlation

with ER, but no correlation with PR. HER2 overexpression had a negative correlation with both types of hormone receptors. We counted 79% of patients with positive ER. Other authors report a similar share of ER-positive tumours.[38,39] PR-positive tumours were present in only 56% of our patients. The cause for the lack of correlation between uPA and PAI-1 with PR may lie in the missing PR values.

Lymphovascular invasion is an important morphological prognostic factor. Breast cancer develops metastases into regional and distant lymph nodes by lymphatic dissemination, and metastases into other parenchymal organs by hematogenous spread. Lymphovascular invasion was more frequently present in the tumour in patients with high uPA levels. There was no such correlation with PAI-1. Čufer *et al.*[36] did not find any correlation between uPA and PAI-1 and lymphovascular invasion. In cases of lymphovascular invasion, HER2 overexpression was more often present.

High levels of one or both proteolytic enzymes were found in 53% of our patients and HER2 overexpression was present in 25%. A slightly higher percentage of HER2 overexpressing tumours was found in patients with high uPA, PAI-1 or both. A positive relationship between HER2 and proteolytic enzymes has been reported for other types of cancer[24,25] but it has not been confirmed in breast cancer.[28,29] This is the subject of our further research.

The results of our study on the correlation between uPA and PAI-1 and classic prognostic factors in primary breast cancer are concordant with the results of a meta-analysis of 8377 patients on the prognostic effect of uPA and PAI-1 in primary breast cancer.[7] Correlation between uPA and PAI-1 and tumour size, pathological tumour type and hormone receptors and between uPA and axillary lymph node involvement are in line.

Further research of the characteristics of patients with primary breast cancer and their tumours as well as the definition of the role of proteolytic enzymes uPA and PAI-1 as prognostic and predictive factors in breast cancer is required.

Conclusions

Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) play a key role in invasion and metastases of malignant tumours. High levels of both proteolytic enzymes are related to poor prognosis in patients with breast cancer. By conducting this study we established that primary breast cancer patients with high values of uPA and PAI-1 usually have tumours that are larger, higher malignancy grade, invasive ductal pathohistological type and hormone independent. In cases of higher uPA lymphovascular invasion is more often present. We also established that HER2 overexpressing tumours occur more often in younger, premenopausal patients, are usually larger, hormone independent, of higher malignancy grade and invasive ductal histology, and they often show lymphovascular invasion.

Despite these significant correlations, it seems that uPA and PAI-1 values may help to additionally stratify especially node-negative breast cancer patients into different prognostic subgroups. In order to form a solid recommendation for or against routinely performing uPA/PAI-1 testing in breast cancer patients, further research about the prognostic and predictive impact of these factors in patients with primary breast cancer is required. The role of uPA and PAI-1 in survival of node-negative breast cancer patients is the subject of our ongoing research.

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research article

Prognostic value of some tumor markers in unresectable stage IV oropharyngeal carcinoma patients treated with concomitant radiochemotherapy

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Background. The aim of the study was to investigate how the expression of tumor markers p21, p27, p53, cyclin D1, EGFR, Ki-67, and CD31 influenced the outcome of advanced inoperable oropharyngeal carcinoma patients, treated with concomitant radiochemotherapy.

Patients and methods. The pretreatment biopsy specimens of 74 consecutive patients with inoperable stage IV oropharyngeal squamous cell carcinoma treated with concomitant radiochemotherapy were in retrospective study processed by immunochemistry for p21, p27, p53, cyclin D1, EGFR, Ki-67, and CD31. Disease-free survival (DFS) was assessed according to the expression of tumor markers.

Results. Patients with a high expression of p21 (≥10%), p27 (>50%), Ki-67 (>50%), CD31 (>130 vessels/mm2) and low expression of p53 (<10%), cyclin D1 (<10%) and EGFR (<10%) (favorable levels - FL) had better DFS than patients with a low expression of p21 (<10%), p27 (≤50%), Ki-67 (≤50%), CD31 (<130 vessels/mm2) and high expression of p53 (≥10%), cyclin D1 (≥10%) and EGFR (≥10%) (unfavorable levels - UL). However, statistical significance in survival between FL and UL was achieved only for p27 and cyclin D1. DFS significantly decreased with an increasing number of markers with an unfavorable level per tumor (1–4 vs. 5–7) (78% vs. 32%, respectively; p = 0.004). The number of markers per tumor with UL of expression retained prognostic significance also in multivariate analysis.

Conclusions. Statistical significance in survival between FL and UL emerged only for p27 and cyclin D1. The number of markers per tumor with UL of expression was an independent prognostic factor for an adverse outcome.

Key words: oropharynx; radiochemotherapy; tumor markers

Introduction

Prognostic evaluation of patients with unresectable squamous cell carcinoma in the head and neck (SCCHN) is currently based on the performance status of the patient and the tumor stage. Recently, the presence of human papillomavirus (HPV) DNA in tumor cells has also been identified as a strong predictor of survival in patients with oropharyngeal primaries.[1] However, these classical prognostic factors often do not provide sufficient information for the selection of the optimal therapy. The changes in the genes or their products which can be traced biochemically or immunohistochemically may serve to estimate the aggressiveness of the tumor and, consequently, to adapt treatment accordingly.

On the basis of the favorable results of our pro-

TABLE 1. Antibodies and preconditioning applied for immunohistochemistry

Antibody	Clone	Producer	Dilution	Preconditioning
p21	SX118	DAKO	1:10	MW*, 6 min, EDTA buffer, pH 8.0 cooling 10 min
p27	1B4	Novocastra	1:10	MW*, 6 min, EDTA buffer, pH 8.0 cooling 10 min
p53	DO7	DAKO	1:50	MW*, 6 min, EDTA buffer, pH 8.0 cooling 10 min
Ciklin D1	P2D11F11	Novocastra	1:10	MW*, 6 min, EDTA buffer, pH 8.0 cooling 10 min
EGFR	H11	DAKO	1:10	Proteinase 1 (Ventana) 12 min
Ki-67	MIB1	DAKO	1:20	MW*, 6 min, EDTA buffer, pH 8.0 cooling 10 min
CD31	JC/70A	DAKO	1:15	MW**, 7 min, 96°C, citr. buffer, pH 6.0 + Proteinase 1 (Ventana), 2 min

EGFR = epidermal growth factor receptor; EDTA = ethylene-diamine-tetraacetic acid; MW* = common microwave oven; MW** = microwave oven Polar Patent

spective randomized clinical study[2], concomitant radiochemotherapy with mitomycin C and bleomycin was introduced in the 1990s as a routine treatment for patients with unresectable squamous cell carcinoma of the oropharynx (SCCOP) in our department. Because the treatment morbidity was rather severe also in those patients who were not cured[3], the identification of patients from a homogenous group (in regard to the primary tumor origin, stage and treatment), who will or will not respond to aggressive radiochemotherapy would help in sorting patients into various treatment programs of different intensity, to spare some of them from unnecessary toxicity. Therefore, the primary aim of our study was to investigate how the expression of growth promoting (cyclin D1, epidermal growth factor receptor [EGFR], Ki-67) and growth suppressing (p21, p27, p53) tumor markers and CD31 in the primary tumor tissue influenced the outcome of patients with unresectable SCCOP.

Patients and methods

Patients

The 95 consecutive patients with previously untreated, technically inoperable SCCOP were treated with curative intent in the period 1991–1998 with irradiation and concomitant application of mitomycin C and bleomycin. To achieve maximal possible homogeneity of the studied group, 21 patients with disease of UICC TNM stage III were excluded for further evaluation. Patients were irradiated five times weekly with one fraction of 2 Gy/day with a planned total dose of 66–70 Gy. The physical dose was converted into a biologically effective dose (BED) according to the formula: BED = TD x (1 + d/α/β) – K x (Tt – Td), where TD = total tumor dose in Gy, Tt = total treatment time in days, K = daily dose equivalent of repopulation in units of $Gy_{\alpha/\beta}$ per day (K = 0.6, and α/β = 10), and Td = lag time in days to the onset of effective repopulation during the treatment (4). It was assumed that Td = 28 days.

The chemotherapy regimen consisted of intramuscular applications of bleomycin 5 mg twice a week with the planned dose being 70 mg and one application of mitomycin C 15 mg/m^2 applied intravenously after delivery of 9–10 Gy of irradiation. Radiotherapy was considered intensive if the BED was ≥65Gy10. Chemotherapy was considered intensive if the dose of mitomycin C was ≥14.1 mg/m2 and of bleomycin ≥35mg. The whole treatment was considered intensive, if chemotherapy or radiotherapy or both were intensive.

Methods

Immunohistochemistry

The pretreatment biopsy specimens of the primary tumors were in retrospective clinical study processed by immunochemistry for p21, p27, p53, Cyclin D1, EGFR, Ki-67, and CD31. Immunohistochemistry was performed on 4 μm paraffin sections mounted on silicon-coated glass slides. The antibodies and preconditioning applied for immunohistochemistry are presented in Table 1.

To determine the level of expression of the tested markers, semi-quantitative scoring of immune reactivity was performed according to the percentage of positivity in the tumor cells as follows: 0 = less than 10%, 1 = 10–50%, 2 = more than 50% of tumor cells with a positive reaction (for p21, p27, p53, Ki-67, cyclin D1 and EGFR). Microvascular density (MVD) was assessed quantitatively with the CD31 antibody. Stained microvessels were counted and expressed as the number of microvessels per mm^2 in the areas of maximal neovascularization of the tumor stroma.

Statistical methods

Disease free survival (DFS) was defined as the time interval from the beginning of the treatment to the appearance of local and/or regional progression

TABLE 2. The distribution of patients according to the expression of tumor markers

Intensity of expression	P21	P27	P53	Cyclin D1	EGFR	Ki-67	CD31
< 10%	20	22	28	31	13	13	Microvascular density in stroma: Median 118 (48–206) per mm^2
10%–50%	26	23	9	21	15	33	< 130 (n = 40)
> 50%	13	14	22	7	31	13	> 130 (n = 19)
Total	59	59	59	59	59	59	59

EGFR = epidermal growth factor receptor

and/or distant metastases. The survival curves were plotted by using the method of Kaplan-Meier[5] and a log rank test was used to test the differences in survival between subgroups.[6] Survival was calculated in subgroups defined by the level of expression of tumor markers, performance status (PS) and intensity of treatment. For multivariate analysis, a Cox proportional hazards model was used.[7]

Ethical consideration

The study was carried out according to the Helsinki Declaration (1964, with later amendments) and of the European Council Convention on Protection of Human Rights in Bio-Medicine (Oviedo 1997). It was approved by the Institutional Review Board Committee and by the National Committee for Medical Ethics, Ministry of Health, Republic of Slovenia

Results

Because of severe mucositis, some patients could not be irradiated to the planned dose or receive the full dose of bleomycin. Some patients had mild liver damage and the dose of mitomycin had to be below the planned dose. So, the intensity of the treatment was not the same for all patients.

Only in 59 out of 74 patients with UICC stage IV of SCCOP was there sufficient biopsy tissue in paraffin blocks for the analysis of all seven tumor markers under investigation. Among them, there were 2 females and 57 males with a median age of 52 years (39–67 years). In 42 patients the WHO PS was 0, and in 17 it was assessed as 1–2. The treatment intensity was low in 13 patients and high in 46.

The distribution of patients according to the expression of markers in the tumor tissue is presented in Table 2

The median follow-up time of patients was 6 years (3–10 years). Twenty nine (49%) patients had

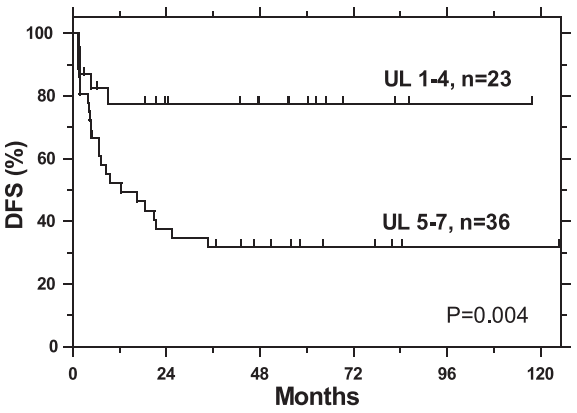

FIGURE 1. The disease-free survival (DFS) of patients according to the number of markers with unfavorable level of expression per tumor (UL).

a local, regional or distant relapse; at the last follow-up 15 (25%) were still alive and 44 (75%) were dead. The probability for DFS at five years was 48%. Intensive treatment (high vs. low) and good PS (0 vs. ≥1) were associated with statistically significantly better DFS: 59% vs. 9%, p = 0.000; 58% vs. 19%, p = 0.005, respectively. In the subgroup of intensively treated patients and good PS, DFS was 65 %, while for those with a poor performance status it was 30% (p = 0.08).

Patients with a favorable expression profile (FL) of studied markers, *i.e.* a high expression of p21 (≥ 10%), p27 (> 50%), Ki-67 (> 50%), CD31 (> 130 vessels/mm^2) and low expression of p53 (< 10%), cyclin D1 (< 10%) and EGFR (< 10%), had better DFS than patients with an unfavorable expression of these markers, *i.e.* p21 (< 10%), p27 (≤ 50%), Ki-67 (≤ 50%), CD31 (< 130 vessels/mm^2) and a high expression of p53 (≥ 10%), cyclinD1 (≥ 10%) and EGFR (≥ 10%). However, statistical significance in DFS between FL and UL was achieved only in the case of p27 and cyclin D1. DFS significantly decreased with an increasing number of markers with UL per tumor (1–4 vs. 5–7): 78% vs. 32%, p = 0.004 (Table 3,

TABLE 3. Disease-free survival at 5 years according to the expression of tumor markers

Marker		Expression	n	DFS %	p
p21	UL	< 10%	20	34	0.204
	FL	≥ 10%	39	55	
p27	UL	≤ 50%	45	40	0.040
	FL	> 50%	14	77	
p53	FL	< 10%	28	59	0.177
	UL	≥ 10%	31	38	
Cyclin D1	FL	< 10%	31	66	0.020
	UL	≥ 10%	28	30	
EGFR	FL	< 10%	13	77	0.093
	UL	≥ 10%	46	41	
Ki-67	UL	≤ 50%	46	42	0.131
	FL	> 50%	13	68	
CD31*	UL	< 130	40	40	0.100
	FL	> 130	19	69	
Number of UL per patient 1-4 vs 5-7		1–4	23	78	0.004
		5–7	36	32	
Number of UL only for P27 & cyclin D1		0–1	39	65	0.002
		2	20	17	

DFS = disease free survival; UL = unfavorable level of expression of tumor marker; FL = favorable level of expression of tumor marker; EGFR = pidermal growth factor receptor; n = number of patients; * in micro-vessels per mm^2.

Figure 1). Considering the expression of only p27 and cyclin D1, apart from other markers, DFS was significantly worse for those patients whose tumor had unfavorable expression levels of both markers: (0–1 vs. 2): 65% vs. 17%, respectively (p = 0,002) (Table. 3). Intensively treated patients in poor PS with FL of expression of p27 survived significantly better than those with a low expression of p27 (75% vs. 0%, p = 0.017). In the case of cyclin D1, corresponding analysis was not possible due to a small number of intensively treated patients with FL of this marker.

In a Cox proportional regression model, PS, intensity of treatment and p27 retained statistical significance (HR = 2.363, 95% CI = 0.999–5.589, p = 0.050; HR = 2.550, 95% CI = 1.105–5.886, p = 0.028; HR = 3.743, 95% CI =1.064–13.169, p = 0.040, respectively) while cyclin D1 was marginally significant (HR = 1.070, 95% CI = 1.000–1.145, p = 0.051). Compared to p27, the number of all markers per tumor with UL of expression had a stronger statistically significant influence on the prognosis (HR = 3.614, p = 0.009), along with the intensity of treatment (HR = 3.150, p = 0.005) and PS (HR = 2.352, p = 0.031).

Discussion

Information on the prognostic value of different tumor markers in patients with unresectable SCCOP, treated with concomitant radiochemotherapy is scarce. Out of several known tumor markers we choose some growth promoting (cyclin D1, EGFR, Ki-67) and growth suppressing (p21, p27, p53) markers, and CD31. In addition to PS, which is one of the stronger prognostic factor in different malignant diseases[8], and treatment intensity, only the number of markers with FL of expression in a particular tumor and p27 (as an individual marker) were recognized as independent prognosticators for DFS.

The published results on the value of these tumor markers in SCCHN vary. For example, in the case of p27, a negative cell-cycle regulator that blocks progression from late G1 to S phase[9], its protein expression was found to positively correlate with disease outcome in some studies[9-12], although a negative relationship was also described.[13] Probably the most widely studied marker is the nuclear transcription factor p53, playing a role in the control of cell proliferation, apoptosis and maintenance of the fidelity of DNA duplication.[14,15] The results of a meta-analysis on the role of upregulated p53 in patients with SCCHN were inconclusive, mainly due to large heterogeneity across the studies.[16] Interestingly, in three rather homogeneous studies with SCCOP[17-19], meta-analysis indicates that p53 overexpression/mutation confers a survival advantage.[16] Discrepancies in the results of different studies can also be found in the case of p21 (G1-phase blocker)[20-23], cyclin D1 (promotes progression of cells throughout the cycle)[24-26], Ki-67 (a measure of the proliferative capacity of the tumor)[27-29], EGFR (cell growth promoter)[30,31], and CD31 (microvascular density indicator).[32-34] In our study, the HPV testing was not performed. However, because 90% of the patients from our series were heavy smokers (*i.e.* with lifetime tobacco exposure of one pack of cigarettes per day for ≥10 years) - a fact that negatively influences immune system activity, which is crucial for the favorable outcome observed in HPV-positive tumors - the tumor HPV status in our patients would be less likely to play a significant role.[35,36]

Survival of our patients, who represent a rather homogeneous group regarding histology, primary tumor localization, stage, and treatment, depended primarily on the intensity of the applied therapies, their PS and also on the biological characteristics of the tumor. The latter was determined by studying dysregulation in the expression of seven tumor markers, and was influenced also by several other pathologic processes taking place in the tumor, not considered in our study. It was found that a

low expression of p21, p27, Ki-67, CD31 and high expression of p53, cyclin D1 and EGFR negatively influenced DFS. In general, when analyzing separately the expression of each of the seven markers, the difference in their expression showed no statistically significant correlation with survival probability; the two exceptions were p27 and cyclin D1. In addition to the lack of prognostic potential as an intrinsic characteristic of an individual marker, another reason for negative results could be the low number of patients in our series. However, the influence of studied markers on survival increased above the level of statistical significance, when the sum of only those markers with UL per tumor was taken into account (Figure1). By ranking the patients according to this criterion, we found a significantly lower DFS in the group with the increased number of markers with UL of expression. In the multivariate analysis, the sum of UL of expression of markers per tumor remained an independent prognostic factor for DFS, along with PS and intensity of treatment. It appears that the prediction of the outcome of the disease on the basis of expression of only one marker, even in a homogeneous group of patients, is not necessarily successful. The expression profiles of different genes are interdependent and none of the known tumor markers can play independently inside this network. Accordingly, the expression level of a particular maker resulted from the sum of influences exerted by a variety of other markers and vice versa. This might also explain why the opinions on the prognostic value of individual markers in the literature differ to such a great extent.

As expected, the survival of our patients with poor PS was low. Among them we tried to identify those who did not benefit from rather toxic concomitant radiochemotherapy. Intensively treated poor PS patients with ≤ 4 UL markers and those who had FL of expression of one or both of p27 and cyclin D1 had comparable DFS (67% and 60% respectively) to intensively treated patients with good PS. On the other hand, in spite of intensive treatment, patients with poor PS with > 4 UL markers and those with UL of expression of one or both of p27 and cyclin D1 had poor DFS (17% and 0% respectively). It seems that these patients should not be treated that vigorously and are candidates for palliative treatment programs.

In conclusion, in a series of 59 SCCOP patients, uniformly treated with concomitant radiochemotherapy with mitomycin C and bleomycin, a set of seven markers, determined immunohistochemically, was recognized as a significant predictor of DFS only when the number of markers per tumor with UL of expression was considered. Of the individual markers, only p27 correlated with survival on multivariate analysis, in addition to the PS of the patients and the intensity of the applied therapies. Despite intensive treatment, poor PS patients with > 4 markers with UL of expression as well as those with UL of expression of p27 and cyclin D1, had unfavorable survival rates: these patients should be treated with palliative intent.

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research article

Clinical results of proton beam therapy for twenty older patients with esophageal cancer

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Background. In an aging society, increasing number of older patients are diagnosed with esophageal cancer. The purpose of this study was to assess the clinical efficacy and safety of proton beam therapy for older patients with esophageal cancer.

Patients and methods. Older patients (age: ≥ 65 years) newly diagnosed with esophageal cancer between January 2009 and June 2013 were enrolled in this study. All patients underwent either proton beam therapy alone or proton beam therapy with initial X-ray irradiation. Toxicities were evaluated using the Common Terminology Criteria for Adverse Events version 4.0.

Results. Twenty patients were eligible for this study and all completed the treatment. The median age was 78 years (range: 65–89 years) and the median follow-up time was 26.5 months (range: 6–62 months). Seven patients had lymph node metastases and 10 had stage II/III cancer. The median dose of proton beam therapy was 72.6 Gy relative biological dose effectiveness (RBE) (range: 66–74.8 Gy [RBE]) for proton beam therapy alone and 33 Gy (RBE) (range: 30.8–39.6 Gy [RBE]; total dose range: 66.8–75.6 Gy [RBE]) for proton beam therapy with initial X-ray irradiation. The 2-year overall survival rate was 81.8% (95% confidence interval [CI]: 62.4%–100%), and the 2-year local control rate was 89.4% (95% CI: 75.5%–100%). Grade 2 or 3 toxicities occurred in some cases; however, no grade 4 or 5 toxicity was observed.

Conclusions. High-dose (66–75.6 Gy [RBE]) proton beam therapy without chemotherapy was an efficacious and safe treatment for older patients with esophageal cancer.

Key words: proton therapy; aged; esophageal neoplasms; radiotherapy

Introduction

Esophageal cancer is the sixth leading cause of cancer death and the eighth most common cancer worldwide.[1] In eastern Asia, esophageal cancer is the fourth most common cause of cancer death.[2] Surgery remains the main treatment choice for resectable esophageal cancer. However, following reports from the Radiation Therapy Oncology Group[3,4] and studies of the efficacy of chemoradiotherapy (CRT)[5-7], CRT has become another choice for the treatment of esophageal cancer.

In an aging society, an increasing number of older patients are diagnosed with esophageal cancer. Not all of these patients can be treated with CRT or surgery because of their age, general condition and/or complications, although there are some reports regarding the use of CRT or surgery in older patients with esophageal cancer.[8,9] Other studies have reported the efficacy of radiotherapy alone for older patients.[9,10] However, compared with CRT or surgery, X-ray irradiation alone has not shown satisfactory results for the treatment of esophageal cancer.[3,9]

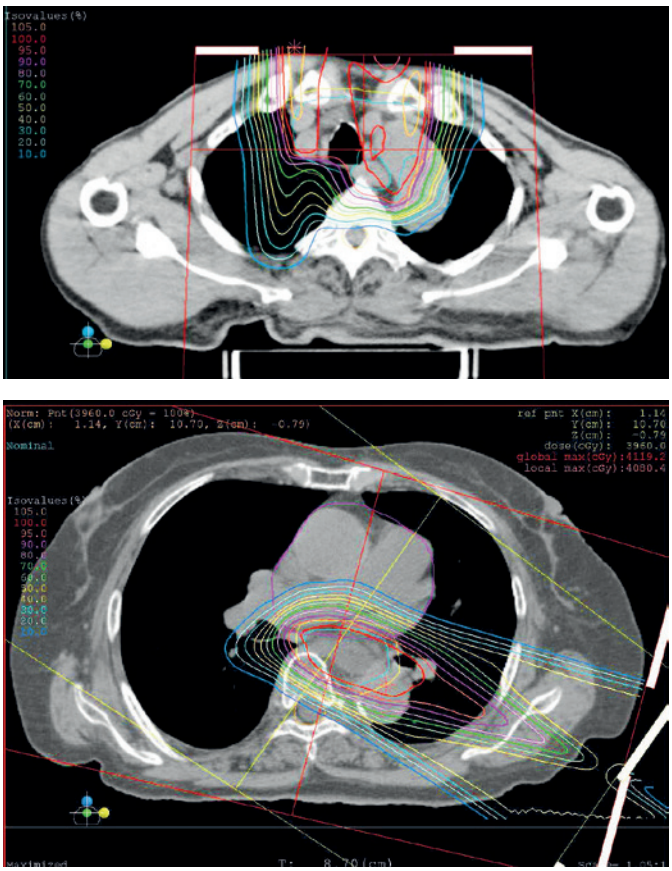
New radiotherapy treatments, such as intensity-modulated radiotherapy and proton beam therapy (PBT), deliver concentrated doses to the target volume, avoiding the organs at risk.[11-13] These therapies may thus be suitable for treating older patients with esophageal cancer. Despite the increased use of PBT for esophageal cancer[14-18], few data are available regarding the efficacy of PBT in older patients with esophageal cancer. In this study, we treated older patients with esophageal cancer using PBT without chemotherapy. We retrospectively evaluated the efficacy and safety of PBT in these older patients.

Patients and methods

Patients

Patients newly diagnosed with esophageal cancer treated with PBT without chemotherapy between January 2009 and June 2013 at the Southern Tohoku Proton Therapy Center were recruited from our database retrospectively. All patients were histologically confirmed to have esophageal cancer based on a biopsy before each treatment. Every patient was assessed, and the clinical stage of esophageal cancer was determined using endoscopy, computed tomography (CT) and positron emission tomography (PET)-CT. Written informed consent was obtained from all patients and the investigators followed recommendations of the Helsinki Declaration.

The inclusion criteria were as follows: a histologically confirmed diagnosis of esophageal cancer, age of ≥ 65 years, World Health Organization performance status of 0–2 and no distant organ metastasis or other sites of uncontrolled cancer.



A

B

FIGURE 1. Dose distribution map for proton beam therapy following initial X-ray irradiation. The region outside the outermost line received <10% radiation. **(A)** Dose distribution map for cephalic esophageal cancer. **(B)** Dose distribution map for thoracic esophageal cancer.

Proton beam therapy

Treatment planning for PBT was based on three-dimensional CT images taken at 2 mm intervals in the exhalation phase while using a respiratory gating system (Anzai Medical, Tokyo, Japan). The gross tumor volume (GTV) included the primary tumor and lymph node metastases. The primary tumor volume was determined from markers implanted using endoscopy at the cranial and caudal ends of the tumor. Lymph nodes over 1.0 cm in the short axis or exhibiting a high ^{18}F-fluorodeoxyglucose uptake on PET-CT were considered metastases. The clinical target volume (CTV) was defined as GTV plus longitudinal margins of ≥ 2.5 cm and lateral margins of 0.5 cm. The planning target volume (PTV) was CTV plus 0.5 cm margins. The daily PBT fraction was 2.2 Gy relative biological dose effectiveness (RBE). Proton energy levels of 150 MeV or 210 MeV for 1–2 portals, and spread-out Bragg peak were tuned as much as possible until the PTV was exposed to a 90% isodose of the prescribed dose (Figure 1). The PBT system at our institute (Proton beam system, Mitsubishi, Tokyo, Japan) used synchrotron, and scattering methods. Treatment was administered during the exhalation phase using a respiratory gating system. Daily front and lateral X-ray imaging was used for positioning. The PBT schedule was 33.0 Gy (RBE) in 15 fractions over 3 weeks in the combination therapy group and 72.6 Gy (RBE) in 33 fractions over 7 weeks in the PBT-only group. The PBT dose was modulated appropriately considering the response of the primary tumor as determined using endoscopy and PET-CT images. If the reduction in the maximal diameter of the primary tumor was < 50%, 1 to 3 fractions of PBT were performed without replanning. On the other hand, PBT was stopped without administer-

ing 1 to 3 fractions if the degree of tumor reduction was adequate and the patient had esophageal ulcer.

Initial X-ray irradiation

Treatment planning for X-ray irradiation was also based on three-dimensional CT images taken at 2.5 mm intervals. The patients received PBT along with the initial X-ray irradiation (combination therapy) if they had ≥ T2 disease or lymph node metastases. However, patients were treated with PBT without initial X-ray irradiation if they had severe cardiopulmonary complications, such as interstitial pneumonitis or myocardial infarction, their performance status was 2 or they refused X-ray irradiation.

The cephalic and caudal borders of the initial X-ray irradiation fields included the bilateral supraclavicular nodes and cephalic plexus for thoracic or abdominal esophageal cancer. For cephalic esophageal cancer, we irradiated the region from the laryngopharynx to the carina. 10-MV X-ray irradiation was used with anteroposterior fields. The field number was generally two, whereas three fields were used for the field within a field technique when there was a large hot area. The daily X-ray irradiation fraction was 1.8 Gy, and the irradiation schedule was 36.0 Gy in 20 fractions delivered over 4 weeks.

Evaluation and follow-up

All patients underwent endoscopy and PET to evaluate the initial tumor response within three months of the completion of treatment. The follow-up interval was every 2–3 months for the first year and every 3–6 months thereafter. Endoscopy, CT and PET-CT were performed if necessary.

Complete response was defined as the complete disappearance of all detectable tumors, partial response was defined as a ≥ 50% reduction in the maximal diameter of the tumor and stable disease was defined as no decreases or increases in the tumor diameter. Progressive disease was defined as enlargement of the primary tumor or the appearance of new lesions, including lymph node and distant metastases. Toxicities were evaluated using the Common Terminology Criteria for Adverse Events version 4.0.[19]

Statistical analysis

The statistical tests were performed using the IBM SPSS Statistics version 22 software package (SPSS

TABLE 1. Patient characteristics

Characteristics	Patients
Age (years)	
Median	78
Range	65–89
65–69	2 (10%)
70–74	5 (25%)
75–79	6 (30%)
80–89	7 (35%)
Gender	
Male	14 (70%)
Female	6 (30%)
Performance status	
0	7 (35%)
1	11 (55%)
2	2 (10%)
Follow up time (months)	
Median	26.5
Range	6–62
T category*	
T1	8 (40%)
T2	5 (25%)
T3	6 (30%)
T4	1 (5%)
N category*	
N0	13 (65%)
N1	4 (20%)
N2	3 (15%)
Stage*	
I	10 (50%)
II	5 (25%)
III	5 (25%)
Tumor location	
Cervical	3 (15%)
Upper thoracic	4 (15%)
Mid thoracic	9 (45%)
Lower thoracic	4 (25%)
Histopathology	
Squamous cell carcinoma	19 (95%)
Adenocarcinoma	1 (5%)
Proton dose in PBT with initial X-ray irradiation (n=9) (Gy (RBE))	
Median	33.0 (total dose: 69.0)
Range	30.8–39.6 (total dose: 66.8–75.6)
Proton dose in PBT alone (n=11) (Gy (RBE))	
Median	72.6
Range	66.0–74.8

PBT = proton beam therapy; RBE = relative biological dose effectiveness; * Numbers correspond to the tumor-node-metastasis system of classification (International Union Against Cancer criteria)

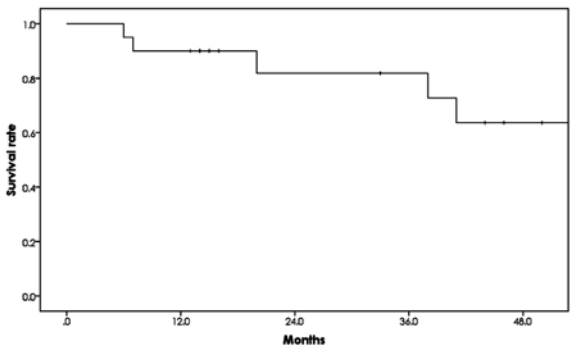


FIGURE 2. Overall survival rate of the patients with esophageal cancer after proton beam therapy. The 1- and 2-year overall survival rates were 90.0% and 81.8%, respectively.

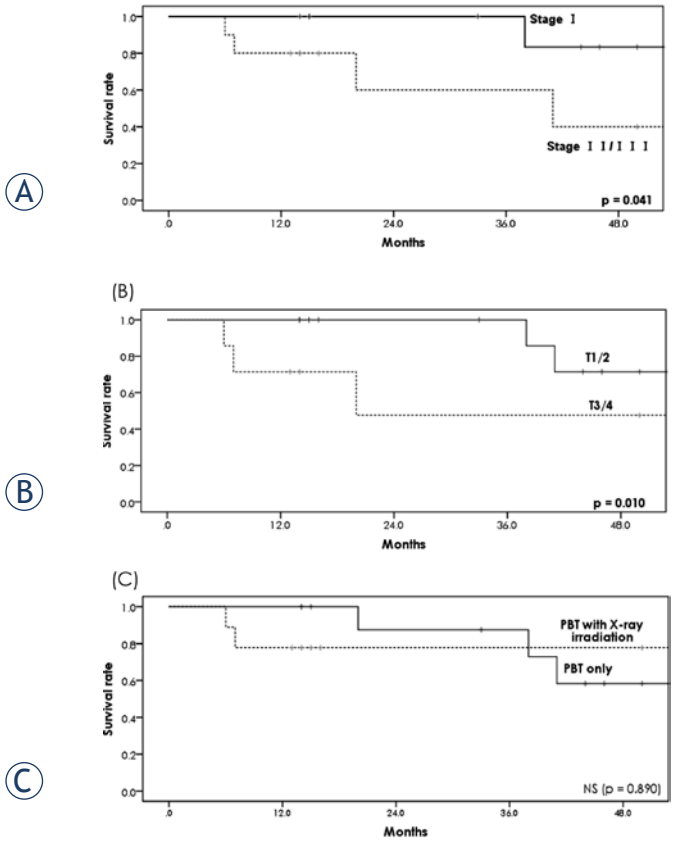


FIGURE 3. (A) Overall survival rate of the patients with stage I and II/III esophageal cancer. The 2-year overall survival rate was statistically different between the two groups (p = 0.041). **(B)** Overall survival rate of the patients in T 1/2 and T 3/4. The 2-year overall survival rate was statistically different between the two groups (p = 0.010). **(C)** Overall survival rate of the patients receiving proton beam therapy alone or proton beam therapy with initial X-ray irradiation. The 2-year overall survival rate was not statistically different between the two groups (p = 0.890).

NS = not significant; PBT = proton beam therapy

Inc., Chicago, IL, USA). The overall survival (OS) time was defined as the time between the start of treatment and the last follow-up. The local control time was defined as the time between the start of treatment and the date on which tumor recurrence was found or the last follow-up. The Kaplan–Meier method and log-rank test were applied to estimate survival probabilities and compare the survival rates, respectively.

Results

Patients

Twenty-six older patients were treated for esophageal cancer using PBT with or without initial X-ray irradiation between January 2009 and June 2013. All of these subjects were treated without any concurrent treatments, including chemotherapy. Of these 26 patients, 2 were excluded from the analysis because of distant metastasis and 4 were excluded for uncontrolled cancer at other sites. The characteristics of the remaining 20 patients, including 9 with inoperable cancer, are summarized in Table 1. All 20 patients completed their treatment. The cohort comprised 14 men and 6 women, with a median age of 78 years (range: 65–89 years). The median follow-up time was 26.5 months (range: 6–62 months). Comorbidities included interstitial pneumonitis owing to collagen disease (2 patients), chronic obstructive pulmonary disease (3 patients), myocardial infarction (5 patients), chronic heart failure (3 patients), chronic renal failure (2 patients) and diabetes mellitus (1 patient). Lymph node metastasis was present in 7 patients, and 10 patients had stage II/III cancer. Eleven patients were treated with PBT alone, and 9 patients were treated with combination therapy. The median dose of PBT

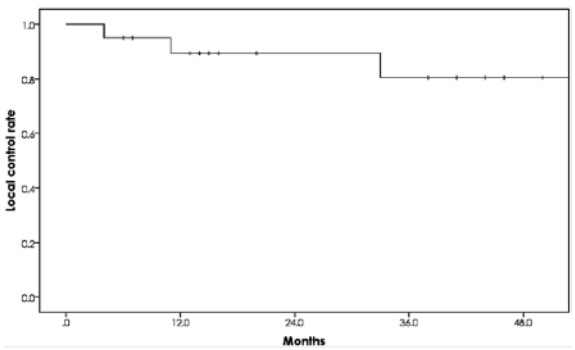


FIGURE 4. Local control rate for the patients with esophageal cancer after proton beam therapy. The 2-year local control rate was 89%.

TABLE 2. Toxicities

Toxicities	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Esophagitis	3 (15%)	3 (15%)	14 (70%)	0	0	0
Esophageal ulcer	13 (65%)	0	6 (30%)	1 (5%)	0	0
Esophageal stenosis	18 (90%)	0	2 (10%)	0	0	0
Esophageal fistula	19 (95%)	0	1 (5%)	0	0	0
Pneumonitis	6 (30%)	11 (55%)	2 (10%)	1 (5%)	0	0
Pleural effusion	12 (60%)	6 (30%)	2 (10%)	0	0	0
Pericardial effusion	17 (85%)	3 (15%)	0	0	0	0

was 72.6 Gy (RBE) (range: 66.0–74.8 Gy (RBE)) for PBT alone and 33.0 Gy (RBE) (range: 30.8–39.6 Gy (RBE)) for the combination therapy. With regard to the dose of X-ray irradiation, all patients received 36.0 Gy (RBE), except for one patient who received 32.4 Gy.

Survival and local control

All patients were followed for at least 13 months or until death. Six patients died, 4 from esophageal cancer and 2 from other causes (1 from bacterial pneumonia and 1 from another cancer). The 1- and 2-year OS rates were 90.0% (95% confidence interval [CI]: 76.9%–100%) and 81.8% (95% CI: 62.4%–100%), respectively (Figure 2). There was a significant difference in the 2-year OS rate between the patients with stage I (100%) and stage II/III (60.0%) cancers (p = 0.041) (Figure 3A). There was also a significant difference in the 2-year OS rate between the patients with T category 1/2 (100%) and T category 3/4 (47.6%) (p = 0.010) lesions (Figure 3B). On the other hand, the OS rate with or without initial X-ray irradiation was not significantly different (p = 0.890) (Figure 3C). Seventeen (85%) patients achieved a complete response and 3 (15%) achieved a partial response. Three patients (1 treated with PBT alone and 2 treated with the combination therapy) had local recurrence. The 2-year local control rate was 89.4% (95% CI: 75.5%–100%) (Figure 4).

Failure patterns

Seven patients had recurrence. One patient had lymph node recurrence within the PBT field, 3 had distant metastases and 3 had local recurrence. There were no primary tumors or sites of lymph node recurrence outside the irradiation field in the PBT-only group.

Toxicities

There were no grade 4 or 5 toxicities after treatment (Table 2). Of the 20 patients, 6 (30%) had grade 2 esophageal ulcers, 2 (10%) had grade 2 pneumonitis and 2 (10%) had grade 2 pleural effusion. One patient (5%) with an esophageal ulcer required intravenous hyperalimentation (grade 3 esophageal ulcer), and the ulcer healed one month later. Two patients (10%) with esophageal stenosis were treated with dilation using endoscopy (grade 2 esophageal stenosis). One of these patients developed an esophageal fistula (grade 2 esophageal fistula) just after dilation and was treated with the insertion of a stent in the esophagus. Neither patient required surgery. One patient (5%) with pneumonitis was treated with oxygenation and steroid administration two years after PBT because of dyspnea (grade 3 pneumonitis). In that case, the dyspnea was relieved 3 days later, and the dose of steroids was gradually reduced.

Discussion

We herein demonstrated that PBT without chemotherapy is efficacious and safe for the treatment of older patients with esophageal cancer. To the best of our knowledge, this is the first report on the use of PBT without chemotherapy in older patients with esophageal cancer.

Radiotherapy alone is one choice for treating older patients with esophageal cancer who cannot receive CRT or surgery. Kawashima *et al.*[10] reported the results of 66 Gy X-ray irradiation without chemotherapy in 51 older patients with no lymph node metastasis: the 1- and 2-year OS rates were 71% and 53%, respectively. Additionally, Cooper *et al.*[3] reported 1- and 2-year OS rates after 64 Gy radiotherapy alone of 34% and 10%, respectively,

TABLE 3. Previous results of radiation therapy without chemotherapy for esophageal cancer and our result

	number of patients	T category	N category	median total dose	1-year OS	2-year OS
Nemoto *et al.*, 2000[20]	78	T1	N0	65.5 Gy	88%	73%
Kawashima *et al.*, 2006[10]	51	T1–3	N0	66 Gy	71%	53%
Cooper *et al.*, 1999[3]	62	T1–3	N0–1	64 Gy	34%	10%
Smith *et al.*, 2009[9]	623	T1–4	N0–1	none	16%	7%
Oto *et al.*, 2015	20	T1–4	N0–2	69.0 Gy (RBE) (PBT with X-ray) 72.6 Gy (RBE) (PBT only)	90%	81.8%

OS = overall survival; PBT = proton beam therapy; RBE = relative biological dose effectiveness

Smith *et al.*[9] reported 1-year and 2-year OS rates for older esophageal cancer patients treated with X-ray irradiation alone of 16% and 7%, respectively and Nemoto *et al.*[20] reported 1- and 2-year OS rates after radiotherapy alone (median total dose: 65.5 Gy) for superficial esophageal cancer (stage I) of 88% and 73%, respectively. Our results, including those for the 7 patients with lymph node metastases, showed superior 1- and 2-year OS rates to those observed in these studies (Table 3). These results may differ because we were able to administer higher CTV doses with less exposure to organs at risk, such as the lungs and heart, although a previous report indicated that higher doses do not improve outcomes in cases of CRT.[4]

Studies of esophageal cancer treated with CRT have reported 5-year OS rates of 11%–75.7%; these cohorts included older patients.[3,5,6,8] In a comparative study of CRT versus surgery alone for the treatment of esophageal cancer, Ariga *et al.*[5] reported a 3-year OS rate of 69.1% for CRT patients in stage II/III and 47.9% for surgery patients in stage II/III; for patients with stage I cancer, the 2-year OS rate was 100% in the CRT group and 90% in the surgery alone group. Ishikura *et al.*[7] reported long-term toxicities after CRT in a study of 139 patients, with grade 4 or 5 esophagitis (7 patients), pneumonitis (4 patients) and pericardial effusion (1 patient). Our results showed an equivalent 2-year OS rate for patients with stage I cancer but an inferior OS rate for patients with stage II/III cancer. This result suggests that PBT without chemotherapy is sufficient for treating stage I esophageal cancer, although patients with stage II/III esophageal cancer have a higher OS when treated with concomitant chemotherapy. However, older patients receiving platinum-based chemotherapy develop significantly more grade 3–5 toxicities than younger patients.[21,22] In addition, patients treated with CRT for esophageal cancer experience more life-threatening acute toxicities than those treated with radiotherapy alone (CRT: 8%; radiotherapy alone: 2%).[3] Higher grade toxicities are more common in patients receiving concomitant CRT, particularly older patients. Therefore, the administration of concomitant chemotherapy is not possible in all older patients; PBT without chemotherapy may be a feasible treatment choice for older patients with stage II/III esophageal cancer, particularly for older patients who have cardiopulmonary comorbidities, renal failure or a bad performance status.

No broad consensus has been established regarding the optimal CTV protocol for elective nodal irradiation in cases of esophageal cancer. Zhao *et al.*[23] evaluated the results of late-course accelerated hyperfractionated involved-field conformal radiotherapy for locally advanced esophageal squamous cell carcinoma, reporting OS rates of 77% at 1 year and 56% at 2 years, although both T4 and N1 patients were included. In addition, the rate of out-field node recurrence alone was only 8%. Similarly, Kawaguchi *et al.*[24] observed a rate of out-field lymph node recurrence alone of 11%. Ji *et al.*[25] reported that lymph nodes located near esophageal cancer lesions receive considerable incidental doses of irradiation to the involved field, which may eliminate subclinical lesions. Zhang *et al.*[26] reported the results of involved-field irradiation for esophageal cancer, including patients with lymph node metastasis (73.4%); they observed that the rate of out-field lymph node recurrence was as high as 30%. In our study, we observed no recurrence outside the irradiation field in patients receiving PBT alone and found no significant differences in the OS rates between the patients treated with PBT alone and those treated with the combination therapy. These results suggest that involved-field irradiation is a sufficient treatment for

esophageal cancer without lymph node metastasis. Furthermore, PBT has advantages over other treatments for esophageal cancer because higher radiation doses can be administered without increasing the toxicity.

High radiation doses reportedly do not improve the OS rate in cases of CRT[4]; however, the optimal dose for radiotherapy alone has not been determined. The OS rate may increase if patients receive a higher dose of radiation. In Japan, even older patients receive 66 Gy for radiotherapy alone[10]; most patients receiving PBT can tolerate doses higher than 66 Gy (RBE). In the current study, our patients who underwent PBT experienced no severe or fatal toxicities within the follow-up period, although they received doses higher than 66 Gy. There were 2 patients who had esophageal stenosis in the current study, however, the esophageal stenosis was severe in both cases because of esophageal cancer before starting treatment and the stenosis remained even after they achieved a complete response. Kawashima *et al.*[10] reported that 3 (5.9%) patients receiving 66 Gy X-ray irradiation developed grade 5 pneumonitis within 90 days of the start of radiotherapy. Mizumoto *et al.*[15] reported the results of PBT (median total dose of combined X-ray and proton beam: 80.0 Gy (RBE); median dose of PBT alone: 79.0 Gy (RBE) without chemotherapy for locally advanced T1-4 N0/1 M0 esophageal cancer. The only toxicity observed was non-healing ulcers in 4 (8%) patients. These results suggest that, compared with X-ray therapy alone (dose: > 60 Gy (RBE)) or PBT (dose: > 80 Gy (RBE)), PBT is a safe and feasible treatment for esophageal cancer when the dose is 66.0 to 75.6 Gy (RBE).

We used initial X-ray irradiation for elective nodal irradiation, because the available field size of PBT at our institute is 15 cm × 15 cm. Some researchers have also reported using combination therapy.[14,15] When the patients received the initial X-ray irradiation at our institute, PBT was performed as shown in Figure 1. Although the proton beam was stopped when it reached a location close to the spinal cord (Figure 1A), the irradiation dose for the spinal cord was adequately reduced and no patients with radiation myelopathy were observed as of the last follow-up. On the other hand, the lung regions received a high dose (Figure 1B), however, the irradiation dose for the lung of PBT was less than the oblique opposed X-ray irradiation following anteroposterior irradiation for elective nodal irradiation. As a result, we think that combination therapy is therefore a practical and safe technique for treatment with esophageal cancer.

There are two limitations associated with this study. First, the number of patients was very small and we only included patients from a single institution. However, the current study revealed the high 1- and 2-year OS rates with following survivors for at least 13 months. Second, the follow-up time was short, as we started using PBT only in 2008. Therefore, longer follow-up is needed to ascertain the long-term OS rate and toxicities.

The high 1- and 2-year OS rates with acceptable toxicity observed in this study indicated that high-dose 66.0–75.6 Gy (RBE) PBT without chemotherapy was an efficacious and safe treatment for older patients with esophageal cancer.

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research article

Cutaneous melanoma frequencies and seasonal trend in 20 years of observation of a population characterised by excessive sun exposure

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Background. Cutaneous melanoma is an aggressive form of skin cancer. It has become an increasingly common neoplasm in the most developed countries, especially among individuals of European origin.

Patients and methods. Anonymous data of patients with cutaneous melanoma were collected from the diagnostic database of the University Hospital of Trieste from 1 January 1990 to 10 December 2013. Our study is based on a population which was constant over the period of observation; it was also well-defined and characterised by unrestrained sun exposure.

Results. The number of cutaneous melanomas increased during the period of observation with a seasonality trend and gender related differences both for anatomical sites distribution and stage of the disease. Moreover, 6% of our cohort developed multiple melanomas.

Conclusions. In a well-defined population devoted to excessive sun exposure the frequencies of skin melanomas roughly doubled from 1990 to 2013 following a seasonal trend. In that population, prevention efforts according to gender specific risk behaviour, as well as follow-up programmes both for evaluation of metastatic spreading and for early diagnosis of additional skin melanomas, are crucial due to gender specific differences and to the occurrence of multiple melanomas.

Key words: cutaneous melanoma; sun exposure; frequencies; multiple melanomas; gender related differences

Introduction

Cutaneous melanoma has become an increasingly common neoplasm in most developed countries, especially among individuals of European origin.[1,2] Different patterns referred to patients' sex and age have been observed worldwide; most recent estimates indicate wide North–South and East–West variation of melanoma incidence in Europe, with the lowest rates in Southern and Eastern countries.[3]

The main possible reason for the general increasing melanoma incidence over the last 40 years is greater exposure of pale Caucasian skin to natural ultraviolet (UV) radiation.[2] Epidemiological studies suggest a relationship between suntan habits and high risk of melanoma. Sun exposure is highly prevalent in all age groups, especially among the young; it is influenced by certain convictions and attitudes towards suntan, and it is stimulated by peer pressure and beauty reasons. Although the

TABLE 1. Frequencies by age range

Calendar period of diagnosis	Age group (years)							
In situ		*20-29*	*30-39*	*40-49*	*50-59*	*60-69*	*70-79*	*80+*
				Women				
1990-1995	0	3	4	6	5	1	4	1
1996-2001	0	2	8	13	7	11	9	1
2002-2007	0	1	8	12	5	10	3	1
2008-2013	1	9	16	25	17	13	11	4
				Men				
1990-1995	0	0	0	3	6	0	4	0
1996-2001	1	1	1	4	9	4	11	1
2002-2007	0	0	2	5	2	5	2	1
2008-2013	0	1	13	22	19	16	21	3
Total *in situ*	2	17	68	90	70	60	65	12
Invasive	*0-19*	*20-29*	*30-39*	*40-49*	*50-59*	*60-69*	*70-79*	*80+*
				Women				
1990-1995	4	10	14	17	28	21	20	13
1996-2001	0	11	20	19	36	31	29	17
2002-2007	0	9	21	35	30	35	24	23
2008-2013	0	7	28	38	36	62	39	29
				Men				
1990-1995	3	5	11	12	21	30	26	17
1996-2001	0	3	10	16	35	32	40	20
2002-2007	0	2	8	21	35	54	42	34
2008-2013	1	6	16	34	39	71	78	38
Total invasive	8	53	112	192	260	336	298	191
Overall (%)	10 (1)	70 (4)	180 (10)	282 (15)	330 (18)	396 (21)	363 (20)	203 (11)

general public is now aware that sunlight exposure has been leading to increased risk of skin cancer over the decades, most people still believe that a tanned person looks healthier.[4] Moreover, also environmental risks referred to climate changes[5] and increased reporting of *in situ* melanomas[6] seem to contribute to the rise of melanoma rates.

The aim of this study is to analyse the frequencies and characterise melanoma patients in one of the Italian cities with the highest incidence of cutaneous melanoma[7] and whose inhabitants sunbathe excessively.

Patients and methods

Anonymous data from patients with cutaneous melanoma were collected from the diagnostic database of the University Hospital of Trieste. Inclusion criteria were a diagnosis of cutaneous melanoma and to be resident in the Italian province of Trieste. Data include gender, age at diagnosis, date of diagnosis and anatomical site of melanoma onset. Data were collected separately for *in situ* and invasive melanoma from 1 January 1990 to 10 December 2013. For patients submitted to BRAF mutation analysis for therapeutic issues also BRAF mutational status was retained. For survival and analyses related to melanoma thickness, histological type and disease progression, we retained those patients who were followed up by the Dermatology unit of the University Hospital of Cattinara. Clinical data criteria as well as results are reported in the Supplementary file. The study was conducted according to the Declaration of Helsinki protocols. Moreover, clinical data were available only for patients who signed an informed consent for research use of their data.

Statistical analyses

The distribution of the clinical, histological, and epidemiological categorical variables was compared by the chi-squared test. ANOVA and t-test were performed for continuous variables depending on the number of categories. Seasonality test was evaluated by means of the Walter and Elwood test.[8] To estimate trend across groups for non-parametric data the Cuzick's test was used. All p-values are two-sided with values <0.05 regarded as statistically significant. Statistical analyses were performed with the Stata/SE 12 package (Stata, College Station, TX).

Results

Frequencies

From 1990 to 2013, 1834 patients had a diagnosis of cutaneous melanoma. They were all Caucasian residents of the province of Trieste between 14 and 98 years of age. Data on *in situ* as well as invasive cutaneous melanoma by age group and gender are reported in Table 1. Overall, mean age at diagnosis was 59 years (± 16.3 years) with 50% gender distribution. Age at diagnosis for men was significantly higher (average age 62 years) than for women (average age 57 years) ($p < 0.001$). The number of melanoma patients has increased over the years both for *in situ* and invasive melanomas and that number was more pronounced in the last 6 years (Figure 1). The diagnosis of melanoma was more frequent for middle-aged and older patients, although an incre-

ment for younger patients (aged < 40) over years has been observed.

When we divided the 24 years of observation into 4 periods of 6 years, we did not find any variation of age at diagnosis over the years (p = 0.1), even by sex stratification (p = 0.06 for men and p = 0.5 for women). However for invasive melanomas a significant increment of age at diagnosis was observed over the 4 intervals of observation (p = 0.02) (Figure 2). This information was maintained for men (p = 0.05), but not for women (p = 0.3) (Figure 2). Age at diagnosis for men increased significantly from 61 (± 18 years) years to 64 (± 15 years) years. Age at diagnosis for *in situ* melanomas did not vary across years (p = 0.3), for both genders (p = 0.6 for men and p = 0.4 for women).

Anatomical sites

The distribution of primary cutaneous melanomas by anatomical sites is reported in detail in Table 2. Hereafter only significant results are reported. Overall, melanomas of the trunk were more frequent in men (54%) than women (35%) (p < 0.001). This result was maintained considering separately invasive and *in situ* melanomas (p < 0.001 for both); therefore 53% of men and 36% of women developed an invasive melanoma of the trunk. With regard to *in situ* neoplasm the proportion of melanoma of the trunk was 60% for men and 32% for women. Melanomas of the head and neck were also more frequent in men (p = 0.003), who had that localization in 12% of cases compared to 8% of women. The distribution of invasive melanomas of head and neck was different between genders (p = 0.02), although it was comparable for *in situ* (p = 0.07).

The percentage of melanomas of the lower limbs was higher in women (29% *vs.* 10%) (p < 0.001), both for invasive melanomas (p < 0.001) and *in situ* ones (p < 0.001) (Table 2).

Invasive melanomas of hands and foot were more frequent in women (p = 0.05) with a proportion of 4.4% of cases in comparison to 2.5% of men; this observation was not confirmed for *in situ* melanoma (p = 0.7).

When we divided the 24 years of observation into 4 periods of 6 years the proportions of melanomas by anatomical sites remained essentially the same (p = 0.1), both for men (p = 0.2) and women (p = 0.4).

Seasonality

The overall monthly diagnosis of invasive melanomas showed significant excess from the cyclic variation with the maximum around the month of June (represented by θ = 171°) and another lower peak in October (p < 0.001) (Figure 3). The same pattern was present for males (θ = 162°- early June, p < 0.001) and females (θ = 181°- late June, p = 0.0002) (Figure 3). No statistically significant cyclic trend was evidenced for *in situ* melanomas (p = 0.2), either for men (p = 0.5) or women (p = 0.3).
According to the anatomical site of invasive melanoma onset, head and neck did not show cyclic trend (p=0.3), but all the other sites did so: invasive melanomas of the hands and foot peaked in late May (θ = 147°, p = 0.002), those that were developed on the trunk and lower limbs peaked in June (θ = 157°, p = 0.03; θ = 178°, p = 0.0001, respectively) and invasive melanomas of the upper limbs peaked in July (θ = 205°, p = 0.004).

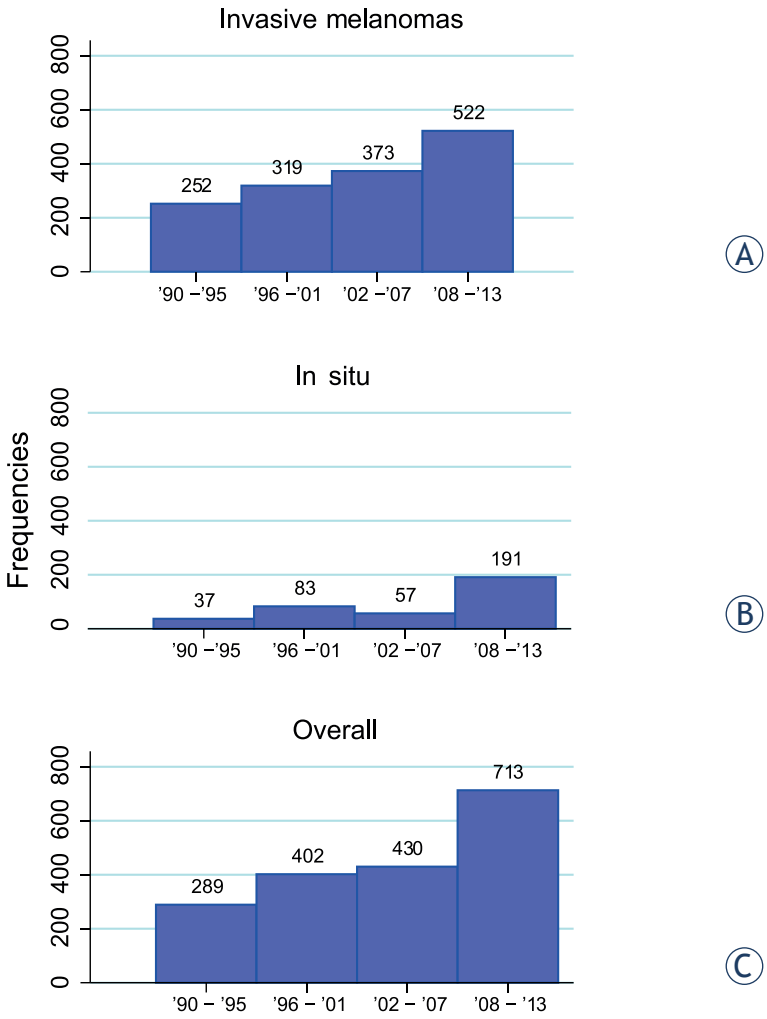

FIGURE 1. Frequencies of cutaneous melanoma over years of observation: **(A)** invasive melanomas; **(B)** *in situ* melanomas; **(C)** invasive and *in situ* melanomas.

TABLE 2. Distribution of melanomas by sex and anatomical sites, percentage is included in brackets

Anatomical sites	Female, n (%)	Male n (%)	Total n (%)
***In situ* melanoma**			
Head and neck	10 (4.7)	15 (9.6)	25 (6.8)
Hands and feet	2 (1.0)	2 (1.3)	4 (1.1)
Back	21 (10.0)	35 (22.3)	56 (15.2)
Chest	48 (22.7)	60 (38.2)	108 (29.3)
Trunk (back+chest)	69 (32.7)	95 (60.5)	164 (44.5)
Upper limbs	50 (23.7)	20 (12.7)	70 (19.0)
Lower limbs	64 (30.3)	18 (11.5)	82 (22.3)
NOS	16 (7.6)	7 (4.5)	23 (6.3)
Total	211 (100)	157 (100)	368 (100)
Invasive melanoma			
Head and neck	60 (8.5)	92 (12.1)	152 (10.4)
Hands and feet	31 (4.4)	19 (2.5)	50 (3.4)
Back	86 (12.2)	156 (20.5)	242 (16.5)
Chest	165 (23.4)	244 (32.1)	409 (27.9)
Trunk (back+chest)	251 (35.6)	400 (52.6)	651 (44.4)
Upper limbs	107 (15.2)	125 (16.4)	232 (15.8)
Lower limbs	201 (28.5)	74 (9.7)	275 (18.8)
Other	2 (0.3)	0 (0.0)	2 (0.1)
NOS	54 (7.6)	50 (6.6)	104 (7.1)
Total	706 (100)	760 (100)	1466 (100)

NOS = not otherwise specified

Multiple melanomas

In our cohort 112 patients developed multiple melanomas: 26 of them had synchronous melanomas and 86 had metachronous melanomas.

Synchronous

With respect to synchronous melanomas all patients, except one, had diagnosis of *in situ* melanomas for both neoplasms.

There was no difference between genders for age at diagnosis. Distribution of anatomical sites was significantly different between genders (p = 0.04), mainly because melanomas of the lower limbs were more frequent among women (p = 0.03; 21% *vs.* 0); the other anatomical sites were similarly distributed between genders (p = 0.3). No patient belonging to this group of multiple melanomas developed the neoplasm on hands and foot.

Metachronous

Of the 86 patients who developed metachronous melanomas 53 were males and 33 were females, therefore men developed multiple metachronous melanomas (p = 0.02) more frequently. The appearance of the second melanoma did not follow any seasonal trend (p = 0.07). Most patients had a diagnosis of invasive melanomas for both neoplasms. On average, the second primary melanoma developed 3.7 years after the first one (min-max = 0–17 years), without any significant difference in time of appearance among genders (p = 0.8) and anatomical sites of the primary and secondary melanomas (p = 0.8 and p = 0.5, respectively). Overall, the location of primary melanomas was not significantly different between genders (p = 0.07), although a higher frequency of primary melanomas of the trunk was observed for men (62% *vs.* 33%, p = 0.02) and a higher frequency of primary melanoma of the lower limbs was observed in women (24% *vs.* 9%, p = 0.05) in agreement with data obtained from the entire cohort. In 30 patients (35%) the second melanoma developed in the same anatomical region as the first one, more frequently on the back (40%) and lower limbs (23%). The site of second primary melanomas differed between genders (p = 0.03) with a prevalence of the melanoma of the trunk (62% *vs.* 36%; p = 0.01) for men and lower limbs for women (27% *vs.* 4%; p = 0.002). Breslow's depth of primary melanomas was significantly higher than that of secondary melanomas (p = 0.001) (Figure 4). Similarly, the mean number of mitoses of primary melanomas was higher than that of the second ones (1.5 *vs.* 0.6, p = 0.01) and on average the stage of the first lesion was also higher than the second one (p = 0.01).

BRAF mutational status:

BRAF mutational status was assessed in 40 patients for therapeutic issues due to disease progression. Of those 25 had mutations at BRAF gene while 15 were wild type (see supplementary file for details). Patients with mutant BRAF gene were significantly younger (average age 52 years) than those with wild type BRAF melanoma (average age 64 years; p = 0.03), in particular this observation was confirmed in females (p = 0.04), but not in males (p = 0.2) as shown in supplementary Table 2.

BRAF mutational status was also related to anatomical sites (p = 0.03): in our sub-group of patients BRAF mutations prevail in melanoma of trunk compared to melanoma of the hand and foot that were all wild type for BRAF.

Clinical data and cancer specific survival

Elaboration of clinical data and cancer specific survival did not add anything new to cutaneous mela-

noma research, because they confirm already published evidence. Therefore, they are reported in the supplementary file.

Discussion

This is a population-based study referred on data collection from cutaneous melanoma patients resident in Trieste, a seaside town of about 250.000 residents in North-eastern Italy. This population is stable and well-defined, and it was constant over the years of observation.[9] Residents in this area are mainly fair skinned and blue eyes because of their Celtic.[10] and Austro-Hungarian origins. The peculiarity of the inhabitants of this town referred to cutaneous melanoma is their unrestrained sun exposure, mainly for traditional and cultural reasons. For that particular reason any increment in cutaneous melanoma frequencies over the years is mainly due to environmental changes and suntan habits. The frequencies of cutaneous melanoma retrieved in this study are in agreement with the data reported by the Cancer registry of Friuli-Venezia Giulia region.[11] The number of cutaneous melanoma increased during the period of observation, showing higher rates particularly for middle-aged and for elderly residents, who probably had not used any sun protection in their childhood and adult life, as reported by other authors.[12] During our period of observation, a steep increment in cutaneous melanoma cases occurred; in addition in the last six years cutaneous melanoma frequencies doubled in comparison to 1990-1995 in a homogeneous and stable population. That increment may be due in part to improved registration of melanoma as well as to over-diagnosis[12], but it's unlikely that the frequency growth could be solely ascribed to those factors. Although there are no data supporting this hypothesis, a possible explanation could be the cumulative effect of air pollution and sun exposure, since the air pollution index has been correlated with skin cancer.[13] Nonetheless, other individual factors such as the use of artificial sunbed, cosmetics including sunscreen, photosensitising drugs, and exogenous hormones could be additional risk factors for the development of cutaneous melanoma.[14]

In our cohort seasonality of cutaneous melanoma diagnosis was detected with a higher peak in June probably as a consequence of increased patient awareness and self-detection of suspected lesions due to summer clothing.[15] The presence of a lower seasonality peak in October could find a reasonable possible explanation in the effect of intense sun exposure on the visibility of melanocytic proliferation after intense ultraviolet exposure. Consequently, in summer the highlighted pigmented lesions may alert the patients themselves or the physician.[15] No seasonality has been detected for *in situ* lesions in agreement with Asken *et al*. as different explanations seem to work for invasive and *in situ* melanomas.[12]

Six percent of our cohort developed multiple melanomas, which are not uncommon in cutaneous melanoma patients.[16] In agreement with Savoia *et al*. in our series of patients there are no differences in clinical characteristics or histopathological features of the first cutaneous melanoma between patients with single or multiple metachronous

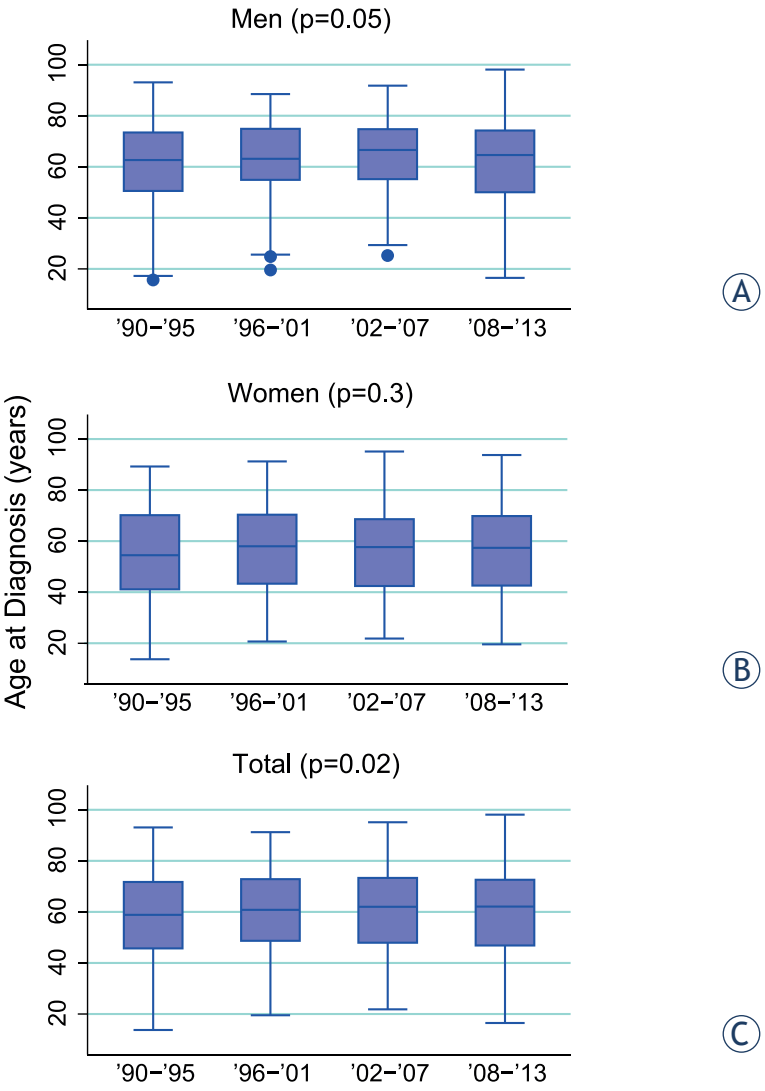

FIGURE 2. Age at diagnosis over years of observation: **(A)** men; **(B)** women; **(C)** total.

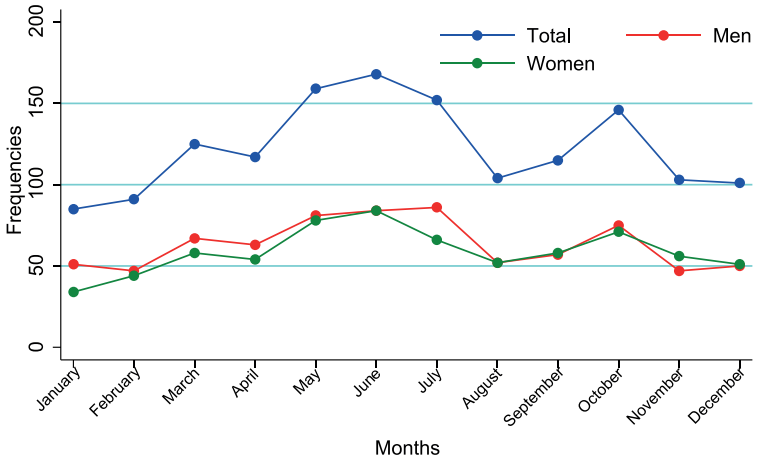

FIGURE 3. Seasonality of invasive cutaneous melanoma.

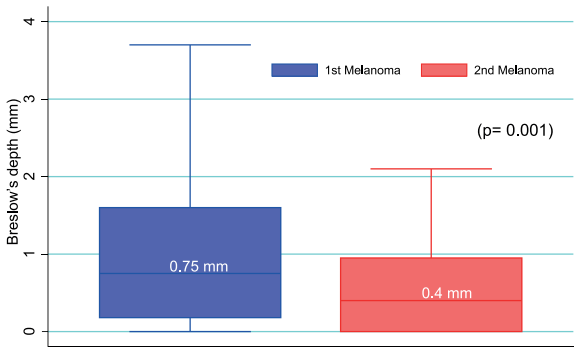

FIGURE 4. Box plot representing the Breslow's depth variation between 1st and 2nd cutaneous melanoma in patients with metachronous melanomas. Median values are reported in boxes

melanomas; the distribution of the first melanoma sites also follows the same pattern as single melanomas.[16] The significant decrease in the mean Breslow's thickness as well as in the number of mitoses and stage for the second metachronous melanoma is mainly due to the follow-up in those patients and to their increased awareness of pigmented lesion after having a melanoma.[16] Even for early melanoma lesions the importance of scheduled and well defined follow-up procedures was stressed.

Regarding melanomas mutated at BRAF gene we observed a correlation between BRAF mutations and being young at the time of primary melanoma diagnosis in agreement with other authors[17], and our observation was confirmed particularly in women. BRAF mutations seem to be associated also to anatomical sites of the primary lesion, with melanomas on the trunk presenting higher rate of mutations at BRAF gene, as already shown.[18]

Gender-related differences in the anatomical distribution and stage of cutaneous melanoma were found in our cohort. At diagnosis women present with thinner lesions and show up before men as shown by the lower age at diagnosis and lower Breslow's depth for female (Results in Supplementary file). Women show *in situ* or stage I lesions, while men have stage II and locally advanced cutaneous melanoma as shown by an Austrian report which found similar results.[19] Moreover, as already pointed out[20], cutaneous melanoma predominated at lower limb and hand-foot for women and trunk for men. In our cohort invasive melanomas of head and neck were significantly more frequent in men as observed in England after the early 1990s.[21] In agreement with others[22] men also tended to develop multiple metachronous melanomas more frequently.

Overall those observations underline that prevention efforts should be increased taking into account gender-specific risk behaviour. As it was shown in the female population, less aggressive cutaneous melanoma are most likely diagnosed due to prevention.

Conclusions

In a well-defined population excessively exposing to sunlight the frequencies of cutaneous melanoma have roughly doubled from 1990 to 2013. In that population gender specific differences as well as a seasonality trend have been observed, stressing the importance of prevention efforts taking into account gender-specific risk behaviour.

The fact that multiple melanomas are not really uncommon, especially in men, highlights the need for follow-up programmes not only for evaluation of metastatic spread but also for early diagnosis of additional cutaneous melanoma.

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research article

Release of growth factors after mechanical and chemical pleurodesis for treatment of malignant pleural effusion: a randomized control study

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Background. Growth factors are key inducers of fibrosis but can also mediate inflammatory responses resulting in increasing pleural effusion and acute respiratory distress syndrome. The primary aim of the study was to analyse growth factors release after performing chemical and mechanical pleurodesis in the first 48 hours at the patients with malignant pleural effusion. The secondary endpoints were to evaluate the effectiveness of the both pleurodeses, symptoms release and the quality of life of patients after the treatment.

Patients and methods. A prospective randomized study included 36 consecutive female patients with breast carcinoma and malignant pleural effusion in an intention-to-treat analysis. We treated 18 patients by means of thoracoscopic mechanical pleurodesis and 18 patients by chemical pleurodesis with talcum applied over a chest tube. We gathered the pleural fluid and serum samples in the following 48 hours under a dedicated protocol and tested them for growth factors levels. A quality of life and visual analogue pain score surveys were also performed.

Results. Median measured serum vascular endothelial growth factor (VEGF) level after chemical pleurodesis was 930.68 pg/ml (95% CI: 388.22–4656.65) and after mechanical pleurodesis 808.54 pg/ml. (95% CI: 463.20-1235.13) (p = 0.103). Median pleural levels of transforming growth factor (TGF) β1 were higher after performing mechanical pleurodesis (4814.00 pg/ml [95% CI: 2726.51–7292.94]) when compared to those after performing chemical pleurodesis (1976.50 pg/ml [95% CI: 1659.82–5136.26]) (p = 0.078). We observed similar results for fibroblast growth factor (FGF) β; the serum level was higher after mechanical pleurodesis (30.45 pg/ml [95% CI: 20.40–59.42]), compared to those after chemical pleurodesis (13.39 pg/ml [95% CI: 5.04 – 74.60]) (p = 0.076). Mechanical pleurodesis was equally effective as chemical pleurodesis in terms of hospital stay, pleural effusion re-accumulation, requiring of additional thoracentesis, median overall survival, but, it shortened the mean thoracic drainage duration (p = 0.030) and resulted in a higher symptoms release and in a better quality of life (p = 0.047).

Conclusions. We recorded an increase in serum VEGF levels after chemical pleurodesis, however on the contrary, an increase in the pleural fluid level of TGFβ1 and FGFβ] after mechanical pleurodesis with respect to compared group. Although the differences did not reach statistical significance, VEGF, TGFβ1 and FGFβ remain the most interesting parameters for future research. Considering the mechanisms of growth factors action, we conclude that in our study group mechanical pleurodesis might be more efficient in terms of growth factors release, thoracic drainage duration and resulted in a higher symptoms release and in a better quality of life than chemical pleurodesis.

Key words: malignant pleural effusion; pleurodesis; growth factors; quality of life

Introduction

A large number of different methods for pleurodesis used throughout the world tells us that an ideal procedure is still undetermined. Defining the best palliative treatment for malignant pleural effusion has been elucidated many times.[1-3] In our clinical practice, we use two distinct pleurodesis procedures. The most common choice is chemical pleurodesis with talc. We have also established thoracoscopic mechanical pleurodesis as an alternative method to treat malignant pleural effusions.[4] By observation, we speculate that chemical pleurodesis causes a systemic inflammatory response whereas mechanical pleurodesis only local tissue response with fewer side effects. Considering the known facts of tissue regeneration, scaring, and healing[5] we see growth factor release as an important link in these processes.

The primary aim of the study was to analyse growth factors release after performing chemical and mechanical pleurodesis in the first 48 hours. The secondary endpoints were to evaluate the effectiveness of the both pleurodeses, symptoms release and the quality of life of patients after the treatment.

Growth factors in pleurodesis

Inflammatory cells are the main source of growth factors.[6] At the same time, we find them attached to the glycoproteins of the extracellular matrix. Different events in the healing process are triggered by their action and interaction.[7-9] Their role in the process of pleurodesis was studied many times.[10-12] A research for their differential diagnostic power has exposed the vascular endothelial growth factor (VEGF) being typically higher in malignant pleural effusion.[13] We must also take into account and explore the impact of their systemic effects, particularly the role of VEGF in relation to the acute respiratory distress syndrome.[14,15]

VEGF induces angiogenesis as well as increases vascular permeability and stimulates the tumour growth and metastasis.[16] It promotes the formation of pleural effusion and is a crucial factor for the growth of malignant tissue and formation of metastases.[17-19] A study on an animal model showed the transforming growth factor (TGF) β1 to be an ideal inductor of pleurodesis with a better performance than talc but minimal to no side effects.[12] Fibroblast growth factor (FGF) β is clearly linked to the success of pleurodesis.[10] After damaging the integrity of extracellular matrix, it is released from the binding sites and serves as the first inductor of fibroblast proliferation and collagen synthesis.

Patients and methods

The study included 36 female patients with breast carcinoma and cytologically confirmed malignant pleural effusion whose lungs re-expanded after thoracic drainage and were eligible for surgery. The patients had the Eastern Cooperative Oncology Group (ECOG) performance status 0-2. The study was approved by the National Ethics Committee with number 40/09/09 and conducted at the Department of Thoracic Surgery at the University Medical Centre Maribor, Slovenia, between July 2010 and August 2013. All patients signed a written consent to participate in the study. Laboratory tests and statistical analysis were carried out at the same centre in cooperation with the Biochemistry Division of Medical Faculty, University of Maribor.

Overall, 81 female patients with breast carcinoma and malignant effusion were presented by the oncologists, of whom 36 met the inclusion criteria. We excluded from the study patients who were due to an underlying disease or concomitant diseases not fit to undergo surgery under general anaesthesia or had a trapped lung. Their demographic data and history as well as prognostic factors that could affect the treatment outcome in different oncological patients (age[20,21], type of tumour[16,22], time interval from previous surgical treatment till pleurodesis, specific systemic oncological therapy[22,23], performance status[22,24], maximal volume of previous thoracentesis[25]) were collected (Table 1).

A random numbers were assigned to the patients at admission and they were divided into two groups. Group with chemical pleurodesis with talc (n = 18) and group with thoracoscopic mechanical pleurodesis (n = 18).

Patients with chemical preurodesis were treated with a 5 g of talcum (Ph.Eur.7.0, Caesar Loretz GmbH, Austria, EU) and 100 ml of 0.9% NaCl slurry over the chest drain. We administered 40 ml of 1% lidocaine intrapleuraly, 20 to 30 minutes prior to talk application. Next, we clamped the drain for 2 hours and then reattached it to the active suction (-15 cm H_2O) drainage system for 24 hours. After the first day, the drainage system was switched to underwater seal gravity drainage until the daily amount of drained fluid was less than 200 ml. A favourable chest x-ray report was a requirement for chest tube removal.

TABLE 1. Demographical and specific data related to preoperative conditions for chemical pleurodesis with talc (CPT) and thoracoscopic mechanical pleurodesis (TMP) group group

	CPT (n = 18)	TMP (n = 18)	p-value
Age (years) [±SD]	66 [± 12]	63 [±11]	0.418
Positive hormone receptors (estrogen/progesterone)	55.6%	50.0%	0.757
HER2-positive	31.4%	23.9%	0.741
Time interval from surgery till pleurodesis (months) [±SD]	63 [± 45]	66 [± 53]	0.884
Time interval from chemotherapy till pleurodesis (months) [±SD]	6.5 [± 4.0]	7.0 [± 5.0]	0.837
Received chemotherapy before pleurodesis	94.4%	95.0%	1.000
Received radiotherapy before pleurodesis	33.3%	55.5%	0.210
Median ECOG performance status	1	1.5	0.635
Time from diagnosis of malignant pleural effusion to pleurodesis (months) [±SD]	3 [± 1]	5 [± 2]	0.112
Max. volume of previous thoracocentesis (ml) [±SD]	1297 [±354)	1375 [±441]	0.555
Pleural dissemination (median value)	not measurable	3	-
pH [±SD]	7.27 [±0.09]	7.22 [±0.06]	0.072

ECOG = Eastern Cooperative Oncology Group; HER2 = human epidermal growth factor receptor 2; SD = standard deviation

TABLE 2. Median values of growth factors in serum and pleural fluid after chemical pleurodesis with talc (CPT) and thoracoscopic mechanical pleurodesis (TMP)

	CPT (n = 18)	TMP (n = 18)	p-value
Maximum value of serum VEGF (pg/ml) [95% CI]	930.68 [388.22 - 4656.65]	808.54 [463.20 – 1235.13]	0.103
Median value of pleural TGFβ1 (pg/ml) [95% CI]	1976.50 [1659.82 – 5136.26]	4814.00 [2726.51 – 7292.94]	0.078
Median value of pleural FGFa (pg/ml) [95% CI]	108.35 [73.29 – 162.62]	66.265 [60.71 – 153.12]	0.364
Median value of pleural FGFβ (pg/ml) [95% CI]	13.39 [5.04 – 74.60]	30.45 [20.40 – 59.42]	0.076

CI = confidence interval; FGF = fibroblast growth factor; TGF = transforming growth factor; VEGF = vascular endothelial growth

Patients with mechanical pleurodesis were treated with a two-port video-assisted thoracoscopic surgery (VATS)[26] during which we performed the mechanical abrasion of parietal pleura using A.M.I.-dock reusable applicator for disposable (DLU) tips (A.M.I. GmbH, Austria, EU).

During VATS, we assessed the coverage of the pleura with malignant tissue:

0 = no obvious lesions;
1 = isolated lesions;
2 = diffuse (covering the majority of the pleura) lesions;
3 = massive (normal pleura could not be seen).

The result was calculated by adding the data from visceral, parietal and diaphragmal pleura and ranged from 0 to 9 (Table 1).

After 24 hours of active suction (-15 cm H_2O) drainage, we disconnected the drains to allow underwater seal gravity drainage of effusion and removed them after a favourable chest x-ray when the daily amount of drained fluid was less than 200 ml.

Post procedural analgesia was kept the same for both study arms. We used a combination of parenteral metamizole, piritramide and after the first post procedural day, we switched to per orally administered combination of paracetamol and tramadol.

To assess the growth factors release we used a specially designed protocol based on research data of our own unpublished pilot study. The time frame for sampling the pleural fluid and blood samples was: prior to the procedure (time 0) and 3, 12, 24, 36 and 48 h after procedure. For both groups we used only one 20F silicone chest drainage catheter (Portex, Smiths Medical, USA) oriented posteriorly and caudally, through which we collected pleural fluid samples. Blood samples taken at 24 and at 48 hours were tested for two general indicators of inflammation: leukocyte count, C-reactive protein (CRP) level. All pleural samples were also tested for lactate dehydrogenase (LDH) level and pH level of the effusion was determined before the pleurodesis. The supernatant prepared from the pleural fluid samples was centrifuged for 10 min at 3000 revolutions per minute (RPM) at 4°C. Alongside with that of the blood serum it was frozen at -70°C and stored for later ELISA testing to determine FGFα and β, VEGF and TGFβ1 levels.

To evaluate the effectiveness of both methods of pleurodesis we used chest X-ray and pleural cavity ultrasound examination. The length of thoracic drainage, hospital stay and pleural effusion re-accumulation were recorded (Table 2). A need for additional procedures due to the recurrence of dispnoic problems and pleural effusion accounted for an unsuccessful pleurodesis. The overall survival was calculated from the day of pleurodesis till the death for any causes. Follow-up lasted up to 12 months.

To describe better the immediate effects of pleurodesis, we measured the visual analogue pain score (VAS) at 0, 12, 24, and 48 hours after pleurodesis. Additionally, we used the questionnaire of European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30, version 3.0[27] to determine the impact of treatment on patients' quality of life. Global health status, physical functioning, fatigue, pain and dyspnoea measure-

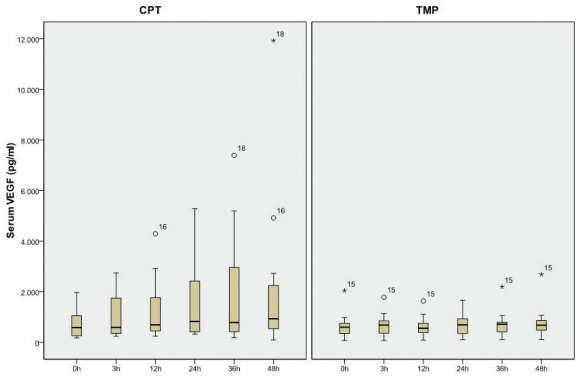

FIGURE 1. Comparison of serum VEGF values during 48 hours after chemical (CPT) and mechanical (TMP) pleurodesis (p = 0.103).

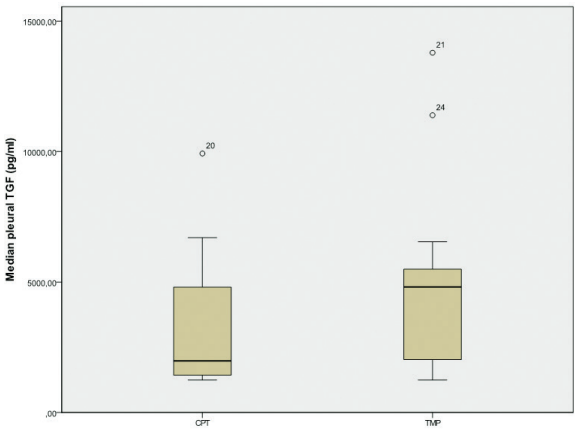

FIGURE 2. Comparison of median values of pleural TGF β1 during 48 hours after chemical (CPT)-1 and after mechanical (TMP) pleurodesis – 2 (p = 0.078).

ments were selected. Patients completed them with the data for the week before the procedure and for the week and month thereafter. These questionnaires served us as a reflection of patient tolerance towards pleurodesis.

Statistical analysis

Statistical analysis was performed by using IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp. When comparing groups of patients, $\chi 2$ test was used to test the frequency counts, t-test was used to test the means of continuous variables and when the value distribution was not normal, Mann-Whitney U test was used to test their median values. Kolmogorov-Smirnov test was used for assessing the normality of data. When dealing with repeated measures, mixed ANOVA was used to compare the mean values between and within groups. Kaplan-Meier survival analysis was used for estimating the survival function for both groups. When performing multiple comparisons Bonferroni correction was used to adjust p-values. Statistical significance was set at $p < 0.05$.

Results

Patients

The two observed groups did not differ significantly with respect to demographic data and prognostic factors (Table 1). The age, hormonal status of tumours, HER2 status, and time interval from previous surgical treatment, last chemotherapy, time from diagnosis of malignant pleural effusion until pleurodesis, specific systemic oncological therapy, performance status and maximal volume of previous thoracentesis were well balanced (Table 1).

The average coverage of the pleura in the group with mechanical pleurodesis was 3.4 out of 9 points and the median value was 3. The pH values of pleural effusions did not statistically differ (Table 1).

Growth factors release after performing chemical and mechanical pleurodesis

The focal point of our study was serum VEGF, specifically the maximum value for a given patient. Median serum VEGF level, measured after chemical pleurodesis, was 930.68 pg/ml and after mechanical pleurodesis, it was 808.54 pg/ml (Table 2, Figure 1). Maximal serum VEGF level after chemical pleurodesis was 11930 pg/ml and after mechanical it was 2687 pg/ml (Figure 1).

Other key values that showed intergroup difference were that of the pleural TGFβ1 and FGFα and β. Median pleural levels of FGFα and β and TGFβ1 were higher after performing mechanical pleurodesis, when compared to those after performing chemical pleurodesis (Table 2, Figure 2).

Levels of VEGF in pleural fluid samples and TGFβ1 in serum were similar in both groups.

We have not recorded any specific impact of any technique of pleurodesis on the serum levels of FGFα and β, most of the time they were undetectable being below the lower limit of sensitivity of ELISA.

We found the average largest increase in serum VEGF, TGFβ1 pleural value, FGFα and β pleural values between the 24th and 36th hour post pleu-

TABLE 3. Serum inflammatory parameters and biochemical parameters of pleural effusion before and after chemical pleurodesis with talc (CPT) and thoracoscopic mechanical pleurodesis (TMP)

	CPT (n=18)	TMP (n=18)	p-value
Mean increase in serum leucocyte value (x10⁹/l) [± SD] (median value of increase)	3.8 [±3,3] (3.0)	2.9 [±3.1] (2.3)	0.456
Mean increase in serum CRP value (mg/l) [± SD] (median value of increase)	54 [±46] (36.5)	61 [±59] (34.5)	0.977
Mean increase in pleural LDH value (µkat/l) [± SD] (median value of increase)	5.3 [±7.6] (2.8)	4.6 [±18.5] (3.5)	0.826

CRP = C-reactive protein; LDH = lactate dehydrogenase; SD = standard deviation

TABLE 4. Results of post pleurodesis (PD) outcomes for chemical pleurodesis with talc (CPT) and thoracoscopic mechanical pleurodesis (TMP) group

	CPT (n = 18)	TMP (n = 18)	p-value
Mean thoracic drainage duration (days)	4.5	3.8	**0.030**
Median hospital stay (days)	5	5	0.126
Pleural effusion re-accumulation	38.9%	20.0%	0.288
Additional thoracentesis required	11.1%	5.0%	0.595
Median survival post PD (months) [95% CI]	6.8 [5-9]	7.0 [4-10]	0.060

CI = confidence interval

TABLE 5. Median visual analogue scale (VAS) score for pain before and after chemical pleurodesis with talc (CPT) and thoracoscopic mechanical pleurodesis (TMP)

	CPT (n=18)	TMP (n=18)	p-value
Median VAS before pleurodesis [95% CI]	2 [2-3]	3 [2-3]	0.311
Median VAS 12 hour [95% CI]	5 [3-6]	4 [2-4]	**0.039**
Median VAS 24 hour [95% CI]	5 [4-6]	4 [3-5]	0.085
Median VAS 48 hour [95% CI]	3 [3-5]	3,5 [3-4]	0.881

CI = confidence interval

rodesis. VEGF levels in the serum were still growing up to 48th hour post pleurodesis when we stopped taking samples.

There was a significant elevation of blood leukocytes in the first 24 hours, it was more pronounced for the group with chemical pleurodesis. The same was established for the CRP levels. The LDH levels in serum and pleura correlated well with the levels of inflammation and they show a tendency to be lower after mechanical pleurodesis compared to those after chemical pleurodesis (Table 3).

We made no relevant correlation between pH value of pleural effusion and success of pleurodesis or level of growth factors.

The effectiveness of pleurodesis

Both methods were successful in preventing the recurrence of malignant pleural effusion. One patient in the group with mechanical pleurodesis and two patients in the group with chemical pleurodesis needed additional interventions due to the re-accumulation of MPE. The difference in the pleurodesis success between the groups was not significant as in other previous studies on small samples, except in terms of length of post procedural thoracic drainage (Table 4). Mean thoracic drainage duration after mechanical pleurodesis was significantly shorter than after chemical pleurodesis (p = 0.030).

We found no relevant correlation between pleurodesis success and the level of growth factors in our study groups.

One patient in the group with mechanical pleurodesis had a larger drainage of bloody effusion. Haemoglobin in the pleural fluid sample was 4.6 g/dL - 800 ml on the first postoperative day. The volume of drainage has significantly decreased on the post-operative day one. One patient from the group with chemical pleurodesis formed an empyema by the end of the first week after pleurodesis. We treated her successfully using negative-pressure wound therapy (V.A.C. freedom, K.C.I., USA) over the thoracostomy at the site of the chest drain for 25 days.

The median survival of patients was 6.8 months after performing chemical and 7.0 months after performing mechanical pleurodesis (p = 0.060) These patients were evenly distributed between both observation groups and Kaplan-Meier survival analysis showed similar survival curves for both groups. Five patients survived for 1 year or more, which shows more than 10% of one-year survival. There was also no mortality associated with the reported procedures (Table 4).

Symptoms release and the quality of life

The measured score through a pain visual analogue scale (VAS) showed lower pain-load for patients in the TMP group (Table 5). There was significant less pain load after mechanical pleurodesis compared to chemical pleurodesis 12 hours post procedure (p = 0.039). This difference was lost after 48 hours.

We got similar results for quality of life (Table 6). The quality of life questioners were filed at the three time points: (1) "pre" – one week before pleuodesis, (2) "post" - 1 week after pleurodesis, and (3) "end" – 1 month after pleurodesis. Tables 6 and 7 summarize the important results to express

TABLE 6. Comparison of quality of life questionnaire results for global health, physical functioning, fatigue, pain, and dyspnoea between groups at 3 different points in time according to the pleurodesis (PD) - chemical pleurodesis with talc (CPT) and thoracoscopic mechanical pleurodesis (TMP)

	CPT (n = 18)			TMP (n = 18)		
	pre PD	post PD	end PD	pre PD	post PD	end PD
Global health status (mean ± SD/median)	15.7 ± 9.0/16.7	20.4 ± 10.0/16.7	28.7± 14.1/25.0	20.0 ± 11.6/16.7	31.3 ± 7.1/33.3	43.8± 12.9/41.7
Physical functioning (mean ± SD/median)	38.9 ± 11.5/40.0	47.0 ± 20.6/46.7	48.9 ± 21.1/53.3	45.0 ± 14.8/40.0	51.3 ± 12.3/53.3	64.0 ± 13.4/66.7
Fatigue (mean ± SD/median)	77.2 ± 15.9/77.8	68.5 ± 18.4/66.7	64.2 ± 20.4/66.7	70.6 ± 17.0/77.8	56.7 ± 16.5/55.6	41.1 ± 19.8/38.9
Pain (mean ± SD/median)	67.6 ± 20.2/66.7	63.0 ± 14.6/66.7	52.8 ± 21.6/58.3	64.2 ± 16.5/66.7	55.8 ± 13.5/50.0	35.0± 17.0/33.3
Dyspnoea (mean ± SD/median)	68.5 ± 21.3/66.7	61.1 ± 26.2/66.7	63.0 ± 25.3/66.7	76.7 ± 21.9/66.7	65.0 ± 20.2/66.7	40.0 ± 20.5/33.3

pre PD = 1 week prior to pleurodesis; post PD = 1 week after the pleurodesis; end PD = 1 month after pleurodesis

TABLE 7. Comparison of the quality of life questionnaire results between the chemical pleurodesis with talc and thoracoscopic mechanical pleurodesis group

	Main effect of time p - value	Main effect of treatment p - value	Interaction effect between time and treatment (pre to post treatment) p - value	Interaction effect between time and treatment (pre to end result) p - value
Global health status*	< 0.001	0.001	**0.032**	**0.047**
Physical functioning	< 0.001	0.057	0.742	0.083
Fatigue	< 0.001	0.008	0.271	**0.014**
Pain	< 0.001	0.040	0.477	0.057
Dyspnoea	< 0.001	0.536	0.631	**< 0.001**

* Mauchly's test indicated that the assumption of sphericity had been violated for global health status; therefore, Greenhouse-Geiser correction was used

how the observed interventions effect patients and the impact of the treatment on the quality of life when the effect of pleurodesis is maximal. For specific indicators we took global health, physical functioning, fatigue, pain, and dyspnoea.

In both groups, we saw an improvement in quality of life over time. For the majority of targeted questions, the comparison between the two groups showed a statistically significant improvement of quality of life in the group with mechanical pleurodesis. A distinctly beneficial effect of mechanical pleurodesis was present as a reduction in the form of dyspnoea (Table 7).

Discussion

It is our strong belief that studies oriented towards pleural space can help us understand many problems we encounter in our daily work. An ideal agent that would produce effective pleurodesis in the shortest possible time with little side effects and would be affordable remains vague. Light wrote in an article summarizing his research on pleural space that it is the lack of interest of industry that turns researchers away from dedicated research.[28]

Our wish was to set up new, additional standards on how to determine the best agent for this palliative procedure. We are focused on the culprits for small but significant differences between two methods of pleurodesis and try to explain the reasons for rare but serious side effects after chemical pleurodesis with talcum. For patients with malignant pleural effusion, a pleurodesis efficiency of more than 90% achieved by the chemical pleurodesis is satisfactory and does not necessarily require further studies. However, the process of healing and scarring is extremely important for understanding the impact of surgical procedure on the body and opens up many opportunities for improvement. Progress in methods of pleurodesis can be used as an advantage for patients with recurrent primary pneumothorax and other cases where chemical pleurodesis is often used although

many thoracic surgeons believe that this is not the best choice of treatment.

Besides the usual indicators of inflammation, such as leukocyte number, LDH or CRP[14], we are establishing growth factors as additional parameters to determine the safety and efficiency of different methods of pleurodesis.

VEGF is the most extensively studied cytokine related to pleural effusion and pleurodesis outcome; it has numerous actions crucial to our understanding of the effects of pleurodesis, central being the fact that it is 50,000 times more potent vasodilator than histamine.[29] Previous research data show that an ideal pleurodesis should have limited excretion of VEGF into the pleural space, but should not elevate its serum levels.[15,17-19,28,29] VEGF is related to the increased permeability of pleura and promotes growth and metastasis of malignancies.[15,16,18,19] We believe that accelerated release of VEGF after chemical pleurodesis with talc gives serious concerns for its use.[18,29] This is especially important for patients with increased risk of respiratory problems and maybe generally for patients with malignancies. Extremely high serum levels of VEGF were observed in a small proportion (10%) of patients following chemical pleurodesis. These values of serum VEGF significantly exceed those for which it has been shown that are dangerously high.[15]

TGFβ1 is another multifunctional cytokine strongly connected to success of pleurodesis; in areas of inflammation, it stimulates cell proliferation, increases mesothelial permeability, and the production of fibrin, collagen, and tissue fibrosis.[12] Experimental studies have also shown that corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the extent of talcum or doxycycline pleurodesis, but, these drugs have no influence on pleurodesis induced with TGF β1.[30] FGFβ is another potent inductor of angiogenesis and fibrosis we see relevant.

Female patients with breast cancer and associated malignant pleural effusions are the most appropriate population to answer our research question. Their survival is relatively good and the patients are at the presentation of pleural effusions in a good performance status.[31] A large caseload enables us to strictly adhere to the main inclusion criteria: good performance status, positive cytology and re-expanded lung after evacuation of pleural effusion. The need for many repeated thoracenteses, regardless of positive cytology in a patient with breast cancer, is usually an indication for chemical pleurodesis. However, mechanical pleurodesis is another possible method for the prevention of malignant pleural effusion recurrence, but, not widely used. In our study, the two methods of pleurodesis show distinctly different features. Mechanical pleurodesis has on the contrary to chemical considerably less side effects and is proven more effective in advanced malignancy.[4]

Our data was collected on a small sample, but, we can plan for broader studies with targeted sample collection between 24 and 48, or up to 72 hours after the procedure. Monitoring and comparison of specific biochemical parameters is reasonable on a small number of well-comparable patients as well[29], which we guaranteed with the specified inclusion criteria. The timelines were set based on previous studies[4] and a pilot study that we conducted at our department prior to the study that is presented. A measurable increase of growth factors levels in blood serum and pleural fluid is unlike in animal studies[14] observed a little later. We recorded the largest increase of serum VEGF and pleural TGFβ1 and FGFα and β values between the 24th and 36th hour. After this period, the values were still gradually increasing up to 48 hours post pleurodesis when we stopped recording them. These processes might be lengthier in humans compared to small animals, which should be considered for future research. The collection of samples after 48 hours is difficult as pleurodesis is usually already quite strong and the amount of fluid drained through catheters, that may already be clogged, is minimal.

There was also no relevant correlation between success of pleurodesis and the level of growth factors in our study groups. We believe this is due to the high efficiency of both methods. Therefore, it is difficult to estimate the impact growth factors have in these few unsuccessful cases. We made our conclusions based on our study in the light of several other reports that describe the role of grow factors in the setting of pleural effusion and pleurodesis.

The two observed methods, chemical and mechanical pleurodesis, are the most commonly used for treatment of patients with primary spontaneous pneumothorax. Small amounts of drained pleural effusion after pleurodesis in this group prevent an insight into the release of cytokines and other components that contribute to fibrosis. In addition, we chose patients with malignant pleural effusion as a more appropriate observation group as we do not perform chemical pleurodesis on young patients or for benign diseases with long-term survival, such being the primary spontaneous pneumothorax.

The coverage of the pleura with malignant tissue is another factor that affects the success of pleurodesis, which we could verify only in the group with mechanical pleurodesis. The fact that the lungs re-expanded, normal width of the mediastinum on the chest x-ray and positive cytology allows us to conclude that in the patients group with chemical pleurodesis the pleura was also covered with a comparable proportion of malignant tissue. A small number of unsuccessful pleurodesis in our study is attributed to the selection criteria, since the fibrosis process is more pronounced in healthier pleura. Judging by the data collected in the group with mechanical pleurodesis, an average coverage is only 3.4 from a total of 9 points.

Our protocol included questionnaires on pain load and quality of life. The data are relevant, so we included them in this paper. Patients reported greater sensation of pain and dyspnoea after performing chemical compared to the mechanical pleurodesis. We see pleural inflammation as the main source of pain after pleurodesis. Additionally, we are connecting the effect, which talcum has on the lung parenchyma, with the sensation of dyspnoea. After being absorbed to some extent into tissue, talc causes pneumonitis, pulmonary oedema and signs of acute respiratory distress syndrome. This effect of pleurodesis is less pronounced after mechanical pleurodesis.

There was a significant main effect of time on all assessed quality of life scales (global health status, physical functioning, fatigue, pain and dyspnoea) with "post" scores being significantly better than "pre" scores and "end" scores being better than "post" scores with only one exception, there was no significant difference between "pre" and "post" dyspnoea score. There was also a significant main effect of treatment group on the global health status, fatigue and pain score with better average scores across all points in time in the TMP group. We were especially interested in the interaction effect between time and treatment group, broken down to comparing "post" and "end" scores to "pre" scores across both groups. These comparisons revealed significant interactions when comparing chemical and mechanical pleurodesis global health status scores (both "post" vs. "pre" and "end" vs. "pre"), "end" vs. "pre" fatigue scores and "end" vs. "pre" dyspnoea scores. Looking at the interaction graphs, this suggests that those scores improved more substantially from "pre" to "end" point in the group with mechanical pleurodesis than in the group with chemical pleurodesis as well as from "pre" to "post" point in the case of global health status (Tables 6, 7).

By testing our ideas and views of treatment of malignant pleural effusion, we tried to look into the effect our treatment has on tissue or on the whole body. By giving some answers as to what is important in view of better and faster healing and what should be omitted when testing new methods, we try to help the dedicated laboratories. These parameters can be easily recorded in laboratory animals and can help in minimising the needed numbers. We are also directing the interest of professional public towards the growth factors when trying to explain other differences between surgical treatments.[31] New solutions for many clinical issues can be developed by examining the process of healing and fibrosis.

We believe that the main limiting factor for the clinical use of growth factors is the cost. It is especially limited for terminal patients. Nevertheless, for further experimental studies on pleurodesis and other procedures that boost or stimulate fibrosis growth factors represent an important indicator of efficiency and safety. It is also interesting that TGF β1 works better in an environment with a lower pH.[32] Again this experimental data could not be clarified in our study groups, mainly because the success rate was very high. It would probably be necessary to perform autopsies to reveal the extent of fibrosis and connect this data to the level of pH and the TGF β1 level. In cases of malignant pleural effusion, a low pH value is an indicator of an advanced disease, such patients accumulate effusion faster. In this case, an agent that triggers abundant secretion of TGF β1 should enable faster pleurodesis.

Surgeons use extremely expensive devices and procedures to promote healing and fibrosis. We believe that further exploration of clinical application or promotion of topical secretion of growth factors might be useful in many cases. One option is by supplying the growth factors to the place where we want their activities. Another would be the promotion of targeted growth factors secretion that could accelerate healing. Both should be useful on the resection surfaces and reduce the time of tissue leakage and bleeding. Achieving this might have a significant impact on the main factors for the postoperative complications and duration of hospitalization.

Conclusions

We recorded an increase in serum VEGF levels after chemical pleurodesis with talcum and an increase

in the pleural fluid level of FGFβ and TGFβ1 after thoracoscopic mechanical pleurodesis with respect to compared group. The differences did not reach statistical significance; however, TGFβ1, FGFβ and VEGF remain the most interesting parameters for future research. Considering the mechanisms of growth factors action, we conclude that in our study group mechanical pleurodesis might be more efficient in terms of growth factors release, better-tolerated and safer method than chemical pleurodesis.

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case report

Giant solitary fibrous tumour of the pleura. Case report and review of the literature

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Background. Solitary fibrous tumours of the pleura (SFTP) are rare tumours. They are mostly benign. Only around 12% of them are malignant. In the initial stage they are mostly asymptomatic and by growing they cause chest pain, irritating cough and dyspnoea on account of the pressure created on the surrounding structures. Rare giant tumours have compression symptoms on the mediastinal structures. The condition requires tiered diagnostic radiology. Preoperative biopsy is not successful in most cases. The therapy of choice is radical surgical tumour removal. Malignant or non-radically removed benign solitary fibrous tumours of the pleura additionally require neoadjuvant therapy.

Case report. A 68-year old patient was hospitalized for giant solitary fibrous tumour of the pleura in the right pleural cavity. With its expansive growth the tumour caused the shift of the mediastinum by compressing the lower vena cava, right cardiac auricle as well as the intermediate and lower lobe bronchus. Due to cardiac inflow obstruction and right lung collapse, the patient's life was endangered with signs of cardio-respiratory failure. After preoperative diagnostic radiology, the tumour was surgically removed. Postoperatively, the patient's condition improved. No disease recurrence was diagnosed after a year.

Conclusions. Giant solitary fibrous tumour of the pleura may cause serious and life-threatening conditions by causing compression of the pleural cavity with its expansive growth. Early diagnosis of the condition enables less aggressive as well as video-assisted thoracic surgery in patients with significantly better state of health. Large tumour surgeries in cardio-respiratory affected patients are highly risk-associated procedures.

Key words: solitary fibrous tumour of the pleura; expansive growth; mediastinum shift; surgical treatment

Introduction

Solitary fibrous tumours of the pleura (SFTP) are rare mesenchymal tumours representing less than 5% of all tumours of the pleura.[1] Around 800 cases of such tumour types have been mentioned in global literature up to date.[2,3] In 1870 Wagner was the first to describe a localized primary tumour of the pleura[4], Klemperer and Rabin first classified them in 1931 into a diffuse and localized form and set a hypothesis that localized mesothelium, covering the intact layer of mesothelial cells, stems from structures under the mesothelial layer.[5] With the introduction of electronic microscopic and immunohistochemical examinations it was finally confirmed that SFTP grow from deeper-lying mesenchymal structures of the thoracic wall.

Historically, SFTP are thought to derive from subpleural mesenchymal cells with fibroblasts or myofibroblast differentation.[6] However, negative staining for smooth muscle markers and diffuse positivity for CD34 led van de Rijn *et al.* to propose an origin of this tumour from ubiquitous dispersed dendritic interstitial cels.[7] Recent ultrastructural observations have highlighted that SFTP may originate from peculiar perivascular multipotent mesenchymal elements displaying features akin to pericytes and submesothelial fibroblasts.[8] To differentiate these tumours from other soft tissue tumours, immunohistochemical examinations are required.

TABLE 1. Malignancy criteria for SFTP

A. Abundant cellularity with crowding and overlapping of nuclei
B. High mitotic activity of more than four mitotic figures per 10 high power fields
C. Pleomorphism with cytonuclear atypia
D. Large necrotic or haemorrhagic areas
E. Associated pleural effusion
F. Atypical location and inversion of adjacent structures

TABLE 2. Classification of SFTP into stages

Stage 0 –peduculated SFTP without signs of malignancy
Stage 1- sessile or inverted SFTP without signs of malignancy
Stage 2- pedunculated SFTP with histological signs of malignancy
Stage 3- sessile or inverted SFTP with histological signs of malignancy
Stage 4- multiple synchronous metastatic tumours

Tumours with similar histological characteristics are described by certain authors also in extrathoracic organs, such as liver, peritoneum, meninges, orbits, thyroid gland, salivary gland, breast.[9-12]

SFTP affect male and female patients equally; however, they may develop in all age periods (5 to 87 years) with the highest incidence between 60 and 70 years of age.[13,14] Generally, there is no evidence of correlation with the genetic predisposition for the tumour, and in contrast with mesothelioma[15], there is no relationship to the exposure to asbestos, tobacco or any other environmental agents.[16]

Most SFTP are benign, but may alter in malignancy with age. They usually develop in lower part of pleural cavity; from the visceral pleura in around 80%.[16] Individual tumours grow over 10 cm; however, only individual cases of giant tumours measuring over 20 cm are described.[1] Cytological and histological diagnosis as well as differential diagnosis by defining the benign or malignant biological potential is difficult to perform with the bioptic material acquired by means of fine-needle aspiration biopsy or with large-core needle biopsy. The final diagnosis is usually made after the tumour has been removed. England *et al.* set the basis for differentiating benign from malignant SFTP (Table 1).[13] Based on the histological and morphological characteristics of SFTP, De Perrot *et al.* classified tumours into five stages and thus enabled easier planning of therapy and expected outcome of treatment (Table 2).[2] Most minor SFTP are asymptomatic. They are usually discovered incidentally during chest X-ray examination.[17] By growing and pressuring the surrounding structures they become symptomatic. The most common clinical signs are coughing, dyspnoea, and chest pain, especially in tumours growing from parietal pleura. Rarely are they manifested with the signs of haemoptysis, obstructive pneumonitis or atelectasis.[18] In larger tumours, digital clubbing and hypertrophic pulmonary osteoarthropathy (Pierre Marie Bamberg syndrome)[19] may be present or signs of refractory hypoglycaemia on account of insulin-like growth hormone release (Doege Potter syndrome).[20] Extremely large tumours cause a variety of clinical signs of pressure on the mediastinum or mediastinal shift.

Diagnostic radiology plays a very important role in discovering SFTP. Chest radiographs of patients demonstrate a well-defined, solitary nodule or mass, which may appear at the lung periphery and typically abuts the pleural surface or is located within a fissure.[21] Pedunculated tumours may show mobility within the pleural space.[14] Computer tomography (CT) of the chest shows a homogenous, well-defined and lobulated soft tissue mass.[20] In cases with suspected infiltrative tumour growth into the mediastinal structures, like in others pleural tumours, magnetic resonance imaging (MRI) is required.[22,23] Because such tumours are well-circulated, it seem sensible to perform angiography.[24] Lately, it has been recommended to perform a PET-CT scan, especially when suspecting malignant SFTP or to confirm the presence of potential metastases.[25]

Radical surgical resection is the optimal way of treating patients with SFTP. Aggressive surgery is recommended due to the high probability of their recurrence.[26] The safety margin of healthy tissue after resection should be 1-2 cm wide. Wedge resection of the lung and limited pleurectomy may suffice in peripheral tumours. For sessile tumours it is necessary to perform a lobectomy or pneumonectomy as well as extensive pleurectomy, sometimes even partial resection of the chest wall.[27] Smaller, especially pedunculated tumours, can also be radically removed with minimally invasive thoracoscopic surgical procedure (VATS), which is routinely use in different thoracic pathologies.[28] In cases of larger SFTP, the continuation of surgical treatment with adjuvant chemotherapy is indicated.[27,29] Park *et al*. have found that the combination of temozolomide and bevacizumab had high rates of overall response and long term disease control.[30] In their study, patients received temozolomide 150 mg/m^2 orally on days 1-7 and days 15-21 and bevacizumab 5 mg/kg intravenously on day 8 and day 22 on a 28-day cycle.[30] The role of brachytherapy

and photodynamic therapy, a method in treating diffuse mesotheliomas, has not been sufficiently studied.[31]

Case report

68-year old female patient was admitted to the Department of Lung Disease with signs of severe cardio-respiratory failure. One month prior to being admitted, the patient's breathing was getting heavier, she was tired, weak, had poor appetite and increasing pain in the right hemithorax. She also had arterial hypertension and atrial fibrillation. She has never smoked.

Written informed consent of patient was obtained for the treatments and for the scientific use of clinical data, according to Declaration of Helsinki and Slovenian law requirements.

At examination the patient was cyanotic, tachypnoic with the breathing frequency 22/min. With the administration of oxygen by a nasal catheter a peripheral capillary oxygen saturation (SpO2) was 91%, the patient had signs of heart failure with atrial fibrillation. Breathing was weakened and audible only apically on the right side. Pulmonary function test showed a significant decrease in values of the forced vital capacity (FVC, 42% of the norm), the forced expiratory volume in 1 second (FEV1, 35% of the norm), and the FEV1/FVC ratio or Tiffeneau index (TI, 68% of the norm). Gas analysis of arterial blood showed signs of chronic hypercapnic respiratory failure.

Chest X-ray showed a large tumour mass in the right part of the thorax with mediastinal shift to the left (Figure 1). CT scan of the chest showed an extensive expansive process, larger than 20 cm. The tumour was heterogeneous, lobulated and practically extended over the entire right pleural cavity and shifted mediastinal structures to the left (Figure 2). An MRI examination did not confirm tumour infiltration of the surrounding mediastinal structures (Figure 3).

Bronchoscopy showed a visibly compressed trachea from the right side, shift of the carina and the main right bronchus into the left and closed bronchus for the right lower lung lobe. Transbronchial tumour biopsy was negative; the material collected by means of transthoracic needle biopsy did not suffice for histological tumour confirmation.

For purposes of further diagnosis and surgical treatment the patient was transferred to the Department of Thoracic Surgery. In the preoperative phase we performed an ultrasound (US) of the

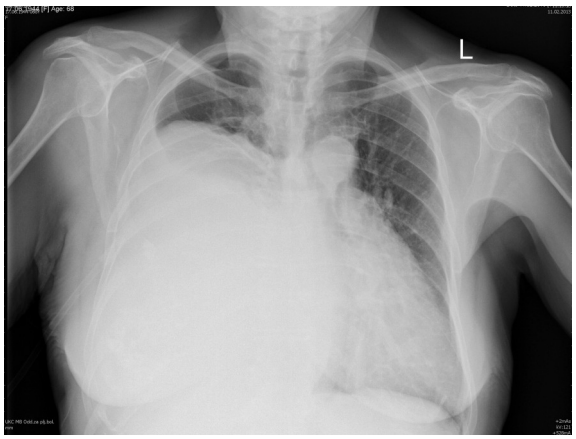


FIGURE 1. Chest X-ray - a large tumorous process of the right hemithorax with mediastinal shift to the left.

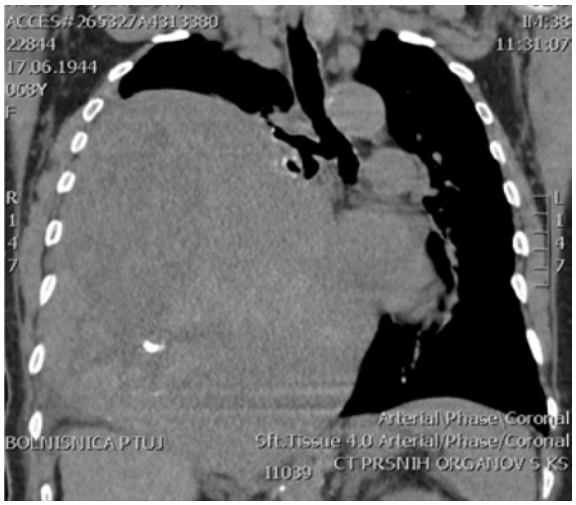


FIGURE 2. Chest CT scan - non-homogeneous, extensive tumour of the right chest side.

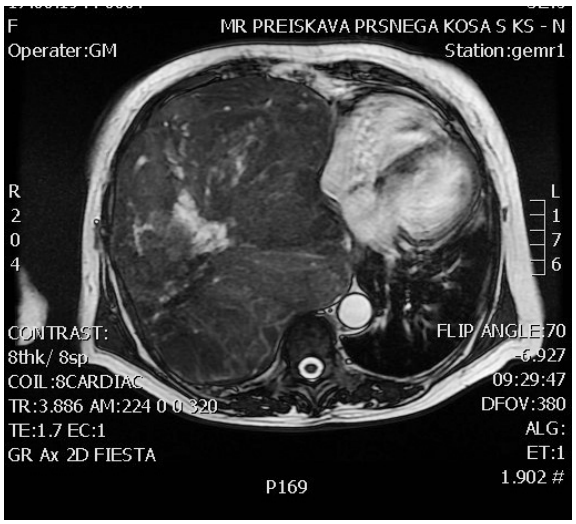


FIGURE 3. Same tumour displayed using MRI - no signs of overgrowth in the structure of the mediastinum.

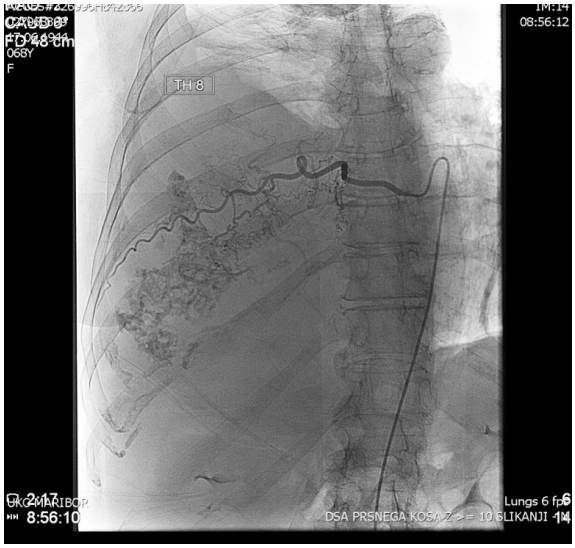


FIGURE 4. The angiography - perfusion of the tumour from the intercostal arteries and right inferior phrenic artery.

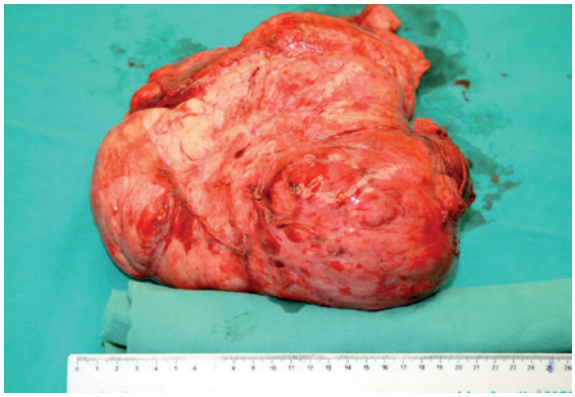


FIGURE 5. Removed solitary fibrous tumour of the pleura; size 22 x 16 x 15 cm.

heart and angiography of the tumour. Heart US showed a compressed right atrium and lower vena cava as well as increased pressure in the right atrium and ventricle. Angiography displayed an extensive blood circulation of the tumour from the intercostal arteries 8, 9, 11, and 12 and inferior phrenic arteries from the right side (Figure 4).

Because of the clinical status of the patient with the signs of respiratory failure, cardiac inflow obstruction and the possibility of massive bleeding, all together representing a high-risk surgery, a detailed plan was designed. A wide approach for safe and radical tumour removal was enabled with the right thoracosternotomy (hemiclamshell). By continuous ligation of blood vessels nourishing the tumour, the blood loss during surgery was only 1.5 l of blood, which was recycled by a cell-saver**.** Surgical preparation in the mediastinal area was difficult because of compressed structures and numerous postinflammatory adhesions**.** A fully removed tumour was sent for pathohistological examination (Figure 5).

Macroscopic examination of the resected specimen showed firm lobulated and bosselated white-grey tumour measuring 25 cm x 16 cm x 13 cm. The surface of the tumour was covered with thin, shiny, smooth capsule. The cut surface was rubbery, vaguely nodular, grey-white, focally glassy and haemorrhagic. On the edges tiny calcifications were present. Macroscopically no necrotic areas were identified.

Microscopically the bland appearing tumour cells were arranged in a "patternless pattern" (storiform and fascicular pattern), the hypercellular regions were mixed with hypocellular areas with hyalinised stroma. In some areas stromal myxoid change and degeneration of collagen was present, too. Stroma was highly vascularized with angiofibromatous and haemangiopericytic vascular pattern. The tumour cells were spindle and oval with scant cytoplasm and nuclei with dense chromatin. Focally spindle cells showed wavy nuclei, resembling schwanian cells and also there were some areas with pleomorphic and giant cells population. The nuclei of the pleomorphic cells were larger, hyperchromatic, and different in shape. Very rare mitotic figures (<2/10 high-power fields [HPF]) were present and there was no necrosis (Figure 6).

Immunochistochemically tumour cells were reactive for CD34, CD99 and bcl2 and typically no immunoreactivity was observed with S-100, WT-1, Desmin, CEA, CK AE1/AE3, CK5/6 and calretinin (Figure 7).

According to the morphology and cellular immunophenotype the diagnosis of benign giant pleural SFT was signed out.

The patient was placed for seven days into a room with perioperative intensive care and extubated after two days. Longer intubation was required to ventilate a long time collapsed right intermediate and lower lung lobe. She left the hospital on day 22 after surgery. At the follow-up after a year, no recurrence of the disease was present.

Discussion

SFTP are a rare pathology of the pleural cavity, which most of the time develop from submesothe-

lial fibroblasts of the visceral pleura and usually in the lower parts of the chest. Prior to the introduction of immunohistochemical examinations and electronic microscopy, they were classified into a large group of mesotheliomas as a localized form of this dangerous, asbestos-related pathology.[16] Despite the relatively benign disease course, questions remain open in the field of diagnostics, preoperative histological verification and final treatment.

Due to their non-characteristic clinical picture, SFTP are usually diagnosed in the later stages of the development, when causing pressure on the surrounding structures on account of their size. Smaller, accidentally discovered SFTP are relatively easy to remove surgically. A much more significant issue are radical surgical procedures of giant SFTP in patients affected by the pressure of the tumour on the mediastinal structures and lungs. Only a few cases of giant SFTP that cover almost the entire pleural space are described in literature. SFTP that we removed belongs to the largest so far described cases in global literature. Certain authors report of having performed the surgery *via* one or two thoracotomies at two different levels[1], others via sternotomy.[3] Our approach *via* right thoracosternotomy (hemiclamshell) provides an optimal view of the pleural cavity and mediastinal structures.

Preoperative diagnostics needs to be systematic to enable the surgeon a precise estimation of the scope of the surgical procedure, tumour operability, blood flow of the tumour and the relation to neighbouring structures.

A CT scan provides valuable data on the exact location of SFTP, its relation to surrounding structures, tumour homogeneity or potential bleeding areas or necrosis, chest wall destruction and the presence of pleural effusion.[21,32,33] However, a CT scan cannot differentiate between benign and malignant SFTP cases. Large tumours are more likely to be malignant, with distinct heterogeneous structure, not clearly separated from the surrounding environment and in potential presence of pleural effusion.[21] A part of the diagnostic preoperative examinations performed in our patient was an MRI examination to exclude tumour infiltration into mediastinal structures and angiography to display the feeding arteries. Similar guidelines are also supported by other authors.[22,24] Pathological arteries usually arise from chest arteries and we found an interesting description of the arterial circulation in SFTP from the celiac and hepatic arteries.[34]

Preoperative histological confirmation of such tumour changes in the chest remains a big problem. The diagnosis of SFTP is rarely reached before surgical excision and pathological examination of the mass.[16] Sometimes preoperative diagnosis can be made with large-core needle biopsies. The risk of pneumothorax could be minimal with avoiding aerated lung on the introduction of the needle.[35] Although fine-needle aspiration biopsy may yield characteristic and diagnostic morphological features, it was difficult to reach a histological di-

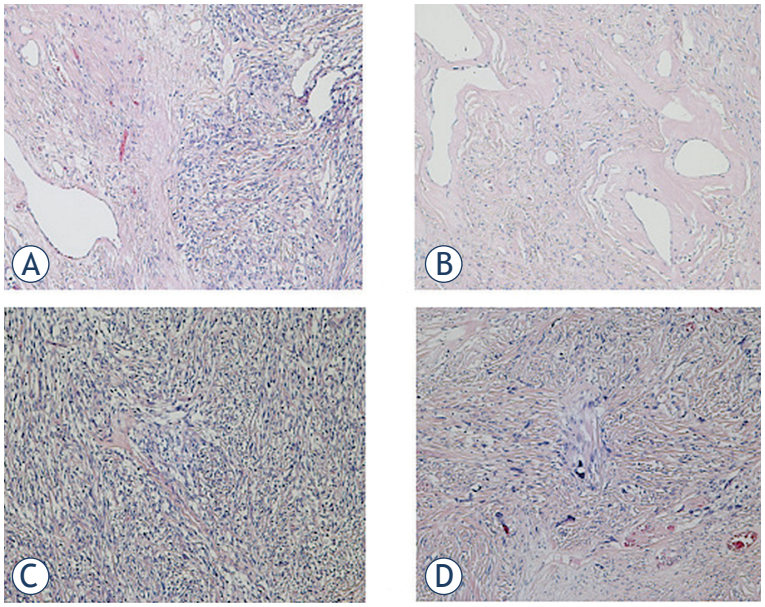


FIGURE 6. Microscopic features of the tumour. (**A**) Haemagioperycytic pattern in hypercellular and hypocellular sclerotic setting; (**B**) Hyalinised vascular structures; (**C**) Patternless pattern of growth; (**D**) Pleomorphic cells. (A,B,C,D: HE, 100x).

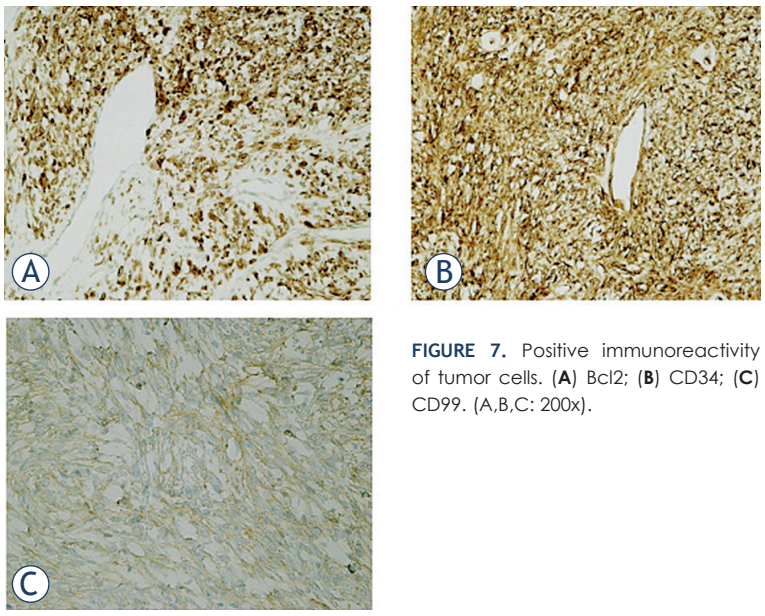
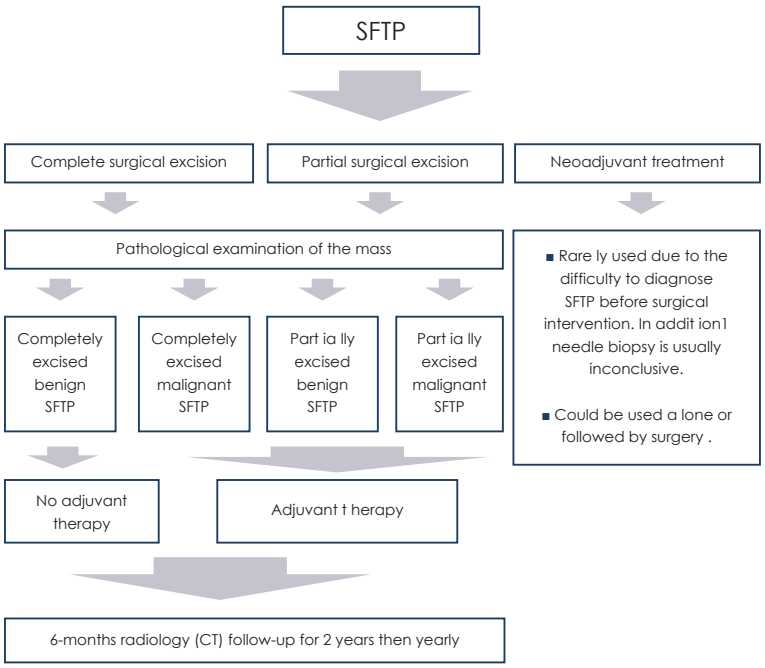


FIGURE 7. Positive immunoreactivity of tumor cells. (**A**) Bcl2; (**B**) CD34; (**C**) CD99. (A,B,C: 200x).

TABLE 3. The guidelines provided by De Perrot *et al*.



SFTP = solitary fibrous tumours of the pleura

agnosis in most studies.[19] Cutting needle biopsy is probably preferable because of wider tissue sampling.[35] Thoracoscopic procedure, an effevtive diagnostic and therapeutic method, have also be to considered.[36] In our case it was not possible to collect suitable material for histological analysis with transthoracic and transbronchial biopsy.

Because in most cases the biological potential of SFTp is not preoperatively histologically confirmed, neoadjuvant therapy is not appropriate. After removing the tumour surgically we opt for adjuvant therapy in malignant or non-radically removed benign SFTP in accordance with the guidelines provided by De Perrot *et al*. (Table 3).[16] Because the benign SFTP was removed radically, our patient did not receive adjuvant therapy.

It is possible that the tumour will recur, which mainly depends on the histological characteristics of SFTP and radical nature of the surgical procedure. The possibility of benign pedunculated tumours recurring is around 2%, for benign sessile tumours around 8%, malignant pedunculated tumour around 14%, and for malignant sessile tumours around 63%.[31,37] It is necessary to follow the patients, usually every 6 months when control CT scan of the chest is performed. No recurrence was established in our patient after a year.

Conclusions

SFTP are rare pleural neoplasms, stemming from submesothelial fibroblast cells and are in more than 80% benign. Initially, they are asymptomatic and by growing they create pressure on the surrounding structures of the chest and cause chest pain, coughing, dyspnoea, and by pressuring on the mediastinum they can cause life-threatening signs of mediastinal shift. Tiered diagnostic radiology is very important and provides valuable data in the most appropriate manner of treatment. Preoperative biopsy is usually not successful and the final diagnosis is obtained in most cases only after the surgical removal of the tumour. Radical surgical resection is the method of choice when treating benign and operable SFTP and need to be upgraded in malignant or non-radically removed benign tumours with adjuvant therapy. Extensive surgical resections can be avoided with timely diagnosis of smaller tumours, which can be radically removed with VATS. Regular check-up are required due to possible disease recurrence.

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research article

Impact of comorbidity on the outcome in men with advanced prostate cancer treated with docetaxel

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Background. Men with metastatic castrate-resistant prostate cancer (mCRPC) may not receive docetaxel in everyday clinical practice due to comorbidities. Here we explore the impact of comorbidity on outcome in men with mCRPC treated with docetaxel in a population-based outcome study.

Methods. Men with mCRPC treated with docetaxel at the Institute of Oncology Ljubljana between 2005 and 2012 were eligible. Comorbidity was assessed by the age-adjusted Charlson comorbidity index (aa-CCI) and adult comorbidity evaluation (ACE-27) index. Hospital admissions due to the toxicity and deaths during treatment with docetaxel were used as a measure of tolerability. Association between comorbidity and overall survival (OS) was tested using the Cox proportional hazards analysis.

Results. Two hundred and eight men were treated with docetaxel. No, mild, moderate and severe comorbidity was present in 2%, 32%, 53% and 13% using aa-CCI and in 27%, 35%, 29% and 8% when assessed by ACE-27. A substantial dose reduction of docetaxel occurred more often in men with moderate or severe comorbidity as compared to those with no or mild comorbidity. At all comorbidity levels about one-third of men required hospitalization or died during treatment with docetaxel. In univariate analysis a higher level of comorbidity was not associated with worse OS (aa-CCI HR 0.99; [95% CI 0.87–1.13], p = 0.93; ACE-27: HR 0.96; [95% CI 0.79–1.17], p = 0.69).

Conclusions. Men with mCRPC, who have comorbidities may benefit from treatment with docetaxel.

Key words: metastatic castration-resistant prostate cancer; comorbidity; chemotherapy

Introduction

Men with advanced prostate cancer are usually treated with hormonal therapy, which increases the risk for the development of comorbid conditions, such as diabetes, osteoporosis and cardiovascular disease.[1-3] Most men with advanced prostate cancer receive hormonal therapy for several years and the average patient with metastatic castrate-resistant prostate cancer (mCRPC) in every-day clinical practice is 70 years old.[4] Observational studies show that at this age more than 75% of cancer patients have at least one comorbid condition, with more than 30% having moderate or severe comorbidity.[5]

Based on improved overall survival (OS) in the TAX 327 and SWOG 9916 trials, docetaxel once every 3-weeks (hereafter Dq3w) in combination with prednisone is the standard treatment for men with mCRPC.[6,7] A post-hoc analysis of the TAX327 showed that tolerability and efficacy of Dq3w appear less favourable with advanced age.[8] Men with mCRPC who are treated with docetaxel in everyday practice may be less selected, older and have

more comorbidities as compared to those treated with docetaxel in the pivotal randomized clinical trials.[4,9] Outside of clinical trials, about 20-40% of men with mCRPC never receive treatment with docetaxel.[10] Presence of comorbid conditions and/or poor performance status may be the reasons that these patients are not treated with docetaxel.[11-15] Although comorbidities are known negative prognostic factors for OS in men with early prostate cancer their prognostic role in men with mCRPC is less clear.[11-13] We hypothesized that comorbidity has detrimental effect on the outcome of men with mCRPC who are treated with docetaxel. Due to the dose reductions, docetaxel may be less effective in comorbid men. Furthermore, treatment with docetaxel may increase the risk for toxic deaths in men with mCRPC. Here we explored the impact of comorbidity on the efficacy and tolerability in men with mCRPC, who were treated with docetaxel in a population-based observational study.

Patients and methods

Study population and data collection

In this population-based observational study we included men with mCRPC who were treated with docetaxel and subsequent systemic therapies at the Institute of Oncology Ljubljana between January 1, 2005 and June 27, 2012. Men with mCRPC, who receive docetaxel in routine clinical practice, have usually performance status (PS) 0–2 and therefore PS was not evaluated retrospectively in this study. Optimal dose intensity of docetaxel was 25 mg/m^2/week, which served as a denominator in the calculation of the relative dose intensity (RDI).

An optimal comorbidity index for prostate cancer patients is not established.[16] We used the age-adjusted Charlson comorbidity index (aa-CCI) which is a composite index of 19 conditions weighted from 1 to 6 points and adjusted for age with each decade above 50 years of age counting for an extra point with a total score of 0-35 points. Point scores can then be classified into prognostic categories.[17] Categories were formed using the same cut-off values as in the original publication and, for easier differentiation between them, tagged as "none" (0 points), "mild" (1-2 points), "moderate" (3-4) and "severe" (> 4 points) comorbidity. The second comorbidity index we used was the adult comorbidity evaluation-27 (ACE-27).[18] The ACE-27 grades specific diseases and conditions into levels of comorbidity from grade 1 to grade 3. An overall level of comorbidity ("none", "mild", "moderate" or "severe") is assigned based on the highest level of comorbidity. Only malignancies other than mCRPC were included in the final score of both comorbidity indices as its inclusion would have assigned a severe level of comorbidity to all patients. We retrieved relevant clinical information from electronic and hard copies of patients' charts and assessed comorbidity by using the aa-CCI and ACE-27 coding protocols.

In this retrospective study the number of hospital admissions due to the toxicity of chemotherapy or deaths, which occurred during or 30 days after treatment discontinuation represented an estimate of tolerability of docetaxel. Dates of death were obtained from the national cancer registry.

The protocol of our study was reviewed and approved for clinical use by the Ethics and Study Protocol Assessment Committee at the Institute of Oncology Ljubljana. Informed consent was obtained from all patients prior to the treatment; however, for including in this retrospective study it was waived.

Statistical analysis

Descriptive statistics were used to describe relevant characteristics of men at baseline. OS was calculated from the date of the first administration of docetaxel to death from any cause. Data were censored for patients who were alive at the cut-off date of March 13, 2013. OS was estimated using the Kaplan-Meier method. A Cox proportional hazard model was used to examine association between comorbidity and OS. Initial assessment was carried out in the univariable setting and subsequently for all significant ($p < 0.1$) variables in the multivariable setting. Comorbidity was analysed as both categorical and dichotomous variable (score 0 vs. ≥ 1). Discriminatory accuracy of the aa-CCI and ACE-27 in predicting death at 12 months or at any time during follow-up was tested by estimating the area under the receiver operating characteristic (ROC) curve (C-statistic). Association between comorbidity, RDI and tolerability was assessed by the Chi-square test. All tests were two-sided and a p-value of ≤ 0.05 was considered statistically significant. No adjustment for multiple analyses was performed.

Results

Study population

Our analysis included 208 men with mCRPC with median age of 69.9 years. Patients were treated

TABLE 1. Patients' baseline characteristics

Age (years)	
Median	69.9
Range	45.7 – 84.8
PSA (ng/mL)	
Median	217
IQR	78.2 – 595.1
Hb (g/L)	
Median	124
IQR	108 – 134
ALP (µkat/L)	
Median	2.9
IQR	1.57 – 7.20
Docetaxel, n (%)	
Dq3w schedule	199 (96)
Weekly schedule	9 (4)
Number of cycles, n	
Median	8
Range	1-21
IQR	5-10
Rechallenge, n (%)	12 (6)
RDI of docetaxel*, n (%)	
> = 95%	103 (52)
85–94%	56 (29)
75–84%	25 (13)
< 75%	12 (6)
RDI of docetaxel, %	
Median	95
IQR	87–99
G-CSF support, n (%)	
Pegfilgrastim	30 (15)
Filgrastim	15 (7)
Primary prophylaxis Secondary prophylaxis	20 (10) 25 (12)
Visceral metastasis, n (%)	34 (16)
Opioid analgesic, n (%)	91 (44)
New agents, n (%)	
Abiraterone acetate	41 (20)
Cabazitaxel	17 (8)
Abiraterone acetate and/or cabazitaxel	46 (22)†

* Data available for 196 patients
† Among these, 53% and 29% had moderate or severe comorbidity when assessed by the aa-CCI and ACE-27
ALP = alkaline phosphatase; CI = confidence interval; G-CSF = granulocyte colony stimulating factor; Hb = haemoglobin; IQR = interquartile range; RDI = relative dose intensity; PSA = prostate specific antigen

with docetaxel between January 2005 and June 2012. Their baseline characteristics are presented in Table 1. At baseline, median PSA, haemoglobin (Hb) and alkaline phosphatase (ALP) were 217 ng/ml (78.2–595.1ng/ml), 124g/l (108–134g/L) and 2.9 µkat/L (1.57–7.2 µkat/L), respectively. Visceral metastases were present in 34 patients (16%) and 91 patients (44%) received opioid analgesia. Dq3w was administered to 199 patients (96%) with the remaining 9 patients (4%) having received weekly docetaxel. Median number of cycles of docetaxel was 8 (1–21). Twelve patients (6%) were re-challenged with docetaxel. Data for calculation of the RDI were available for 196 patients (94%), among these 159 (81%) received a RDI of ≥ 85%. After treatment with docetaxel 46 men (22%) received new agents (i.e. abiraterone acetate and/or cabazitaxel); 29 patients (14%) received abiraterone acetate alone, 5 patients (2%) cabazitaxel alone and 12 patients (6%) both agents.

Association of comorbidity and tolerability

One hundred ninety-four men (93%) with mCRPC for whom information about both the dose intensity of therapy with docetaxel and comorbidity were available were included into the analysis of tolerability. Median RDI of docetaxel was > 90% in all subgroups of men with various level of comorbidity. However, when assessed by the aa-CCI, 34 men (27%) with moderate or severe comorbidity received RDI of less than 85% as compared to only 2 men (3%) with mild or no comorbidities ($p < 0.001$). When assessed by ACE-27, 17 men (24%) with moderate or severe comorbidity received RDI of less than 85% as compared to 19 men (15%) with mild or no comorbidity ($p = 0.14$) (Table 2).

Overall, 77 men (37%) were hospitalized due to the toxicity of docetaxel or died during treatment with docetaxel. Among those for whom information on dose intensity was available 66 men (34%) were hospitalized or died during treatment with docetaxel. In these men, 71 hospitalisations and 8 deaths occurred. When assessed by aa-CCI, 41 men (33%) with moderate or severe comorbidity as compared to 25 men (36%) with no or mild comorbidity were hospitalized or died during treatment ($p = 0.63$). When assessed by ACE-27, 24 men (34%) with moderate or severe comorbidity required hospitalisations or died during treatment as compared to 44 men (34%) with no or mild comorbidity ($p = 0.84$) (Table 3).

TABLE 2. Association of relative dose intensity of docetaxel and comorbidity

		aa-CCI (n = 194)				ACE-27 (n = 194)			
		None (n = 3)	Mild (n = 66)	Moderate (n = 101)	Severe (n = 24)	None (n = 54)	Mild (n = 69)	Moderate (n = 58)	Severe (n = 13)
RDI	<75% n (%)	0	0	10 (10%)	2 (8%)	3 (6%)	3 (4%)	5 (9%)	1 (8%)
	75-84% n (%)	0	2 (3%)	19 (18%)	3 (12%)	3 (6%)	10 (14%)	11 (19%)	0
	85-94% n (%)	1 (33%)	19 (29%)	28 (28%)	8 (33%)	13 (24%)	18 (26%)	19 (33%)	6 (46%)
	≥95 n (%)	2 (66%)	45 (68%)	44 (44%)	11 (46%)	35 (65%)	38 (55%)	23 (40%)	6 (46%)
Median RDI - %		96%	98%	93%	93%	98%	96%	91%	94%

aa-CCI = age-adjusted Charlson comorbidity index; ACE-27 = adult comorbidity evaluation 27; CI = confidence interval; RDI = relative dose intensity

TABLE 3. Association of comorbidity and tolerability during treatment with docetaxel

	aa-CCI (n = 194)				ACE-27 (n = 194)			
	None (n = 3)	Mild (n = 66)	Moderate (n = 101)	Severe (n = 24)	None (n = 54)	Mild (n = 69)	Moderate (n = 58)	Severe (n = 13)
Number of patients hospitalized or deceased during treatment, n	0	25 (38%)	34 (34%)	7 (29%)	20 (37%)	22 (32%)	18 (31%)	6 (46%)

aa-CCI = age-adjusted Charlson comorbidity index; ACE-27 = adult comorbidity evaluation; CI = confidence interval

TABLE 4. Comorbidity evaluation

Comorbidity index	Level of comorbidity			
	None	Mild	Moderate	Severe
aa-CCI, *N* (%)	3 (2%)	67 (32%)	108 (53%)	27 (13%)
ACE-27, *N* (%)	55 (27%)	73 (35%)	61 (29%)	16 (8%)

aa-CCI = age-adjusted Charlson comorbidity index; ACE-27 = adult comorbidity evaluation 27

Association of comorbidity and efficacy

After a median follow-up time of 14 months 133 men died. Median OS for the whole group was 19 months. For 98% of patients (N = 205) information on comorbidity was available. None, mild, moderate and severe comorbidity was present in 2%, 32%, 53% and 13% using aa-CCI and in 27%, 35%, 29% and 8% when assessed by ACE-27 (Table 4).

In univariable analysis, a higher level of comorbidity was not associated with worse OS (ACE-27: HR 0.96; [95% confidence interval (CI) 0.79–1.17], p = 0.69; aa-CCI HR 0.99; [CI 0.87–1.13], p = 0.93) when studied as a categorical variable (Table 5). Similarly, when analysed as dichotomous variable a higher level of comorbidity was not associated with worse OS (ACE-27: HR 0.75 [CI 0.51–1.08], p = 0.12; aa-CCI: HR 1.48 [CI 0.37–6.0], p = 0.58). Both indices were poor at discriminatory accuracy in predicting death at any time (C-statistics 0.45; p = 0.25 for aa-CCI and C-statistics 0.47; p = 0.44

TABLE 5. Association of comorbidity and overall survival

	Univariable model (HR 95% CI); p-value	Multivariable model (HR 95% CI); p-value
Age (for every year)	0.99 (0.97–1.01) p = 0.47	-
Log PSA (≥ vs. than median)	1.20 (1.07–1.35) p < 0.01	1.05 (0.94–1.18) p = 0.38
Hb (per 10 units)	0.79 (0.72–0.86) p < 0.01	0.78 (0.72–0.86) p < 0.01
ALP (≥ vs. < than median)	0.97 (0.87–1.10) p = 0.65	-
Docetaxel (Dqw vs. Dq3w)	6.39 (3.19–12.81) p < 0.01	6.04 (2.77–13.20) p < 0.01
Visceral metastases (yes vs. no)	1.68 (1.08–2.61) p = 0.02	1.73 (1.10–2.73) p = 0.02
Opioids (yes vs. no)	1.38 (0.65–2.92) P = 0.41	-
New agents (yes vs. no)	0.39 (0.19–0.80) p = 0.02	0.36 (0.17–0.74) p < 0.01
aa-CCI (per category)	0.99 (0.87–1.13) p = 0.93	-
ACE-27 (per category)	0.96 (0.79–1.17) p = 0.69	-

aa-CCI = age-adjusted Charlson comorbidity index; ACE-27 = adult comorbidity evaluation 27; Dqw = weekly docetaxel; Dq3w = 3-weekly docetaxel; HR = hazard ratio; CI = confidence interval

for ACE-27) or death within 12 months of start of treatment with docetaxel (C-statistics 0.47; p = 0.49 for aa-CCI and C-statistics 0.47; p = 0.49 for ACE-27).

In multivariable analysis significant independent predictors of poor OS were presence of visceral metastases (HR 1.73; p = 0.02) and weekly docetaxel schedule (HR 6.04; p < 0.01), whereas high Hb level (HR 0.78; p < 0.01) and use of abiraterone and/or cabazitaxel (HR 0.36; p < 0.01) were independent favourable prognostic factors for OS (Table 5).

Discussion

One of the reasons that a substantial proportion of men with mCRPC are never treated with docetaxel is fear of poor tolerability due to comorbid conditions.[10] Life expectancy of men with mCRPC cancer is limited and therefore the impact of comorbidity on the outcome may be less relevant in this setting as compared to men with early prostate cancer. As patients enrolled into clinical trials usually do not have uncontrolled or severe comorbidities, population-based outcome studies may provide a more generalizable evaluation of the impact of comorbidity on the outcome and tolerability of medical intervention compared to post-hoc analyses of randomized trials.

In our cohort of men with mCRPC treated with docetaxel, a substantial proportion had moderate or severe comorbidity. In this cohort, a substantial dose reduction of docetaxel occurred more often in men with moderate or severe comorbidity as compared to those with no or mild comorbidity. At all comorbidity levels about one-third of men required hospitalization or died during treatment with docetaxel. A higher level of comorbidity was not associated with worse OS, irrespective of whether comorbidity was assessed by the aa-CCI or ACE-27 index. Both indices performed similarly poorly at discriminatory accuracy in predicting death. In concordance with our findings, investigators of the population-based outcome study in France, which enrolled elderly (≥ 75 years) men with mCRPC, did not find any association between comorbidity and outcome.[19]

To date several post-hoc analyses of randomized clinical trials, which evaluated the impact of comorbidity on the outcome in patients with advanced prostate cancer or other cancers, were reported. In a randomized phase II trial in which men with mCRPC were treated with docetaxel and prednisone with or without a bcl-2 antagonist AT-101, comorbidity assessed by the CCI did not predict OS, both as a categorical or continuous variable.[15] In this study patients with acute or uncontrollable comorbidity were excluded and 53% had no other comorbidities. In contrast, in the post-hoc analysis of a large phase III randomized trial, which enrolled men with mCRPC receiving docetaxel and prednisone with or without bevacizumab, investigators found an association between the number of comorbidities at baseline and the risk of death.[14] However, in that study comorbidity was not assessed by any comorbidity index. The lack of association between comorbidity assessed by the CCI and OS is also seen in other malignancies.[20,21] Investigators in one of these studies cautioned that physicians must carefully discriminate between PS and comorbidity when assessing patients for therapy.[21] According to the updated guidelines for the management of men with advanced prostate cancer published by the International Society of Geriatric Oncology (SIOG) comorbidity should be put into the context of patient's general well-being and functional reserve.[22]

A higher level of comorbidity is a known risk factor for hospital admission in cancer patients.[23,24] Irrespective of the level of comorbidity about one third of men required hospitalization or died during or shortly after treatment with docetaxel in our study. However, a higher proportion of men with moderate or severe comorbidity had a substantial dose reduction of docetaxel as compared to those with no or mild comorbidity (24-27% vs. 3-15%). Recent data show that patients with mCRPC treated with docetaxel in routine practice experience more toxicity as compared to those treated with docetaxel within clinical trials.[5] Similarly, high admission rates during treatment with chemotherapy, as found in our study, were observed in older patients with other metastatic solid cancers.[25,26] Accumulating evidence shows that anticancer therapies are less tolerable in patients treated in everyday clinical practise as compared to those treated within clinical trials.

Recently, new treatment options with favourable benefit-risk profile such as abiraterone acetate, enzalutamide and radium-223 became available for chemotherapy-naïve men with mCRPC.[27-29] These agents substantially changed the management of mCRPC as treatment with docetaxel may now be deferred or even omitted in some patients with very advanced prostate cancer. However, we believe that docetaxel remains an important treatment option for mCRPC and therefore our findings may still be clinically relevant.

Our study has several limitations. First, our retrospective analysis is based on a relatively small cohort of men with mCRPC, who were all treated at a single institution. However, all patients with mCRPC in Slovenia who are candidates for treatment with docetaxel are referred to the Institute of Oncology Ljubljana. Therefore, our study is less prone to selection and referral biases which often plague traditional institutional retrospective studies. Second, when assessing comorbidity using aa-CCI and ACE-27 coding protocols, we retrieved relevant clinical information retrospectively from electronic and hard copies of patient's charts, which may have limitations. An ideal set-up to study the association between comorbidity and outcome in men with mCRPC would be a prospective study design. Third, although serum PSA and ALP levels and pain are well established prognostic factors in men with mCRPC, they did not independently predict the outcome in our study. Similar larger study might lead into different conclusions and more generalizable results. Fourth, use of alternative comorbidity indexes might lead to different results and conclusions. We used aa-CCI and ACE-27, as these are convenient, widely used and validated tools. Fifth, the rate of hospitalization during treatment might be underestimated as admissions to community hospitals might not always be recorded in patients' charts. Finally, use of abiraterone acetate and/or cabazitaxel after treatment with docetaxel in 22% of patients might impact the results. However, these new agents were not used more often in men with moderate or severe comorbidities as compared to men with no or minor comorbidities (Table 1).

In conclusion, the presence of comorbidity may not be associated with worse outcome in men with mCRPC, who are treated with docetaxel. Irrespective of the level of comorbidity a substantial proportion of men (about one third) required hospitalization or died during therapy with docetaxel. Dose reduction occurred more often in men with moderate or severe comorbidity. It is likely that, men with mCRPC who have substantial comorbidities may still benefit from reduced dose of docetaxel. Comorbid conditions should always be interpreted in the context of other relevant clinical characteristics when deciding about therapy with docetaxel in men with mCRPC.

Author contributions

Concept and design - A. Zist, B. Seruga; data collection - A. Zist; analysis and interpretation of data - A. Zist, E. Amir, A. Ocana, B. Seruga; manuscript writing and approval - A. Zist, E. Amir, A. Ocana, B. Seruga

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research article

Clinical impact of post-progression survival on overall survival in patients with limited-stage disease small cell lung cancer after first-line chemoradiotherapy

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Background. The effects of first-line chemoradiotherapy on overall survival (OS) may be confounded by subsequent lines of therapy in patients with limited-stage disease small cell lung cancer (LD-SCLC). Therefore, we aimed to determine the relationships between progression-free survival (PFS), post-progression survival (PPS) and OS after first-line chemoradiotherapy in LD-SCLC patients.

Patients and methods. We retrospectively analyzed 71 LD-SCLC patients with performance status (PS) 0-2 who received first-line chemoradiotherapy and had disease recurrence between September 2002 and March 2013 at Shizuoka Cancer Center (Shizuoka, Japan). We determined the correlation between PFS and OS and between PPS and OS at the individual level. In addition, we performed univariate and multivariate analyses to identify significant prognostic factors of PPS.

Results. OS is more strongly correlated with PPS (Spearman's $r = 0.86$, $R^2 = 0.72$, $p < 0.05$) than PFS (Spearman's $r = 0.46$, $R^2 = 0.38$, $p < 0.05$). In addition, the response to second-line treatments, the presence of distant metastases at recurrence and the number of additional regimens after first-line chemoradiotherapy were significant independent prognostic factors for PPS.

Conclusions. PPS has more impact on OS than PFS in recurrent LD-SCLC patients with good PS at beginning of the treatment. Moreover, treatments administered after first-line chemoradiotherapy may affect their OS. However, larger multicenter studies are needed to validate these findings.

Key words: chemoradiotherapy; limited-stage disease small cell lung cancer; overall survival; post-progression survival; progression-free survival

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide.[1] In the United States, 14% of people who were diagnosed with lung cancer had small cell lung cancer (SCLC).[2] Approximately 30% of SCLC patients have limited-stage disease small cell lung cancer (LD-SCLC), which is characterized by locoregional tumors in the hemithorax, mediastinum, or supraclavicular lymph nodes, while the rest have extensive-stage disease.[3] Current therapeutic options for LD-SCLC include combi-

nation chemotherapy with etoposide and cisplatin, chest radiotherapy, and prophylactic cranial irradiation (PCI).[4,5] However, due to the limited efficacy of these therapeutic strategies and the aggressive nature of SCLC tumors, the prognosis for SCLC patients is poor; the median survival time for LD-SCLC patients is less than two years.[6-8]

PFS and OS are two common endpoints in cancer trials. OS is usually preferred, because it is reliable, precise, meaningful and easily documented by the date of death.[9] However, the effect of first-line treatments on OS might be confounded by subsequent lines of therapy. In contrast, PFS is quicker to measure, can be measured more conveniently, and therefore, may be easier to assess than OS.[10] If there is a strong correlation between PFS and OS, then PFS may be a surrogate endpoint for OS. In non-small cell lung cancer (NSCLC), increases in PFS do not necessarily increase OS, but post-progression survival (PPS) is strongly associated with OS after first-line treatment.[11-13] We have also demonstrated a strong correlation between PPS and OS after first-line chemotherapy in patients with extensive-stage disease SCLC.[14] In LD-SCLC, though, the relationship between PPS and OS is unknown.

Therefore, we analyzed the correlation between PFS and OS and between PPS and OS after first-line chemoradiotherapy in LD-SCLC patients to determine whether PFS or PPS has more influence on OS. We also investigated the prognostic value of baseline and tumor characteristics for PPS.

Patients and methods

Patients

We retrospectively enrolled 71 consecutive patients with recurrent LD-SCLC after receiving first-line chemoradiotherapy at Shizuoka Cancer Center (Shizuoka, Japan) between September 2002 and March 2013. The inclusion criteria were as follows: (1) histologically or cytologically confirmed SCLC; (2) 20 years of age or older at the time of chemoradiotherapy; (3) Eastern Cooperative Oncology Group performance status (PS) of 0–2 at the beginning of the first-line treatment; (4) first-line treatment with ≥ 40 Gy curative thoracic radiotherapy and platinum doublet chemotherapy, either concurrently or sequentially; and (5) disease recurrence after first-line treatment. The study protocol was approved by the Institutional Review Board of Shizuoka Cancer Center and was conducted in accordance with the Helsinki Declaration of 1964 (revised 2008). Due to the retrospective nature of this study, the need for informed consent was waived.

Treatment

LD-SCLC patients were treated with a combination of chemotherapy and radiotherapy. Several different first-line chemotherapeutic regimens were used; etoposide (80 or 100 mg/m^2) was administered on days 1–3 in combination with cisplatin (80 mg/m^2) on day 1, cisplatin (25 mg/m^2) on days 1–3, or carboplatin (area under the curve = 5) on day 1. These drugs were injected intravenously every 3–4 weeks for maximum 4 courses. Second and third-line treatments included amrubicin, irinotecan, topotecan, gemcitabine, and paclitaxel.

The fractionation schedule for thoracic radiotherapy in LD-SCLC patients was determined by using information from chest computed tomography (CT) to calculate the pretreatment tumor volume. Typically, the total planned dose was 50 Gy when it was fractionated once daily or 45 Gy when it was fractionated twice daily, based on individual physician decision. Furthermore, the maximum spinal cord dose was limited to 45 Gy when the radiation dose was fractionated once daily or to 36 Gy when it was fractionated twice daily. In addition, no more than 35% of the normal lung volume received more than 20 Gy.

Thoracic radiotherapy was started either during the first cycle of chemotherapy or after four cycles of chemotherapy. It was suspended if a patient experienced grade 4 thrombocytopenia, neutropenia, radiation pneumonitis, fever caused by infection, a decrease of more than 10 mmHg in arterial oxygen pressure, or difficulty swallowing liquids. After thoracic radiotherapy, PCI (25 Gy in 10 fractions) was administered to patients with a complete or near-complete response, as shown by a scar-like shadow on a chest CT, if the treating physician recommended it.

Assessment of treatment efficacy

Tumor responses reflect the best overall response and maximum shrinkage. Radiographic tumor responses were evaluated using chest computed tomography at every two courses of chemotherapy according to the Response Evaluation Criteria In Solid Tumors 1.0 as follows: complete response (CR), disappearance of all target lesions; partial response (PR), ≥ 30% decrease in the total diameter of all target lesions relative to the total baseline diameter; progressive disease (PD), ≥ 20% increase in

the total diameter of all target lesions relative to the smallest total diameter observed during the study; and stable disease (SD), insufficient change in the total diameter of all target lesions to qualify as PR or PD.[15]

PFS was defined as the time from the beginning of first-line treatment until documented PD or death. In addition, OS was reported as the time from the beginning of first-line treatment until death or censored at the time of the last assessment of disease status. Similarly, PPS was documented as the time from tumor progression after first-line treatment until death or censored at the time of the last assessment of disease status.

Treatment-free interval

In this study, we defined treatment-free interval (TFI) as the period from the date of completion of first-line treatment to first relapse. When sequential radiotherapy or PCI were performed as first-line treatment, the date of completion of first-line treatment was defined as the last day of these treatments.

Since TFI is known as a predictive factor of second-line chemotherapy, we analyzed patients according to TFI.[16, 17] In many trials, the relapsed SCLC patients with TFI more than 90 days were defined as sensitive relapses. This definition was also used in this study.

Statistical analyses

We used Spearman's rank correlation and linear regression analyses to determine whether PFS or PPS correlated with OS in LD-SCLC patients. We also applied the Cox proportional hazards model with a stepwise regression procedure to determine prognostic factors for PPS and estimate hazard ratios and 95% confidence intervals. The effects of different prognostic factors on PPS were compared using the log-rank test. *P*-values less than 0.05 were considered to be statistically significant for both one-tailed and two-tailed tests. All statistical analyses were performed using JMP (version 11.0; SAS Institute, Cary, NC, USA).

Results

Patient characteristics and treatment efficacy

Between September 2002 and March 2013, 116 patients with LD-SCLC were treated with chemoradio-

TABLE 1. Patient characteristics

Characteristic	Patients (*n*)
Gender	
Male	57
Female	14
Age (years)	
Median	69
Range	45–92
Performance status	
0	32
1	37
2	2
Clinical stage	
II	8
III	63
Tumor histology	
Small cell carcinoma	68
Combined small cell carcinoma	3
Smoking history	
Current or former smoker	70
Never smoked	1
Number of first-line chemotherapy courses	
1	2
2	1
3	4
4	63
5	1
Number of regimens after first-line treatment	
0	18
1	21
2	16
3	8
4	4
5	2
6	2
Radiation dose (Gy)	
Median	45
Range	40–60
Chemoradiotherapy	
Concurrent	56
Sequential	15
First-line chemotherapy regimens	
Cisplatin + etoposide	49
Carboplatin + etoposide	18
Cisplatin + etoposide → Cisplatin + irinotecan	3
Cisplatin + etoposide → Cisplatin + Vincristine + Doxorubicin + etoposide	
Subsequent lines of chemotherapy, total (second-line/third-line or more)	
Platinum combination	25 (15/10)
Amrubicin	36 (22/14)
Irinotecan	25 (9/16)
Topotecan	13 (7/6)
Gemcitabine	7 (0/7)
Paclitaxel	6 (0/6)
Investigational drug	2 (0/2)
Distant metastases at recurrence	
Yes	48
No	23
Prophylactic cranial irradiation	
Yes	27
No	44

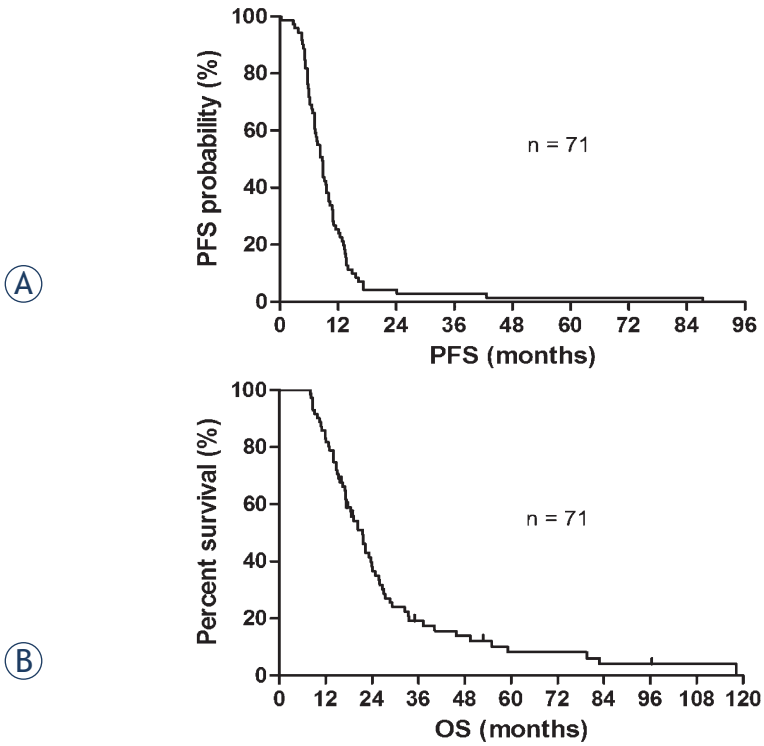

FIGURE 1. Kaplan-Meier survival plots of **(A)** progression-free survival (PFS) and **(B)** overall survival (OS) in 71 limited-stage disease small cell lung cancer (LD-SCLC) patients in this study. Median PFS: 8.8 months, median OS: 21.6 months, median follow-up period: 19.1 months.

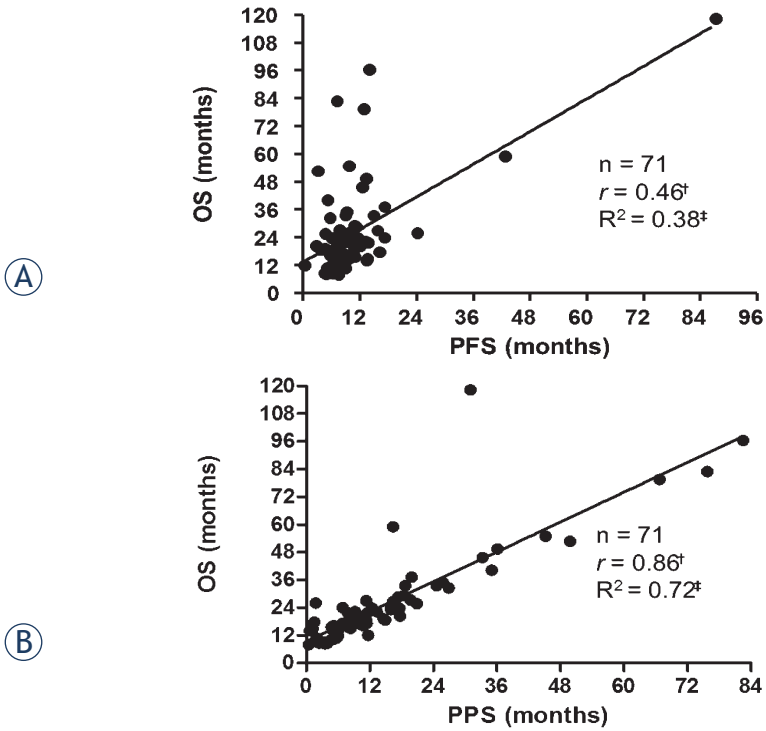

FIGURE 2. Correlations between overall survival (OS) and **(A)** progression-free survival (PFS) and **(B)** post-progression survival (PPS) in 71 limited-stage disease small cell lung cancer (LD-SCLC) patients. †Spearman's rank correlation coefficient. ‡Linear regression correlation coefficient.

therapy, and 71 patients who recurred after first-line treatment were enrolled in this study. Patient characteristics are summarized in Table 1. The majority of patients (80.3%) received concurrent chemotherapy and radiotherapy. Cisplatin plus etoposide combination chemotherapy was the most common first-line treatment. Subsequently, 21/71 (29.6%) patients received a median of one additional regimen (range: 0–6). Twenty-one patients temporarily interrupted RT, but all of them completed previously planned radiation doses. During a median follow-up period of 19.1 months (range: 8.0–118.3 months), 63/71 (88.7%) patients died. Nine patients experienced a CR, 56 patients had a PR, three patients showed SD, and three patients exhibited PD. The overall response rate was 91.5% and the disease control rate was 95.7%. The median PFS and OS were 8.8 months and 21.6 months, respectively (Figures 1A, 1B). The mean OS of other 45 patients who didn't experience recurrence after first-line treatment was 46.5 months (median not reached).

Prognostic factors for post-progression survival

Since OS was more strongly correlated with PPS (Spearman's $r = 0.86$, $R^2 = 0.72$, $p < 0.05$; Figure 2B) than PFS (Spearman's $r = 0.46$, $R^2 = 0.38$, $p < 0.05$; Figure 2A), we assessed the significance of potential prognostic factors for PPS. Univariate analysis showed that six factors, namely, age at the beginning of first-line treatment, relative timing of chemotherapy and radiotherapy (sequential vs. concurrent), response to second-line treatment (non PD vs. PD), the presence of distant metastases at recurrence (yes vs. no), administration of platinum-based chemotherapeutic agents after first-line treatment (yes *vs*. no), and the number of regimens after first-line treatment, were significantly associated with PPS ($p < 0.05$; Table 2). However, multivariate analysis revealed that only the response to second-line treatment (non PD vs. PD), the presence of distant metastases at recurrence (yes *vs*. no) and the number of additional regimens after first-line treatment are significant independent prognostic factors for PPS (Table 3).

We used these three prognostic factors to construct Kaplan-Meier plots of PPS (Figures 3A, 3B and 3C), which showed that the survival distributions for response to second-line treatment (non PD *vs*. PD), the presence of distant metastases at recurrence (yes *vs*. no) and the number of additional regimens after first-line treatment (< 2 *vs*. ≥ 2) are significantly different (log-rank tests, $p <$

TABLE 2. Univariate analysis of factors associated with post-progression survival in limited-stage small cell lung cancer patients

Factors	Post-progression survival		
	Hazard ratio	95% CI	*p*-value
Gender	1.42	0.78–2.81	0.25
Age (years) at the beginning of first-line treatment	1.03	1.00–1.06	**0.03**
Age (years) at the beginning of second-line treatment	1.02	0.99–1.06	0.10
PS at the beginning of first-line treatment	0.90	0.55–1.48	0.69
PS at the end of first-line treatment	0.77	0.47–1.25	0.29
PS at the beginning of second-line treatment	1.31	0.83–2.03	0.23
Tumor histology (small cell carcinoma/combined small cell carcinoma)	1.55	0.63–5.16	0.36
Clinical stage at the beginning of first-line treatment (II/III)	0.55	0.22–1.15	0.12
Chemoradiotherapy (sequential/concurrent)	2.21	1.14–3.99	**0.01**
Number of courses of first-line chemotherapy	1.08	0.75–1.77	0.69
Best response at first-line treatment			
PR/ nonPR	0.98	0.45–2.57	0.97
NonPD /PD	1.33	0.49–5.48	0.61
Best response at second-line treatment			
PR/ nonPR	0.63	0.31–1.21	0.17
NonPD/PD	0.23	0.11–0.45	**< 0.01**
Treatment-free interval Sensitive/refractory	0.87	0.49–1.64	0.65
Distant metastases at recurrence (yes/no)	1.77	1.05–3.10	**0.03**
Administration of platinum-based agents after first-line treatment (yes/no)	0.51	0.28–0.88	**0.01**
Administration of amrubicin after first-line treatment (yes/no)	0.71	0.39–1.28	0.25
Prophylactic cranial irradiation (yes/no)	0.75	0.44–1.25	0.28
Number of regimens after first-line treatment	0.84	0.71–0.98	**0.02**

CI = confidence interval; PD = progressive disease; PR = partial response; PS = performance status. Boldfaced *p*-values are statistically significant ($p < 0.05$).

TABLE 3. Multivariate analysis of factors associated with post-progression survival in limited-stage small cell lung cancer patients

Factors	Post-progression survival		
	Hazard ratio	95% CI	*p*-value
Age (years) at the beginning of first-line treatment	0.98	0.94–1.02	0.47
Chemoradiotherapy (sequential/concurrent)	2.25	0.66–7.04	0.18
Best response at second-line treatment (NonPD/PD)	0.22	0.10–0.47	**< 0.01**
Distant metastases at recurrence (yes/no)	2.42	1.18–5.22	**0.01**
Administration of platinum-based agents after first-line treatment (yes/no)	0.92	0.41–1.98	0.83
Number of regimens after first-line treatment	0.75	0.56–0.98	**0.04**

CI = confidence interval; PD = progressive disease. Boldfaced *p*-values are statistically significant ($p < 0.05$).

0.05). Specifically, the median PPS in patients without PD after second-line treatment (17.5 months) was significantly greater than that for patients with PD (6.9 months; $p < 0.05$). Furthermore, the median PPS in patients without distant metastases (17.3 months) was significantly greater than that for patients with distant metastases (8.7 months; $p < 0.05$). In addition, the median PPS of patients who received two or more regimens after first-line treatment (16.0 months) was significantly greater than that for patients who received less than two additional regimens (6.8 months; $p < 0.05$).

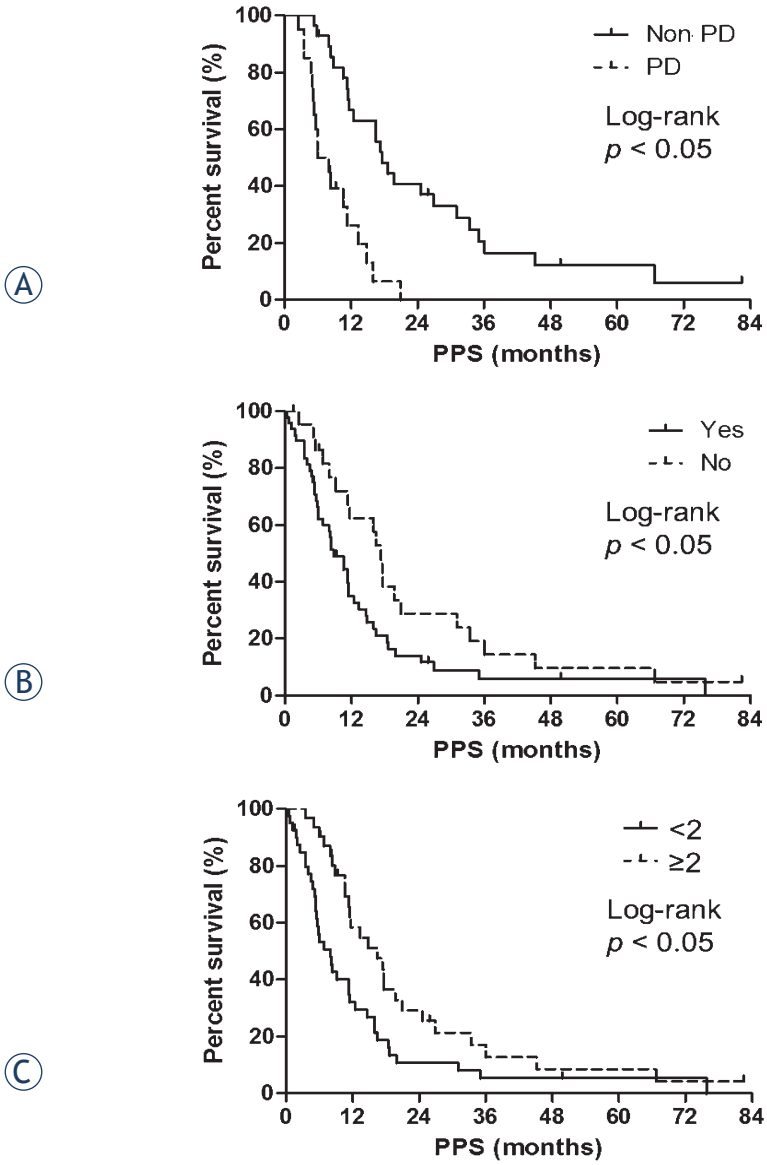

FIGURE 3. Three significant independent prognostic factors of post-progression survival (PPS) (Table 3) result in significantly different PPS distributions in 71 limited-stage disease small cell lung cancer (LD-SCLC) patients (log rank test, $p < 0.05$). **(A)** Response to second-line treatment (progressive disease [PD] vs. non progressive disease [non PD]). Median PPS for non PD: 17.5 months vs. PD: 6.9 months. **(B)** Presence of distant metastases at recurrence (Yes vs. No). Median PPS for Yes: 8.7 months vs. No: 17.3 months. **(C)** Number of regimens after first-line treatment. Median PPS for ≥ 2 additional regimens: 16.0 months vs. < 2 regimens: 6.8 months.

Discussion

In this study, we examined the relationships between OS and PFS or PPS, for recurrent LD-SCLC patients after first-line chemoradiotherapy and found that OS correlates more strongly with PPS than PFS. In addition, we determined that the response to second-line treatment, the presence of distant metastases at recurrence and the number of additional regimens after first-line treatment are significant independent prognostic factors for PPS. To our knowledge, this is the first report of individual-level factors that affect PPS for LD-SCLC patients after first-line chemoradiotherapy.

Several previous meta-analyses have assessed the value of surrogate endpoints, such as time to progression for survival in cancer studies.[18,19] In extensive-stage disease SCLC, tumor response and PFS have been proposed as potential surrogate endpoints for OS, but their appropriateness is controversial in LD-SCLC.[20] Computer simulations have shown that significance of OS may be diluted if PPS is long.[9] Other studies have also demonstrated that PPS is strongly correlated with OS for advanced NSCLC after both first-line chemotherapy and subsequent lines of therapy.[12,13,21] Similarly, we have previously reported that PPS is a potential surrogate marker for advanced NSCLC and extensive-stage disease SCLC.[14,22]

Our finding that OS is more strongly correlated with PPS than PFS implies that subsequent treatments have more effects on OS than the first line treatment. Therefore, LD-SCLC clinical trials should account for factors that may affect PPS to avoid confounding OS. Actually, this recommendation may apply to SCLC in general, because the two of three significant independent prognostic factors associated with PPS for LD-SCLC patients that we identified in this study, namely, response to second-line treatment and the number of additional regimens after first-line chemotherapy, are also associated with PPS in extensive-stage SCLC patients.[14]

These prognostic factors for PPS also suggest that disease stabilization after disease progression following first-line chemoradiotherapy may allow LD-SCLC patients to receive additional lines of treatment, which could prolong PPS, and consequently, OS. Although a number of treatment choices in SCLC are less than that of NSCLC, the large number of treatment regimens that were used after first-line chemoradiotherapy in this study is mainly due to the increasing number of chemotherapeutic options, such as amrubicin, irinotecan, and topotecan, for subsequent-line chemotherapy for LD-SCLC. However, treatments with platinum-based chemotherapeutic agents and amrubicin after first-line treatment were not significant prognostic factors for PPS, which suggest that these drugs do not affect PPS or OS. Likewise, treatment with sequential or concurrent chemoradiotherapy was not a significant prognostic factor for PPS; however, relative few patients in this study were

treated sequentially, so there may have been insufficient statistical power to detect a significant difference.

This study has three major limitations. First, the sample size was relatively small. This limitation is difficult to overcome, particularly in studies that analyze patients with similar backgrounds, because there are relatively few LD-SCLC patients at any given institution. Nevertheless, our institution treats a fair number of these cases and uses unified treatment regimens. Second, the single-center design of our study may limit the generality of our conclusions, so multicenter trials are needed to validate our results in larger patient populations and other clinical settings. Third, since different physicians documented patient responses, the timing of evaluation of PFS and tumor response rates may have been less accurate than if only a single physician had documented all responses. However, this is one of the major limitations of retrospective study, and it is unavoidable. Prospective trials are needed to investigate the validity.

In conclusion, PPS has more impact on OS than PFS in recurrent LD-SCLC patients after first-line chemoradiotherapy. In addition, the response to second-line treatment, the presence of distant metastases at recurrence and the number of additional regimens after first-line treatment are significant independent prognostic factors for PPS. These results suggest that treatments administered after first-line chemoradiotherapy affect OS in LD-SCLC patients. However, larger multicenter studies are needed to validate these conclusions in other patient populations and clinical settings.

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Imunotoksin – nova možnost za zdravljenje bolnikov s ponovljenim ali neodzivnim Hodgkinovim limfomom

Jezeršek Novaković B

Izhodišča. Čeprav je Hodgkinov limfom večinoma ozdravljiva bolezen, se pri nekaterih bolnikih ne odziva na zdravljenje ali pa se ponovi po uspešnem prvem zdravljenju. Pri bolnikih s ponovljenim ali neodzivnim Hodgkinovim limfomom dosežemo dolgotrajen odgovor na zdravljenje in začasno izboljšanje bolezni pri približno polovici bolnikov s t.i. "reševalno" kemoterapijo, ki ji sledi visokodozna kemoterapija in avtologna presaditev krvotvornih matičnih celic. Po drugi strani pa imajo bolniki s ponovitvijo bolezni po takšnem zdravljenju ter tisti, pri katerih je bilo zdravljenje z vsaj dvema redoma kombinirane kemoterapije neuspešno in niso bili primerni za visokodozno kemoterapijo, zelo omejene možnosti nadaljnjega zdravljenja.

Zaključki. Nova možnost za zdravljenje teh bolnikov s ponovljenim ali neodzivnim Hodgkinovim limfomom je imunotoksin brentuksimab vedotin. Sestavljen je iz monoklonalnega protitelesa usmerjenega proti CD30, na katerega je vezana protitubulna učinkovina monometil auristatin E. To zdravilo je bilo učinkovito in je imelo sprejemljivo toksičnost. V registracijski raziskavi je bil celokupni odgovor na zdravljenje 75 % s 34 % popolnih odgovorov. Srednja vrednost trajanja odgovora je bila 20,5 mesecev pri bolnikih s popolnim odgovorom in 6,7 mesecev pri vseh bolnikih, ki so odgovorili na zdravljenje. Srednja vrednost celokupnega preživetja je bila 40,5 mesecev (3-letno celokupno preživetje 54 %) in srednja vrednost brez napredovanja bolezni 9,3 mesecev. Najpogostejši nehematološki neželeni učinki so bili periferna senzorična nevropatija, slabost in utrujenost, najpogostejši resni neželeni učinki pa nevtropenija, trombocitopenija, anemija in periferna senzorična nevropatija.

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Parametri preiskave s [F-18] FDG-PET/CT kot napovedni dejavniki zdravljenja bolnikov z neoperabilnim nedrobnoceličnim rakom pljuč

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Izhodišča. V raziskavi smo ocenili napovedni pomen standardizirane vrednosti privzema (SUVmax), presnovni volumen tumorja in skupno glikolizo v leziji pri [F-18] FDG-PET/CT preiskavi bolnikov z neoperabilnim, nedrobnoceličnim pljučnim rakom.

Bolniki in metode. Pri 103 bolnikih (povprečna starost 65,6 ± 16 let) smo naredili preiskavo [F-18] FDG-PET/CT pred kemoterapijo. Zabeležili smo vrednost SUVmax, presnovni volumen tumorja (cm3; prag 42 %) in skupno glikolizo v leziji. Bolnike smo spremljali do 18 mesecev (razpon 12–55 mesecev). Neodzivnost na zdravljenje brez napredovanja bolezni ter napredovanje bolezni in/ali smrt povezana z boleznijo so pomenili nadomestni končni cilj. Ugotavljali smo, kakšen je optimalni SUVmax, presnovni volumen tumorja in mejna vrednost za skupno glikolizo v leziji, ki bi lahko napovedali izid zdravljenja bolnikov. Rezultate PET/CT smo nato povezali z izidom bolezni (preživetjem brez napredovanja bolezni)

Rezultati. Analiza preživetja po metodi Kaplan-Meier za SUVmax je pokazala znatno krajši čas preživetja brez napredovanja bolezni pri bolnikih, ki so imeli nižje vrednosti v primerjavi s tistimi z višjimi ($p < 0,05$, log-rank test). Presnovni volumen tumorja in skupna glikoliza v leziji nista bila primerna za napovedovanje preživetja brez napredovanja bolezni, razen za podskupino bolnikov s tumorsko zajetimi mediastinalnimi bezgavkami.

Zaključki. Kljub razpoložljivosti novih orodij za kvantitativno oceno aktivnosti bolezni na PET/CT, ostaja SUVmax, ne pa tudi presnovni volumen tumorja in skupna glikoliza v leziji, edini napovedni dejavnik za preživetje brez napredovanja bolezni pri bolnikih z nedrobnoceličnim rakom pljuč. Presnovni volumen tumorja ima vrednost le, če se pojavi sočasna tumorska zajetost bezgavk.

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Optimalni čas slikanja za ovrednotenje obščitničnih adenomov z [^{18}F]-fluoroholin PET/CT

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Izhodišča. Najpogostejši vzrok za primarni hiperparatiroidizem je adenom obščitnice. Adenomi obščitnic so benigni tumorji, ki proizvajajo in izločajo obščitnični hormon. [^{18}F]-fluoroholin, je pozitronski emisijski tomografski (PET) označevalec celične proliferacije. Nedavno so ugotovili, da se [^{18}F]-fluoroholin kopiči v povečanem obščitničnem tkivu, vendar optimalni čas za slikanje z [^{18}F]-fluoroholin PET/CT po aplikaciji [^{18}F]-fluoroholina ni znan. Cilj raziskave je bil ugotoviti optimalni čas za izvedbo preiskave [^{18}F]-fluoroholin PET/CT pri bolnikih s primarnim hiperparatiroidizmom.

Bolniki in metode. V raziskavo smo vključili 43 bolnikov s primarnim hiperparatiroidizmom. Tro-fazno slikanje PET/CT smo izvedli pet minut, eno uro in dve uri po aplikaciji [^{18}F]-fluoroholina. Interesna območja (ROI) smo izbrali v povečanem obščitničnem in ščitničnem tkivu. Standardizirano vrednost privzema (SUV_{mean}), retencijski indeks (RI) in kontrast lezij (LC) smo računali za povečano obščitnično in ščitnično tkivo.

Rezultati. Kopičenje [^{18}F]-fluoroholina je bilo statistično pomembno višje v povečanem obščitničnem tkivu v primerjavi s ščitničnim tkivom, kjer se je pokazal precej višji SUV_{mean} v drugi in v tretji fazi ($p < 0,0001$). Povprečna vrednost RI se je pomembno zmanjšala med prvo in drugo fazo, pomembno povečala med drugo in tretjo fazo v povečanem obščitničnem tkivu ter značilno zmanjšala v vseh fazah v ščitničnem tkivu ($p < 0,0001$). LC med povečanim obščitničnim tkivom in ščitničnim tkivom je bil bistveno boljši v drugi in tretji fazi ($p < 0,05$).

Zaključek. Glede na naše rezultate je optimalni čas slikanja [^{18}F]-fluoroholin PET/CT za lokalizacijo povečanega obščitničnega tkiva eno uro po aplikaciji [^{18}F]-fluoroholina.

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Vloga razmerja deformabilnosti, izmerjenega z ultrazvočno endobronhialno elastografijo v diagnostiki mediastinalnih bezgavk

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Izhodišča. Ultrazvočna elastografija je slikovna metoda za ocenjevanje biomehaničnih lastnosti različnih tkiv. Namen raziskave je bil ugotoviti diagnostično vrednost razmerja deformabilnosti, izmerjenega z endobronhialno ultrazvočno elastografijo na nivoju mediastinalnih bezgavk pri bolnikih s sumom na pljučni rak. Diagnostične vrednosti razmerja deformabilnosti smo primerjali z diagnostičnimi vrednostmi pri pregledu bezgavk z endobronhialnim ultrazvokom v svetlostnem načinu in s citološko diagnozo.

Bolniki in metode. V prospektivno klinično raziskavo, ki smo jo naredili samo v eni ustanovi, smo vključevali bolnike s sumom na pljučni rak in indikacijo za biopsijo. Pri bolnikih smo želeli ugotoviti zamejitev bolezni na nivoju mediastinuma, potem ko smo opravili neinvazivne slikovne preiskave. Pred punkcijo bezgavk pod nadzorom endobronhialnega ultrazvoka smo naredili oceno izbranih bezgavk v svetlostnem načinu in v elastografskem načinu z merjenjem razmerja deformabilnosti.

Rezultati. Vključili smo triintrideset bolnikov z 80-imi sumljivimi mediastinalnimi bezgavkami. Maligno infiltracijo smo potrdili v 34 (42,5 %) bezgavkah. Površina pod krivuljo karakteristik sprejemnika za razmerje deformabilnosti je bila 0,87 ($p < 0,0001$). Pri razmerju deformabilnosti ≥ 8 je bila ocena natančnosti za maligno infiltracijo 86,25 % (občutljivost 88,24 %, specifičnost 84,78 %, pozitivna napovedna vrednost 81,08 %, negativna napovedna vrednost 90,70 %). Razmerje deformabilnosti je bilo natančnejše, kot ocena s konvencionalnim svetlostnim načinom endobronhialnega ultrazvoka pri ločevanju maligno infiltriranih in benignih mediastinalnih bezgavk.

Zaključki. Ocena razmerja deformabilnosti z endobronhialno ultrazvočno elastografijo lahko loči med benignimi in maligno infiltriranimi mediastinalnimi bezgavkami z večjo natančnostjo, kot konvencionalni endobronhialni ultrazvočni pregled bezgavk v svetlostnem načinu. S to novo metodo bi lahko znižali število ultrazvočno vodenih punkcij, vzporedno pa tudi invazivnost in stroške ugotavljanja zamejitve nedrobnoceličnega raka pljuč na nivoju mediastinuma.

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Analiza dejavnikov tveganja za edem možganovine po znotrajžilni embolizaciji nerupturiranih možganskih arterijskih anevrizem

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Izhodišča. Znotrajžilno zdravljenje nerupturiranih možganskih anevrizem je zdravljenje izbora, katerega zaplet je lahko edem možganovine ob anevrizmi. V raziskavi smo želeli ugotoviti dejavnike tveganja za razvoj edema možganovine ob anevrizmi, zdravljeni z znotrajžilnim zapiranjem, njegovo pogostnost in kliničen izhod.

Metode. V raziskavo smo vključili 119 odraslih bolnikov, ki so imeli znotrajžilno zdravljenje nerupturirane anevrizme na Oddelku za intervencijsko nevroradiologijo v Kliničnem centru Kragujevac, Srbija. Za zapiranje anevrizem smo uporabili spirale, ki imajo elektrolitsko nadzorovano sprostitev: spirale iz čiste platine, hidrofilne spirale in kombinirane platinasto-hidrofilne spirale. Z magnetno resonančno preiskavo (MRI) smo ugotavljali razvoj edema možganovine ob zdravljeni anevrizmi 7, 30 in 90 dni po posegu.

Rezultati. Edem smo ugotovili pri 47,6 % bolnikih zdravljenih s hidrofilnimi spiralami, 21,6 % bolnikih zdravljenih s spiralami iz platine in 53,8 % bolnikih zdravljenih s kombiniranimi spiralami. Z multivariatno logistično regresijo smo dokazali povezavo med edemom možganovine ter prostornino anevrizme, zvišanim arterijskim tlakom, sladkorno boleznijo in kajenjem. Zvišan arterijski tlak je najpomembnejši neodvisen napovedni dejavnik za razvoj edema možganovine ob zdravljeni anevrizmi, ki mu sledijo kajenje in sladkorna bolezen.

Zaključki. Rezultati raziskave so pokazali, da imajo največje tveganje za razvoj edema možganovine ob anevrizmi, zdravljeni z znotrajžilnim zapiranjem, starejši bolniki z velikimi, nerupturiranimi anevrizmami, ki so kadilci, imajo sladkorno bolezen in zvišan arterijski tlak.

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Ocena preživetja celic pri frakcionirani radioterapiji z uporabo različnih metod izvedenih iz linerno-kvadratnega modela

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Izhodišča. Namen raziskave je bil uporabiti različne teoretične metode za oceno preživetja celic. Metode smo izvedli iz linearno-kvardartnega modela (LQ). Želeli smo ugotoviti vpliv števila frakcij obsevanja, časovnega intervala med njimi, doze na frakcijo in celotnega časa obsevanja. Načrtovali smo primerjavo rezultatov, temelječih na *in vitro* preživetju melanomskih in adenokarcinomskih celic. Glede na rezultate raziskave smo želeli predlagati najbolj primerno metodo izračuna, ki ustreza *in vitro* rezultatom preživetja celic.

Materiali in metode. Proučili smo najpogosteje uporabljene teoretične modele iz literature avtorjev Keall in sod. in Mu in sod. za izračun preživetja celic po radioterapiji pri različnih režimih obsevanja. Skupni čas obsevanja je bil med 15 in 240 minutami. Za izračun potrebnega števila frakcij obsevanja in doze na frakcijo smo izbrali čas 30, 60 in 240 minut. Poskuse *in vitro* smo naredili pri obsevalni dozi 4 Gy in rezultate primerjali s teoretičnimi izračuni.

Rezultati. Najpomembnejši parameter za preživetje celic je bil skupni čas obsevanja. V teoretičnih modelih je bilo pomembno tudi število frakcij obsevanja, kar nismo statistično značilno potrdili v eksperimentalnih modelih. Spreminjanje števila frakcij pa je statistično značilno pokazalo različne rezultate po izračunih Keall in sod. in Mu in sod. ($p < 0.05$).

Zaključki. Metoda Mu in sod. najbolje napove preživetje celic po frakcionirani radioterapiji. Rezultati nakazujejo na primernost te metode za napoved odgovora celic na frakcionirano radioterapijo in njeno uporabo tudi v kliniče namene.

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Urokinazni aktivator plazminogena (uPA) in inhibitor aktivatorja plazminogena 1 (PAI-1) pri primarnem raku dojk. Povezanost s klasičnimi napovednimi dejavniki

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Izhodišča. Urokinazni aktivator plazminogena (uPA) in inhibitor aktivatorja plazminogena 1 (PAI-1) imata ključno vlogo pri invaziji in zasevanju malignih tumorjev. Visoke vrednosti obeh proteolitičnih encimov so povezane s slabo napovedjo poteka bolezni pri bolnicah z rakom dojk. Namen raziskave je bil preučiti povezanost med klasičnimi napovednimi dejavniki ter uPA in PAI-1 v tkivu primarnega tumorja bolnic z rakom dojk.

Metode. V prospektivno klinično raziskavo na Oddelku za ginekološko onkologijo in onkologijo dojk Univerzitetnega kliničnega centra Maribor smo med leti 2004 in 2010 vključili 606 bolnic s primarnim rakom dojk. Ovrednotili smo klasične napovedne dejavnike (starost, menopavzni status, velikost tumorja, patohistološki tip tumorja, stopnjo malignosti tumorja, prizadetost pazdušnih bezgavk, invazijo v krvne in limfne žile in stanje hormonskih receptorjev) ter vrednosti uPA in PAI-1. Dobljene podatke smo obdelali s Spearmanovo korelacijo rangov, Mann-Whitneyevim U testom in testom χ^2.

Rezultati. Izsledki raziskave nakazujejo pozitivno povezavo med uPA in velikostjo tumorja ($p < 0,001$), stopnjo malignosti tumorja ($p < 0,001$), patohistološkim tipom tumorja ($p < 0,001$), invazijo v krvne in limfne žile ($p = 0,01$) in negativno povezavo s stanjem hormonskih receptorjev ($p < 0,001$). Prav tako nakazujejo pozitivno povezavo med PAI-1 in velikostjo tumorja ($p = 0,004$), stopnjo malignosti tumorja ($p < 0,001$), patohistološkim tipom tumorja ($p < 0,001$) in negativno povezavo s stanjem hormonskih receptorjev ($p = 0,002$).

Zaključki. Raziskava je potrdila povezanost med uPA in PAI-1 ter klasičnimi napovednimi dejavniki. Njuno vlogo kot dejavnikov, ki napovedujejo potek bolezni, in kot dejavnikov, ki napovedujejo odgovor na zdravljenje pri bolnicah z rakom dojke, je potrebno nadalje ovrednotiti.

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Napovedna vrednost nekaterih tumorskih označevalcev pri bolnikih z napredovalim neoperabilnim rakom ustnega žrela, zdravljenih s sočasno radiokemoterapijo

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Izhodišča. Cilj raziskave je bil proučiti, kako tumorski označevalci p21, p27, p53, ciklin D1, receptorji epidermalnega rastnega dejavnika (EGFR), Ki-67 in CD31 napovedujejo izid bolezni pri bolnikih z napredovalim neoperabilnim rakom ustnega žrela, zdravljenih s sočasno kombinacijo obsevanja in kemoterapije.

Bolniki in metode. Tkivo biopsijskih vzorcev 74 zaporedno obsevanih bolnikov z neoperabilnim rakom ustnega žrela, odvzetih pred pričetkom zdravljenja, smo v retrospektivni klinični raziskavi histokemično obarvali za prikaz tumorskih označevalcev p21, p27, p53, ciklin D1, EGFR, Ki-67 in CD31. Ocenjevali smo, kako je izraženost tumorskih označevalcev povezana s preživetjem brez bolezni.

Rezultati. Pri bolnikih z visoko izraženostjo p21 (≥ 10 %), p27 (> 50 %), Ki-67 (> 50 %) in CD31 (> 130 žil/mm2) in nizko izraženostjo p53 (< 10 %), ciklin D1 (< 10 %) in EGFR (< 10 %) (ugodni nivoji) je bilo preživetje brez bolezni boljše kot pri bolnikih z nizko izraženostjo p21 (< 10 %), p27 (≤ 50 %), Ki-67 (≤ 50 %) in CD31 (<130 žil/mm2) ter visoko izraženostjo p53 (≥ 10 %), ciklin D1 (≥ 10 %) in EGFR (≥ 10 %) (neugodni nivoji), a je bila statistična značilna razlika v preživetju med ugodnim in neugodnim nivojem potrjena le za p27 in ciklin D1. Preživetje brez bolezni se je statistično značilno slabšalo z naraščanjem števila označevalcev z neugodnim nivojem v tumorjih (1–4 vs. 5–7) (78 % vs. 32 %; p = 0,004). Število označevalcev z neugodnim nivojem v tumorju je ohranilo napovedno značilnost tudi v multivariatni analizi.

Zaključki. Statistično značilna razlika v preživetju med bolniki z ugodnim in neugodnim nivojem tumorskih označevalcev je bila potrjena le za p27 in ciklin D1. Število označevalcev v tumorju z neugodnim nivojem izraženosti je bilo neodvisen napovedni dejavnik za slab izid bolezni.

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Protonsko obsevanje dvajsetih starejših bolnikov z rakom požiralnika

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Izhodišča. V starajoči se družbi je vedno več starejših bolnikov z ugotovljenim rakom požiralnika. Namen raziskave je bil oceniti klinično učinkovitost in varnost obsevanja s protoni pri starejših bolnikih z rakom požiralnika.

Bolniki in metode. V raziskavo smo vključili starostnike (starost ≥ 65 let) z rakom požiralnika, ugotovljenim med januarjem 2009 in junijem 2013. Vsi bolniki so bili napoteni na zdravljenje bodisi samo s protonskimi žarki bodisi s protonskimi žarki in izhodiščnim obsevanem z žarki x. Neželene učinke smo ocenjevali z lestvico neželenih učinkov zdravljenja po enotnih kriterijih (*angl. Common Terminology Criteria for Adverse Events,* CTCAE), verzija 4,0, ki sta jo izdelala ameriški Nacionalni inštitut za zdravljenje raka in ameriški Nacionalni inštitut za zdravje.

Rezultati. Za raziskavo je bilo primernih 20 bolnikov in pri vseh smo lahko zaključili zdravljenje. Srednja starost je bila 78 let (razpon: 65–89 let) in srednji čas njihovega spremljanja 25,5 mesecev (razpon 6–62 mesecev). Sedem bolnikov je imelo zasevke v bezgavkah in 10 bolnikov je imelo bolezen II. ali III. stadija. Pri obsevanju samo s protoni je bila srednja doza obsevanja s protoni 72,6 Gy relativne biološke dozne učinkovitosti (*angl. ralative biological dose effectiveness*, RBE) (razpon 66–74,8 Gy [RBE]), pri zdravljenju s protoni in izhodiščnem obsevanju z žarki x pa je bila doza s protoni 33 Gy (RBE) (razpon 30,8–39,6 Gy [RBE] ter skupni razpon s protoni in žarki x 66,8–75,6 Gy [RBE]). Dveletno celokupno preživetje je bilo 81,8 % (95% interval zaupanja [CI]: 62,4 % – 100 %) in dveletna lokalna kontrola bolezni je bila 89,4 % (95 % CI: 75,5 % – 100 %). Stranski učinki 2. ali 3. stopnje so se pojavili pri nekaj primerih, ni pa bilo ugotovljene toksičnosti 4. ali 5. stopnje.

Zaključki. Visokodozno obsevanje s protoni (66–75,5 Gy [RBE]) brez kemoterapije je bilo učinkovito in varno zdravljenje pri starejših bolnikih z rakom požiralnika.

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Pogostnost kožnega melanoma in njegova odvisnost od letnega časa. 20-letno obdobje opazovanj v populaciji z prekomernim sončenjem

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Izhodišča. Kožni melanom je zelo agresivna oblika raka, ki je postal vse pogostejši v večini razvitih držav. Posebno številen je pri Evropejcih.

Bolniki in metode. Anonimne podatke bolnikov s kožnim melanomom smo zajeli iz diagnostične baze podatkov Univerzitetne bolnice Trst za obdobje med 1. januarjem 1990 in 10. decembrom 2013. Raziskava obravnava dobro definirano populacijo s karakteristično lastnostjo prekomernega sončenja.

Rezultati. Incidenca kožnega melanoma je v opazovanem obdobju stalno rastla s trendom odvisnosti od letnega časa. Ugotovili smo razlike med spoloma glede anatomske lokacije in stadija bolezni. Poleg tega je 6 % bolnikov razvilo multiple melanome.

Zaključki. V dobro definirani populaciji z navado prekomernega sončenja se je incidenca kožnega melanoma podvojila v obdobju opazovanj od 1990 do 2013. Zato tej populaciji priporočamo preventivne ukrepe glede na navade, ki so odvisne od spola. Priporočamo tudi programe sledenja bolezni za ponovitev bolezni in za zgodnje odkrivanje dodatnih primarnih tumorjev, posebno glede na razlike v pojavnosti pri spolih in pojavnosti multipllih melanomov.

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doi:10.1515/raon-2015-0002

Sproščanje rastnih dejavnikov po mehanski in kemični plevrodezi pri zdravljenju malignih plevralnih izlivov. Prospektivna randomizirana raziskava primerljivih primerov

Hojski A, Leitgeb M, Crnjac A

Izhodišča. Rastni dejavniki so ključni spodbujevalci fibroze, obenem pa sodelujejo v vnetnih odzivih telesa, ki lahko povečajo volumen plevralnega izliva in sprožijo sindrom akutne dihalne stiske. Primarni cilj raziskave je bil primerjava kemične plevrodeze s smukcem in torakoskopske mehanske plevrodeze glede na sproščanje rastnih dejavnikov v prvih 48 urah. Sekundarni cilji raziskave pa so bili ocena učinkovitosti obeh načinov plevrodeze, ugotavljanje lajšanja simptomov in kvalitete življenja po posegu.

Bolniki in metode. V prospektivno randomizirano raziskavo smo vključili 36 zaporednih bolnic z rakom dojke in dokazanim malignim plevralnim izlivom, ki so bile napotene na naš oddelek zaradi zdravljenja. 18 bolnic smo zdravili s kemijsko plevrodezo in 18 bolnic z mehansko plevrodezo. Pri kemijski plevrodezi smo uporabili 5g emulzije smukca, ki smo ga vbrizgali preko torakalnega drena. Po pripravljenem protokolu smo v obdobju 48 ur zbirali vzorce plevralne tekočine in seruma ter v njih ugotavljali rastne dejavnike. Z analogno vizualno skalo smo ocenjevali bolečino, s standardiziranim vprašalnikom pa kvaliteto življenja.

Rezultati. Srednji izmerjeni serumski nivo rastnega dejavnika žilnega endotela (VEGF) po kemični plevrodezi je znašal 930.68 pg/ml (95% interval zaupanja CI: 388.22–4656.65), po mehanski pa 808.54 pg/ml. (95% CI: 463.20-1235.13) (p = 0.103). Nasprotno so bile srednje vrednosti transformirajočega rastnega dejavnika (TGF) β1 v plevralni tekočini višje po mehanski plevrodezi (4814.00 pg/ml [95% CI: 2726.51–7292.94]) v primerjavi s tistimi po kemijski plevrodezi (1976.50 pg/ml [95% CI: 1659.82–5136.26]) (p = 0.078). Podobne rezultate smo zabeležili za fibroblastni rastni dejavnik (FGF) β; serumski nivo je bil višji po mehanski plevrodezi (30.45 pg/ml [95% CI: 20.40–59.42]) kot po kemijski (13,39 pg/ml (13.39 pg/ml [95% CI: 5.04 – 74.60]) (p = 0.076). Bolnice po kemijski in mehanski plevrodezi so imele primerljivo trajanje hospitalizacije, pogostost ponovnega plevralnega izliva in ponovnih torakalnih drenaž ter celokupno preživetje; nasprotno pa so bolnice po mehanski plevrodezi imele krajši čas drenaže po posegu (p = 0.030), manj bolečin in boljšo kakovost življenja (p = 0,047).

Zaključki. Primerjava zdravljenja z različno plevrodezo je pokazala povečanje serumskih vrednosti VEGF pri bolnicah po kemijski plevrodezi. Nasprotno so imele bolnice po mehanski plevrodezi povišane vrednosti TGF β1 in FGF β v plevralni tekočini. Razlike niso dosegle statistične značilnosti; vendar ostajajo VEGF, TGFβ1 in FGF β najbolj zanimivi parametri za nadaljnje raziskave. Ob upoštevanju mehanizmov delovanja rastnih dejavnikov se je v naši raziskavi mehanska plevrodeza nakazovala kot potencialno učinkovitejša, s krajšim časom plevralne drenaže, izrazitejšem zmanjšanjem bolečine in boljšo kvaliteto življenja.

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doi:10.2478/raon-2014-0036

Gigantski solitarni fibrozni tumor plevre. Prikaz primera in pregled literature

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Izhodišča. Mezenhimski solitarni tumorji plevre so redki. Večinoma so benigni, okoli 12 % je malignih. V začetni fazi so največkrat asimptomatski, z rastjo tumorja pa zaradi pritiska na okoliške strukture povzročajo bolečine v prsih, dražeč kašelj in oteženo dihanje. Redko povzročajo simptome pritiska na mediastinalne strukture. Potrebna je stopenjska radiološka diagnostika. Predoperativna biosija je v večini primerov neuspešna. Terapija izbora tovrstne patologije je radikalna kirurška odstranitev tumorja, ki jo je - pri malignih tumorjih ali neradikalno odstranjenih benignih tumorjih - potrebno dopolniti z adjuvantno terapijo.

Prikaz primera. Opisujemo primer 68-letne bolnice, ki smo je sprejeli na Klinični oddelek za torakalno kirurgijo UKC Maribor zaradi gigantskega fibroznega tumorja v desnem plevralnem prostoru. Tumor je s svojo ekspanzivno rastjo povzročal premik mediastinuma in utesnjeval spodnjo votlo veno, desni predvor ter srednji in spodnji pljučni reženj. Zaradi vtočnih motenj in kolapsa desnih pljuč je bila bolnica življenjsko ogrožena z znaki kardiorespiratorne odpovedi. Po predoperativni radiološki diagnostiki smo bolnici kirurško radikalno odstranili tumor. Pooperativno se je bolnici zdravstveno stanje izboljšalo. Po letu dni nismo zasledili ponovitve bolezni.

Zaključki. Veliki fibrozni tumorji plevre lahko zaradi omejenosti plevralnega prostora povzročajo s svojo ekspanzivno rastjo resna, življenje ugrožujoča stanja. Pravočasna prepoznava bolezni omogoča izvedbo manj agresivnega, lahko tudi videotorakoskopskega posega pri bolnikih v bistveno boljšem zdravstvenem stanju. Operacije velikih tumorjev pri kardiorespiratorno prizadetih bolnikih so sicer edini možni, a nadvse tvegan kirurški poseg.

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Vpliv spremljajočih bolezni na izhod bolezni pri bolnikih z napredovalim rakom prostate, zdravljenimi z docetakselom

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Izhodišča. Moškim z metastatskim, proti kastraciji odpornim rakom prostate lahko zdravljenje z docetakselom v vsakodnevni klinični praksi neupravičeno odtegnemo zaradi pridruženih bolezni. Zato smo v raziskavi preučevali vpliv pridruženih bolezni na izid zdravljenja z docetakselom pri bolnikih s takšnim rakom prostate.

Metode. V raziskavo smo vključili vse bolnike z metastatskim, proti kastraciji odpornim rakom prostate, ki smo jih med letoma 2005 in 2012 zdravili z docetakselom na Onkološkem inštitutu Ljubljana. Stopnjo pridruženih bolezni smo ovrednotili s starostno prilagojenim Charlsonovim indeksom pridruženih bolezni (*angl. age-adjusted Charlson comorbidity index*, aa-CCI) in z indeksom ocenjevanja 27 pridruženih bolezni odraslih (*angl. adult comorbidity evaluation-27*, ACE-27). Kako bolniki prenašajo zdravljenje, smo ocenili s številom hospitalizacij in smrti, ki so nastopile zaradi toksičnih sopojavov med zdravljenjem. Povezavo med pridruženimi boleznimi in skupnim preživetjem smo testirali s Cox-ovo analizo.

Rezultati. Z docetakselom smo zdravili 208 moških. Brez pridruženih bolezni ali z blago, zmerno in hudo stopnjo je bilo 2 %, 32 %, 53 % in 13 % bolnikov, če smo jih ocenjevači z aa-CCI ter 27 %, 35 %, 29 % in 8 %, če smo jih ocenjevali z ACE-27. Pomembno zmanjšanje odmerka docetaksela je bilo pogostejše pri bolnikih z zmerno ali hudo stopnjo pridruženih bolezni v primerjavi s tistimi brez ali z blago stopnjo pridruženih bolezni. Ne glede na pridružene bolezni je približno ena tretjina moških potrebovala hospitalizacijo ali je umrla med zdravljenjem z docetakselom. V univariantni analizi višja stopnja pridruženih bolezni ni bila povezana s slabšim skupnim preživetjem (aa-CCI: razmerje obetov [HR] 0,99; 95 % interval zaupanja [CI] 0,87–1,13; p = 0,93 ter ACE-27: HR 0,96; 95 % CI 0,79–1,17; p = 0,69).

Zaključki. Moškim z metastatskim, proti kastraciji odpornim rakom prostate ter pridruženimi boleznimi lahko koristimo, če jih zdravimo z docetakselom.

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doi:10.1515/raon-2015-0037

Klinični pomen preživetja po napredovanju bolezni po primarni kemoradioterapiji na celokupno preživetje pri bolnikih z omejeno obliko drobnoceličnega pljučnega raka

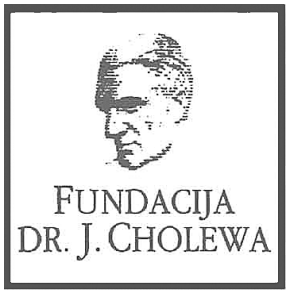
Kasahara N, Imai H, Kaira K, Mori K, Wakuda K, Ono A, Taira T, Kenmotsu H, Harada H, Naito T, Murakami H, Endo M, Nakajima T, Yamada M, Takahashi T

Izhodišča. Pri bolnikih z omejeno obliko drobnoceličnega pljučnega raka lahko učinke primarnega zdravljenja s kemoradioterapije prekrijemo s sekundarnim zdravljenjem. To lahko namreč znatno vpliva na celokupno preživetje. Zato smo želeli ugotoviti povezavo med preživetjem brez napredovanja bolezni oz. preživetjem po napredovanju bolezni in celokupnim preživetjem po primarnem zdravljenju s kemoradioterapijo.

Bolniki in metode. Retrospektivno smo analizirali 71 bolnikov z omejeno obliko drobnoceličnega pljučnega raka in s splošnim stanjem zmogljivosti 0–2. Prejeli so primarno kemoradioterapijo v Shizuoka Cancer Center (Shizuoka, Japonska) in imeli ponovitev bolezni med septembrom 2002 in marcem 2013. Ugotavljali smo korelacijo med preživetjem brez napredovanja bolezni in celokupnim preživetjem ter med preživetjem po napredovanju bolezni in celokupnim preživetjem na individualni ravni. Poleg tega smo izvedli univariatne in multivariatne analize za prepoznavanje pomembnih napovednih dejavnikov preživetja po napredovanju bolezni.

Rezultati. Celokupno preživetje je bolj povezano s preživetjem po napredovanju bolezni (Spearman r = 0,86, R2 = 0,72, p < 0,05) kot s preživetjem brez napredovanja bolezni (Spearman r = 0,46, R2 = 0,38, p < 0,05). Poleg tega smo odkrili, da so pomembni neodvisni napovedni dejavniki za preživetje po napredovanju bolezni odgovor na zdravljenje po sekundarnem zdravljenju, prisotnost oddaljenih metastaz ob ponovitvi bolezni in število dodatnih zdravljenj po primarni kemoradioterapiji.

Zaključki. Preživetje po napredovanju bolezni ima večji vpliv na celokupno preživetje kot pa preživetje brez napredovanja bolezni pri bolnikih z omejeno obliko drobnoceličnega pljučnega raka, z dobrim splošnim stanjem zmogljivosti na začetku zdravljenja in s ponovitvijo bolezni. Poleg tega lahko zdravljenje po primarni kemoradioterapije vpliva na njihovo celokupno preživetje. Vendar pa so potrebne večje multicentrične raziskave za potrditev te ugotovitve.



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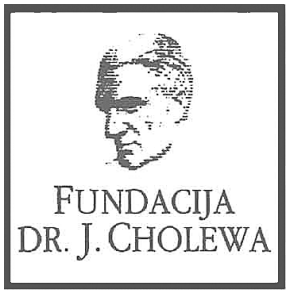

Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the final quarter of 2015

The “Docent Dr. J. Cholewa Foundation for Cancer Research and Education” is named after Dr. Josip Cholewa, one of the first researchers in cancer in Slovenia and the founder of the “Banovinski Inštitut za raziskovanje in zdravljenje novotvorb” in 1937, that later became the Institute of Oncology in Ljubljana, Slovenia. His laboratory and clinical research work was based on an innovative and far-reaching multidisciplinary approach that included studies on prevention, detection and treatment of cancer. This pioneering approach facilitated the understanding of the complexities of all the problems and troubles experienced by cancer patients, their doctors and other medical staff when facing this disease. It could also be regarded as a harbinger of the progress observed in a large part of the world in the last half of the previous century. Therefore, the Foundation is a non-profit, non-political and non-government organisation that helps professionals, institutions and individuals obtaining financial help for cancer research and education in the Republic of Slovenia with the goal of continuing and expanding the great work and efforts of Dr. Josip Cholewa.

The “Docent Dr. J. Cholewa Foundation for Cancer Research and Education” hopes and strives to provide at least part of the financial support needed by qualified individuals and organisations interested in cancer research in the Republic of Slovenia. One of the objectives of the Foundation is to facilitate the transmission of the latest diagnostic and therapy procedures to the clinical environment in Slovenia, thus benefiting the ever increasing number of patients with various types of cancer in Slovenia. With this in mind, it is important to note that the incidence rates of many cancer, like colon, prostate and breast cancer have kept rising in recent decades in Slovenia.

The Foundation continues to provide financial support to "Radiology and Oncology”, an international scientific journal that is edited and published in Ljubljana, Slovenia. It publishes scientific research articles, reviews, and letters to the editor about research and studies in experimental and clinical oncology, supportive therapy, radiology, radiophyics, prevention and early diagnostics of different types of cancer. It is an open access journal freely available in pdf format and with a respectable Science Citation Index Impact factor. All the abstracts in “Radiology and Oncology” are available in Slovenian and the journal can thus provide sufficient scientific information from various fields of high quality cancer research to interested lay public in Slovenia.

The “Docent Dr. J. Cholewa Foundation for Cancer Research and Education” has thus an important role in support of cancer research, cancer education and many of the related fields in the Republic of Slovenia.

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

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

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

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Literatura: 1. Lipton A, et al. Eur J Cancer. 2012; 48: 3082–3092.

Podatki integrirane analize preskušanj III. faze, ki so vključevala bolnike z rakom dojke, rakom prostate, ostalimi solidnimi tumorji ali diseminiranim plazmocitomom, ki so imeli zasevke v kosteh.[1]

*XGEVA® značilno podaljša čas do pojava prvega zapleta kostnih zasevkov (ZKZ) za 8,21 mesecev in zmanjša tveganje za pojav prvega ZKZ za 17% (HR; 0,83 [95% CI; 0,76–0,90]; p < 0,001) v primerjavi z zoledronsko kislino.

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▼Za to zdravilo se izvaja dodatno spremljanje varnosti. Poročati je potrebno o vseh domnevnih neželenih učinkih zdravila. **SESTAVA ZDRAVILA:** Ena viala vsebuje 120 mg denosumaba v 1,7 ml raztopine (70 mg/ml). Pomožne snovi s prepoznavnim delovanjem: 1,7 ml raztopine vsebuje 78 mg sorbitola (E420). **TERAPEVTSKE INDIKACIJE:** Preprečevanje skeletnih dogodkov (patoloških zlomov, obsevanja kosti, kompresije hrbtenjače ali operacije kosti) pri odraslih s kostnimi metastazami solidnih tumorjev. Zdravljenje odraslih bolnikov in skeletno dozorelih mladostnikov z gigantocelularnim kostnim tumorjem, ki ni operabilen, ali pri katerem bi kirurška odstranitev verjetno povzročila hudo obolevnost. **ODMERJANJE IN NAČIN UPORABE:** Vsi bolniki morajo prejemati dodatek vsaj 500 mg kalcija in 400 i.e. vitamina D dnevno, razen če ima bolnik hiperkalciemijo. Bolniki morajo dobiti navodilo za uporabo in opozorilno kartico za bolnika. *Kostne metastaze solidnih tumorjev:* Priporočeni odmerek zdravila XGEVA® za preprečevanje skeletnih dogodkov je 120 mg enkrat na 4 tedne v enkratni subkutani injekciji v stegno, trebuh ali nadlaket. *Gigantocelularni kostni tumor:* Priporočeni odmerek zdravila XGEVA® za zdravljenje gigantocelularnega kostnega tumorja je 120 mg enkrat na 4 tedne v enkratni subkutani injekciji v stegno, trebuh ali nadlaket ter dodaten odmerek 120 mg 8. in 15. dan zdravljenja v prvem mesecu terapije. Bolnike z gigantocelularnim kostnim tumorjem je treba v rednih presledkih ocenjevati, da bi ugotovili, ali jim zdravljenje še koristi. Pri bolnikih, ki imajo bolezen z zdravilom XGEVA® obvladano, niso ocenili učinka prekinitve ali prenehanja zdravljenja, toda omejeni podatki pri teh bolnikih ne kažejo povratnega učinka po prenehanju zdravljenja. *Bolniki z okvaro ledvic:* Prilagoditev odmerka ni potrebna. *Bolniki z okvaro jeter:* Varnost in učinkovitost denosumaba nista raziskani. *Starejši bolniki (stari ≥ 65 let):* Prilagoditev odmerka ni potrebna. *Pediatrični bolniki:* Odmerjanje za zdravljenje skeletno dozorelih mladostnikov z gigantocelularnim kostnim tumorjem, ki ni operabilen, ali pri katerem bi kirurška odstranitev verjetno povzročila hudo obolevnost, je enako kot za odrasle. Zdravilo XGEVA® ni priporočljivo za pediatrične bolnike (stare < 18 let), ker varnost in učinkovitost zdravila XGEVA® pri teh bolnikih nista ocenjeni, z izjemo skeletno dozorelih mladostnikov z gigantocelularnim kostnim tumorjem. Za subkutano uporabo. Zdravilo XGEVA® mora aplicirati zdravstveni delavec. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. Huda, nezdravljena hipokalciemija. Nezaceljene lezije po zobnih ali ustnih kirurških posegih. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Vsi bolniki morajo prejemati dodatek kalcija in vitamina D, razen če ima bolnik hiperkalciemijo. Obstoječo hipokalciemijo je treba odpraviti še pred začetkom zdravljenja z zdravilom XGEVA®. Hipokalciemija se lahko pojavi kadarkoli med zdravljenjem z zdravilom XGEVA®. Kontrolo koncentracije kalcija je treba izvesti (i) pred prvim odmerkom zdravila XGEVA®, (ii) v dveh tednih po prvem odmerku, (iii) če se pojavijo simptomi, sumljivi za hipokalciemijo. O dodatnih kontrolah koncentracije kalcija med zdravljenjem je treba razmisliti pri bolnikih, ki imajo dejavnike tveganja za hipokalciemijo, ali če so takšne kontrole sicer umestne glede na bolnikovo klinično stanje. Bolnikom je treba naročiti, naj zdravnika obvestijo o simptomih, ki kažejo na hipokalciemijo. Če se med prejemanjem zdravila XGEVA® pojavi hipokalciemija, je lahko potrebno dodatno dodajanje kalcija in dodatne kontrole. Bolniki s hudo okvaro ledvic (očistek kreatinina < 30 ml/min) ali bolniki na dializi imajo večje tveganje za pojav hipokalciemije. Tveganje za pojav hipokalciemije in spremljajočega zvišanja paratiroidnega hormona se povečuje s povečano stopnjo okvare ledvic. Pri takšnih bolnikih so redne kontrole koncentracije kalcija posebej pomembne. Pri bolnikih, ki imajo nezaceljene lezije mehkih tkiv v ustih, je treba začetek zdravljenja/nov ciklus zdravljenja odložiti. Pred zdravljenjem z zdravilom XGEVA® je priporočljivo opraviti zobozdravstveni pregled in preventivno zobozdravstveno oskrbo ter individualno oceno koristi in tveganja. Pri ocenjevanju bolnikovega tveganja za pojav osteonekroze čeljustnice je treba upoštevati naslednje dejavnike tveganja: moč zdravila, ki zavira resorpcijo kosti (tveganje je večje z zelo močnimi spojinami), pot uporabe (tveganje je večje v primeru parenteralne uporabe) in kumulativni odmerek zdravila, uporabljenega za zdravljenje resorpcije kosti, rak, sočasne bolezni (npr. anemijo, koagulopatije, okužbo), kajenje, sočasna zdravljenja: kortikosteroide, kemoterapijo, zaviralce angiogeneze, radioterapijo glave in vratu, slabo ustno higieno, periodontalno bolezen, slabo prilegajoče se zobne proteze, že obstoječo zobno bolezen, invazivne zobozdravstvene posege, npr. ekstrakcije zob. Vsem bolnikom je treba naročiti, da morajo med zdravljenjem z zdravilom XGEVA® vzdrževati dobro ustno higieno, redno opravljati zobozdravniške preglede in nemudoma obvestiti zdravnika, če se pojavi kakršen koli simptom v ustih, na primer majanje zob, bolečina, oteklina, rana, ki se ne celi, ali izcedek. Med zdravljenjem je izvajanje invazivnih zobozdravniških posegov dovoljeno le po skrbnem razmisleku in se jim je treba izogniti v bližini termina za odmerjanje zdravila XGEVA®. Načrt vodenja bolnikov, ki se jim pojavi osteonekroza čeljustnice, je treba oblikovati na podlagi tesnega sodelovanja med lečečim zdravnikom in zobozdravnikom ali ustnim kirurgom, ki ima izkušnje z osteonekrozo čeljustnice. Razmisliti je treba o začasnem prenehanju zdravljenja z zdravilom XGEVA®, dokler se to stanje ne razreši in se sovpleteni dejavniki tveganja ublažijo, če je mogoče. Atipični zlomi stegnenice se lahko pojavijo že ob majhni poškodbi ali celo brez poškodbe, in sicer v subtrohanternem in diafiznem predelu stegnenice. Za te dogodke so značilni specifični radiografski izvidi. O njih so poročali tudi pri bolnikih z določenimi sočasnimi bolezenskimi stanji (npr. s pomanjkanjem vitamina D, revmatoidnim artritisom, hipofosfatazijo) in med uporabo določenih zdravil (npr. difosfonatov, glukokortikoidov, zaviralcev protonske črpalke). Ti dogodki so se pojavili tudi brez antiresorpcijskega zdravljenja. Podobni zlomi, opisani v zvezi z difosfonati, so pogosto obojestranski, zato je treba pri bolnikih, ki se zdravijo z denosumabom in so imeli zlom srednjega dela stegnenice, opraviti tudi pregled druge stegnenice. Pri bolnikih, pri katerih obstaja sum na atipičen zlom stegnenice, je treba razmisliti o prenehanju uporabe zdravila XGEVA® ob vrednotenju bolnika glede na individualno oceno koristi in tveganja. Bolnikom je treba naročiti, da morajo med zdravljenjem z zdravilom XGEVA® zdravniku poročati o novi ali nenavadni bolečini v stegnu, kolku ali dimljah. Bolnike s takšnimi simptomi je treba preiskati glede nepopolnega zloma stegnenice. Bolniki, zdravljeni z zdravilom XGEVA®, sočasno ne smejo prejemati drugih zdravil, ki vsebujejo denosumab (za indikacije pri osteoporozi, in difosfonatov. Malignost pri gigantocelularnem kostnem tumorju ali napredovanje do metastatske bolezni je redek dogodek in je znano tveganje pri bolnikih z gigantocelularnim kostnim tumorjem. Bolnike je treba kontrolirati glede radioloških znakov malignosti, nove radiolucentnosti ali osteolize. Razpoložljivi klinični podatki ne kažejo povečanega tveganja za malignost pri bolnikih z gigantocelularnim kostnim tumorjem, zdravljenih z zdravilom XGEVA®. Bolniki z redko prirojeno motnjo intolerance za fruktozo ne smejo uporabljati zdravila XGEVA®. **INTERAKCIJE:** Študij medsebojnega delovanja niso izvedli. V kliničnih preskušanjih sočasna kemoterapija in/ali hormonsko zdravljenje ali predhodna intravenska izpostavljenost difosfonatom niso klinično pomembno spremenili najmanjše koncentracije denosumaba v serumu in farmakodinamike denosumaba (N-telopeptid v urinu, prilagojen na kreatinin, uNTx/Cr). **POVZETEK NEŽELENIH UČINKOV:** Zelo pogosti (≥ 1/10): dispneja, driska, mišično-skeletna bolečina. Pogosti (≥ 1/100 do < 1/10): hipokalciemija, hipofosfatemija, ekstrakcija zoba, hiperhidroza, osteonekroza čeljustnice. Redki (≥ 1/10.000 do < 1/1.000): preobčutljivost na zdravilo, anafilaktična reakcija, atipični zlom stegnenice. **FARMACEVTSKI PODATKI:** Shranjujte v hladilniku (2 °C – 8 °C). Ne zamrzujte. **NAČIN IN REŽIM PREDPISOVANJA TER IZDAJE ZDRAVILA:** Predpisovanje in izdaja zdravila je le na recept s posebnim režimom – ZZ. **IMETNIK DOVOLJENJA ZA PROMET:** Amgen Europe B.V., Minervum 7061, NL-4817 ZK Breda, Nizozemska. **Dodatna pojasnila lahko dobite v lokalni pisarni:** Amgen zdravila d.o.o., Šmartinska 140, SI-1000 Ljubljana. **DATUM ZADNJE REVIZIJE BESEDILA:** Julij 2015. **DATUM PRIPRAVE INFORMACIJE:** Oktober 2015. Podrobne informacije o zdravilu so objavljene na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu/.

SLHR-SLA-1 (v2a-0615-1070101)

Neulasta®:
Zaščitite bolnike, optimizirajte zdravljenje s citostatiki[1]

NEULASTA® 6 mg raztopina za injiciranje (pegfilgrastim) – SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA
Samo za strokovno javnost. Pred predpisovanjem si preberite celoten Povzetek glavnih značilnosti zdravila. SESTAVA ZDRAVILA: Ena napolnjena injekcijska brizga vsebuje 6 mg pegfilgrastima v 0,6 ml (10 mg/ml) raztopine za injiciranje. **TERAPEVTSKE INDIKACIJE:** Skrajšanje trajanja nevtropenije in zmanjšanje incidence febrilne nevtropenije pri odraslih bolnikih, zdravljenih s citotoksično kemoterapijo za maligne bolezni (z izjemo kronične mieloidne levkemije in mielodisplastičnih sindromov). **ODMERJANJE IN NAČIN UPORABE:** Zdravljenje z zdravilom Neulasta® morajo uvesti in nadzorovati zdravniki, izkušeni v onkologiji in/ali hematologiji. Za vsak cikel kemoterapije priporočajo en 6 mg odmerek (eno napolnjeno injekcijsko brizgo) zdravila Neulasta®, ki je dana vsaj 24 ur po citotoksični kemoterapiji. Zdravilo Neulasta® se injicira subkutano. Injekcije se morajo dati v stegno, trebuh ali zgornji del roke. Varnost in učinkovitost zdravila Neulasta® pri otrocih še nista bili dokazani in priporočil o odmerjanju ni mogoče dati. Pri bolnikih z okvaro ledvic in s končno odpovedjo ledvic odmerka ni treba spreminjati. **KONTRAINDIKACIJE**: Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Pri bolnikih z *de novo* akutno mieloično levkemijo omejeni klinični podatki kažejo primerljiv učinek pegfilgrastima in filgrastima na čas do okrevanja po hudi nevtropeniji. Dolgoročni učinki zdravila Neulasta® pri akutni mieloični levkemiji niso ugotovljeni, zato ga je treba pri tej populaciji bolnikov uporabljati previdno. Varnost in učinkovitost zdravila Neulasta® nista raziskani pri bolnikih z mielodisplastičnim sindromom, s kronično mielogeno levkemijo in s sekundarno akutno mieloično levkemijo (AML), zato ga pri takšnih bolnikih ne smete uporabljati. Posebno pozornost je treba nameniti razlikovanju diagnoze blastne transformacije kronične mieloične levkemije od akutne mieloične levkemije. Varnost in učinkovitost uporabe zdravila Neulasta® pri bolnikih z *de novo* AML, mlajših od 55 let in s citogenetiko t(15;17), nista ugotovljeni. Varnosti in učinkovitosti zdravila Neulasta® niso raziskovali pri bolnikih, ki prejemajo kemoterapijo v velikih odmerkih. Tega zdravila ne smete uporabljati za zvečevanje odmerka citotoksične kemoterapije preko uveljavljenih shem odmerjanja. Neželene reakcije na pljučih: Bolj ogroženi so lahko bolniki z nedavno anamnezo pljučnih infiltratov ali pljučnice. Pojav pljučnih znakov, kot so kašelj, zvišana telesna temperatura in dispneja v povezavi z radiološkimi znaki pljučnih infiltratov, in poslabšanje pljučne funkcije skupaj z zvečanim številom nevtrofilcev utegnejo biti preliminarni znaki sindroma akutne dihalne stiske (ARDS -'*Acute Respiratory Distress Syndrome*'). V takih primerih je treba zdravilo Neulasta® po presoji zdravnika prenehati dajati in poskrbeti za ustrezno zdravljenje. Glomerulonefritis: Na splošno so primeri glomerulonefritisa minili po zmanjšanju odmerka ali prenehanju uporabe filgrastima ali pegfilgrastima. Priporočljivo je spremljanje laboratorijskih izvidov urina. Sindrom kapilarne prepustnosti: Bolnike, ki se jim pojavijo simptomi sindroma kapilarne prepustnosti, je treba natančno kontrolirati in deležni morajo biti standardnega simptomatskega zdravljenja, ki lahko vključuje potrebo po intenzivni negi. Splenomegalija in ruptura vranice: Skrbno je treba spremljati velikost vranice (s kliničnim pregledom, ultrazvokom). Na diagnozo rupture vranice moramo misliti pri bolnikih, ki poročajo o bolečini v zgornjem levem delu trebuha ali v predelu lopatice. Trombocitopenija in anemija: Zdravljenje s samim zdravilom Neulasta® ne prepreči trombocitopenije in anemije, ker se hkrati vzdržuje mielosupresivna kemoterapija s polnimi odmerki po predpisani shemi. Priporočajo redno spremljanje števila trombocitov in hematokrita. Posebna previdnost je potrebna med uporabo posameznih kemoterapevtikov ali njihovih kombinacij, za katere je znano, da povzročajo hudo trombocitopenijo. Srpastocelična anemija: Pri bolnikih s srpastocelično dispozicijo ali s srpastocelično anemijo je bila uporaba pegfilgrastima povezana s srpastocelično krizo, zato se mora pri teh bolnikih zdravilo Neulasta® predpisovati previdno in spremljati ustrezne klinične parametre in laboratorijski status in biti pozoren na morebitno povezavo tega zdravila z zvečanjem vranice in vazookluzivno krizo. Levkocitoza: Zaradi kliničnih učinkov zdravila Neulasta® in zaradi možnosti levkocitoze je treba med zdravljenjem redno kontrolirati število belih krvniček. Če število levkocitov po pričakovanem najmanjšem številu preseže 50 x 10^9/l, je treba nemudoma prenehati z zdravljenjem s tem zdravilom. Preobčutljivost: Dokončno prenehajte z zdravljenjem z zdravilom Neulasta® pri bolnikih s klinično signifikantno preobčutljivostjo. Zdravila Neulasta® ne dajajte bolnikom z anamnezo preobčutljivosti na pegfilgrastim ali filgrastim. V primeru resne alergijske reakcije je treba poskrbeti za ustrezno zdravljenje in pazljivo spremljanje bolnika še nekaj dni. Imunogenost: Kot pri vseh terapevtskih beljakovinah obstaja možnost imunogenosti. Stopnja nastajanja protiteles proti pegfilgrastimu je na splošno nizka. Vezavna protitelesa se pojavijo po pričakovanjih pri vseh bioloških zdravilih, vendar jih doslej niso povezali z nevtralizacijskim delovanjem. Varnosti in učinkovitosti zdravila Neulasta® za mobilizacijo matičnih krvotvornih celic pri bolnikih ali zdravih dajalcih niso primerno ovrednotili. Pokrovček igle pri napolnjeni injekcijski brizgi vsebuje suho naravno gumo (derivat lateksa), ki lahko povzroča alergične reakcije. Povečana hemopoetična aktivnost kostnega mozga zaradi zdravljenja z rastnimi dejavniki je bila povezana s prehodnimi pozitivnimi izvidi pri slikanju kosti, kar je treba upoštevati pri interpretaciji izvidov na podlagi slikanja kosti. Zdravilo Neulasta® vsebuje sorbitol. Bolniki z redko prirojeno motnjo intolerance za fruktozo ne smejo dobiti tega zdravila. Zdravilo Neulasta® vsebuje manj kot 1 mmol (23 mg) natrija na 6 mg odmerek, kar v bistvu pomeni "brez natrija". Za izboljšanje sledljivosti granulocitne kolonije spodbujajočih faktorjev (G-CSF) je treba v bolnikovi dokumentaciji jasno zabeležiti zaščiteno ime uporabljenega zdravila. **MEDSEBOJNO DELOVANJE ZDRAVIL IN DRUGE OBLIKE INTERAKCIJ:** Zaradi možne občutljivosti hitro se delečih mieloidnih celic za citotoksično kemoterapijo je treba zdravilo Neulasta® dati vsaj 24 ur po aplikaciji citotoksične kemoterapije. Sočasne uporabe zdravila Neulasta® s katerimkoli kemoterapevtskim zdravilom pri bolnikih niso ovrednotili. **NEŽELENI UČINKI:** Podatki opisujejo neželene učinke, zabeležene v kliničnih preskušanjih in med spontanim poročanjem. Zelo pogosti (≥ 1/10): glavobol, navzea, bolečina v kosteh. Pogosti (≥ 1/100 do < 1/10): trombocitopenija, levkocitoza, mišično-skeletna bolečina (mialgija, artralgija, bolečina v okončini, bolečina v hrbtu, mišično-skeletna bolečina, bolečina v vratu), bolečina na mestu injiciranja, bolečina v prsih, ki ne izvira od srca. Občasni (≥ 1/1.000 do < 1/100): srpastocelična kriza, splenomegalija, ruptura vranice, preobčutljivostne reakcije, anafilaksija, zvišanje sečne kisline, sindrom kapilarne prepustnosti, sindrom akutne dihalne stiske, pljučne neželene reakcije (intersticijska pljučnica, pljučni edem, pljučni infiltrati in pljučna fibroza), Sweetov sindrom (akutna febrilna dermatoza), kožni vaskulitis, reakcije na mestu injiciranja, zvišanje laktat-dehidrogenaze in alkalne fosfataze, prehodno zvišanje jetrnih funkcijskih testov za ALT ali AST, glomerulonefritis. **FARMACEVTSKI PODATKI:** Shranjujte v hladilniku (2°C – 8°C). Ne zamrzujte. Zdravilo Neulasta® sme biti izpostavljeno sobni temperaturi (ne nad 30°C) za enkratno obdobje, ki ne sme preseči 72 ur. Zdravilo Neulasta® ni kompatibilno z raztopinami natrijevega klorida. **NAČIN IN REŽIM PREDPISOVANJA TER IZDAJE ZDRAVILA**: Predpisovanje in izdaja zdravila je le na recept s posebnim režimom – H/Rp. **IMETNIK DOVOLJENJA ZA PROMET:** Amgen Europe B.V., 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:** Maj 2015. Podrobni podatki o tem zdravilu so na voljo na spletni strani Evropske agencije za zdravila http://www.ema.europa.eu/. **Literatura: 1.)** Povzetek glavnih značilnosti zdravila Neulasta®, Amgen, 2015.

SLHR-SLP-003-0615-107089(1)

AMGEN® 35 LET

Neulasta® (pegfilgrastim)

Zaščitite bolnike, optimizirajte zdravljenje s citostatiki.

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Individualizirano zdravljenje za bolnike z metastatskim kolorektalnim rakom

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

Sestava: En ml raztopine za infundiranje vsebuje 5 mg cetuksimaba in pomožne snovi. Cetuksimab je himerno monoklonsko IgG_1 protitelo. **Terapevtske indikacije:** Zdravilo Erbitux je indicirano za zdravljenje bolnikov z metastatskim kolorektalnim rakom z ekspresijo receptorjev EGFR in nemutiranim tipom RAS v kombinaciji s kemoterapijo na osnovi irinotekana, kot primarno zdravljenje v kombinaciji s FOLFOX in kot samostojno zdravilo pri bolnikih, pri katerih zdravljenje z oksaliplatinom in zdravljenje na osnovi irinotekana ni bilo uspešno in pri bolnikih, ki ne prenašajo irinotekana. Zdravilo Erbitux je indicirano za zdravljenje bolnikov z rakom skvamoznih celic glave in vratu v kombinaciji z radioterapijo za lokalno napredovalo bolezen in v kombinaciji s kemoterapijo na osnovi platine za ponavljajočo se in/ali metastatsko bolezen. **Odmerjanje in način uporabe:** Zdravilo Erbitux pri vseh indikacijah infundirajte enkrat na teden. Pred prvo infuzijo mora bolnik prejeti premedikacijo z antihistaminikom in kortikosteroidom najmanj 1 uro pred uporabo cetuksimaba. Začetni odmerek je 400 mg cetuksimaba na m^2 telesne površine. Vsi naslednji tedenski odmerki so vsak po 250 mg/m^2. **Kontraindikacije:** Zdravilo Erbitux je kontraindicirano pri bolnikih z znano hudo preobčutljivostno reakcijo (3. ali 4. stopnje) na cetuksimab. Kombinacija zdravila Erbitux s kemoterapijo, ki vsebuje oksaliplatin, je kontraindicirana pri bolnikih z metastatskim kolorektalnim rakom z mutiranim tipom RAS ali kadar status RAS ni znan. **Posebna opozorila in previdnostni ukrepi:** Pojav hude reakcije, povezane z infundiranjem, zahteva takojšnjo in stalno ukinitev terapije s cetuksimabom. Če pri bolniku nastopi blaga ali zmerna reakcija, povezana z infundiranjem, lahko zmanjšate hitrost infundiranja. Priporočljivo je, da ostane hitrost infundiranja na nižji vrednosti tudi pri vseh naslednjih infuzijah. Če se pri bolniku pojavi kožna reakcija, ki je ne more prenašati, ali huda kožna reakcija (≥ 3. stopnje po kriterijih CTCAE), morate prekiniti terapijo s cetuksimabom. Z zdravljenjem smete nadaljevati le, če se je reakcija izboljšala do 2. stopnje. Če ugotovite intersticijsko bolezen pljuč, morate zdravljenje s cetuksimabom prekiniti, in bolnika ustrezno zdraviti. Zaradi možnosti pojava znižanja nivoja elektrolitov v serumu se pred in periodično med zdravljenjem s cetuksimabom priporoča določanje koncentracije elektrolitov v serumu. Pri bolnikih, ki prejemajo cetuksimab v kombinaciji s kemoterapijo na osnovi platine, obstaja večje tveganje za pojav hude nevtropenije. Takšne bolnike je potrebno skrbno nadzorovati. Pri predpisovanju cetuksimaba je treba upoštevati kardiovaskularno stanje in indeks zmogljivosti bolnika in sočasno dajanje kardiotoksičnih učinkovin kot so fluoropirimidini. Če je diagnoza ulcerativnega keratitisa potrjena, je treba zdravljenje s cetuksimabom prekiniti ali ukiniti. Cetuksimab je treba uporabljati previdno pri bolnikih z anamnezo keratitisa, ulcerativnega keratitisa ali zelo suhih oči. Cetuksimaba ne uporabljajte za zdravljenje bolnikov s kolorektalnim rakom, če imajo tumorje z mutacijo RAS ali pri katerih je tumorski status RAS neznan. **Interakcije:** Pri kombinaciji s fluoropirimidini se je v primerjavi z uporabo fluoropirimidinov, kot monoterapije, povečala pogostnost srčne ishemije, vključno z miokardnim infarktom in kongestivno srčno odpovedjo ter pogostnost sindroma dlani in stopal. V kombinaciji s kemoterapijo na osnovi platine se lahko poveča pogostnost hude levkopenije ali hude nevtropenije. V kombinaciji s kapecitabinom in oksaliplatinom (XELOX) se lahko poveča pogostnost hude driske. **Neželeni učinki:** Zelo pogosti (≥ 1/10): hipomagneziemija, povečanje ravni jetrnih encimov, kožne reakcije, blage ali zmerne reakcije povezane z infundiranjem, mukozitis, v nekaterih primerih resen. Pogosti (≥ 1/100 do < 1/10): dehidracija, hipokalciemija, anoreksija, glavobol, konjunktivitis, driska, navzeja, bruhanje, hude reakcije povezane z infundiranjem, utrujenost. **Posebna navodila za shranjevanje:** Shranjujte v hladilniku (2 °C - 8 °C). **Pakiranje:** 1 viala z 20 ml ali 100 ml raztopine. **Način in režim izdaje:** Izdaja zdravila je le na recept-H. **Imetnik dovoljenja za promet:** Merck KGaA, 64271 Darmstadt, Nemčija.

Datum zadnje revizije besedila: november 2014.

Pred predpisovanjem zdravila natančno preberite celoten Povzetek glavnih značilnosti zdravila.

Samo za strokovno javnost.

Podrobnejše informacije so na voljo pri predstavniku imetnika dovoljenja za promet z zdravilom:
Merck d.o.o., Ameriška ulica 8, 1000 Ljubljana, tel.: 01 560 3810, faks: 01 560 3830, el. pošta: info@merck.si
www.merckserono.net
www.Erbitux-international.com

PM-ONC-05/2015 /13.10.2015

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GAZYVARO® ▼ je edino monoklonsko protitelo z dokazano večjo učinkovitostjo kot MabThera® pri predhodno nezdravljenih bolnikih s kronično limfocitno levkemijo.[1]

ZASNOVAN ZA **NAPREDEK**

GAZYVARO®
obinutuzumab

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

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

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

Ime zdravila: Gazyvaro 1000 mg koncentrat za raztopino za infundiranje **Kakovostna in količinska sestava:** Ena viala s 40 ml koncentrata vsebuje 1000 mg obinutuzumaba; to ustreza koncentraciji 25 mg/ml pred razredčenjem. Obinutuzumab je humanizirano anti-CD20 monoklonsko protitelo tipa II podrazreda IgG1, pridobljeno s humanizacijo parentalnega mišjega protitelesa B-Ly1 in proizvedeno v celični liniji jajčnika kitajskega hrčka s tehnologijo rekombinantne DNA. **Terapevtske indikacije:** Zdravilo Gazyvaro je v kombinaciji s klorambucilom indicirano za zdravljenje odraslih bolnikov s predhodno nezdravljeno kronično limfocitno levkemijo (KLL), pri katerih zdravljenje s polnim odmerkom fludarabina zaradi pridruženih bolezni ni primerno. **Odmerjanje in način uporabe:** Dajanje zdravila Gazyvaro mora potekati pod natančnim nadzorom izkušenega zdravnika in v okolju, kjer je takoj na voljo vse potrebno za oživljanje. Odmerjanje: *Profilaksa in premedikacija sindroma razpada tumorja:* Bolniki z velikim tumorskim bremenom in/ali velikim številom limfocitov v krvnem obtoku in/ali ledvično okvaro imajo večje tveganje za sindrom razpada tumorja in morajo prejeti profilakso. To naj sestavljata ustrezna hidracija in dajanje urikostatikov ali primerno alternativno zdravljenje, kot je uratna oksidaza. *Profilaksa reakcij, povezanih z infuzijo, in premedikacija zanje:* Med intravenskim infundiranjem zdravila Gazyvaro se lahko kot simptom reakcij, povezanih z infuzijo, pojavi hipotenzija. Zato je treba uporabo antihipertenzivnih zdravil zadržati v obdobju 12 ur pred infundiranjem zdravila Gazyvaro, med celotnim infundiranjem in še prvo uro po koncu dajanja zdravila. *Odmerek:* 1. ciklus: Priporočeni odmerek zdravila Gazyvaro je 1000 mg, ki ga dajemo na 1. in 2. dan ter 8. in 15. dan prvega 28-dnevnega ciklusa zdravljenja. Za infundiranje 1. in 2. dan je treba pripraviti dve infuzijski vrečki (100 mg za 1. dan in 900 mg za 2. dan). Če med dajanjem prve vrečke ni bilo treba prilagoditi hitrosti infundiranja ali infundiranja prekiniti, je mogoče drugo vrečko uporabiti isti dan. Pogoj je, da so na voljo ustrezen čas, okoliščine in zdravniški nadzor med celotnim infundiranjem. Če je med dajanjem prvih 100 mg treba spremeniti hitrost infundiranja ali infundiranje prekiniti, je treba drugo vrečko dati naslednji dan. Od 2. do 6. ciklusa: Priporočeni odmerek zdravila Gazyvaro je 1000 mg, uporabljen 1. dan. *Trajanje zdravljenja:* Šest ciklusov zdravljenja, od katerih vsak traja 28 dni. *Zapozneli ali izpuščeni odmerki:* V primeru, da bolnik načrtovani odmerek zdravila Gazyvaro izpusti, mu ga je treba dati čim prej; ne čakajte do naslednjega načrtovanega odmerka. Med odmerki je treba ohranjati načrtovani terapevtski interval za zdravilo Gazyvaro. *Prilagoditev odmerka med zdravljenjem:* Zmanjšanja odmerka zdravila Gazyvaro niso priporočljiva. Posebne skupine bolnikov: Starejšim bolnikom in bolnikom z blago do zmerno ledvično okvaro odmerka ni treba prilagoditi. Varnost in učinkovitost zdravila Gazyvaro pri bolnikih z okvarjenim delovanjem jeter in pri otrocih in mladostnikih, mlajših od 18 let, nista ugotovljeni. Podatkov ni na voljo. Način uporabe: Zdravilo Gazyvaro je namenjeno za intravensko uporabo. Po razredčenju ga je treba dati kot intravensko infuzijo po posebni liniji. Infuzij zdravila Gazyvaro se ne sme dati v obliki hitre intravenske infuzije ali kot bolus. Navodila glede redčenja in hitrosti infuzije zdravila Gazyvaro pred uporabo so opisana v SmPC. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Za izboljšanje sledljivosti bioloških zdravil je treba zaščiteno ime in številko serije uporabljenega zdravila jasno zabeležiti v bolnikovi dokumentaciji. Reakcije, povezane z infuzijo: Najpogosteje opaženi neželeni učinki pri bolnikih, ki so prejemali zdravilo Gazyvaro, so bile reakcije, povezane z infuzijo, ki so se pretežno pojavile med infundiranjem prvih 1000 mg. Za zmanjšanje reakcij, povezanih z infuzijo, je treba upoštevati ukrepe za ublažitev. Incidenca in izrazitost simptomov reakcij, povezanih z infuzijo, se je bistveno zmanjšala po infundiranju prvih 1000 mg in večina bolnikov med nadaljnjo uporabo zdravila Gazyvaro reakcij, povezanih z infuzijo, ni imela. Pri večini bolnikov so bile reakcije, povezane z infuzijo, blage do zmerne in jih je bilo mogoče obvladati z upočasnitvijo ali začasno prekinitvijo prvega infundiranja. Vendar pa so poročali tudi o hudih in življenje ogrožajočih reakcijah, povezanih z infuzijo, ki so zahtevale simptomatsko zdravljenje. Reakcije, povezane z infuzijo, so lahko klinično nerazločljive od alergijskih reakcij, posredovanih z imunoglobulini E (IgE). Bolniki z velikim tumorskim bremenom in bolniki, ki imajo hkrati kumulativno oceno bolezni po lestvici CIRS > 6 in očistek kreatinina < 70 ml/min, imajo večje tveganje za reakcije, povezane z infuzijo, vključno s hudimi reakcijami, povezanimi z infuzijo. Med uporabo zdravila Gazyvaro so opisani tudi primeri sindroma sproščanja citokinov. Če se pri bolniku pojavi reakcija, povezana z infuzijo, je treba v zvezi z infundiranjem ukrepati glede na stopnjo reakcije. Bolniki ne smejo dobiti nadaljnih infuzij zdravila Gazyvaro, če se pri njih pojavi oziroma pojavijo: akutni smrtno nevarni simptomi na dihalih, z infuzijo povezana reakcija 4. stopnje (tj. življenje ogrožajoča reakcija, povezana z infuzijo), ali ponovna z infuzijo povezana reakcija 3. stopnje (podaljšana/ponovna) po ponovnem začetku prve infuzije ali med katero od nadaljnjih infuzij. Bolnike z obstoječo boleznijo srca ali pljuč je treba skrbno nadzorovati ves čas infundiranja in v obdobju po njem. Pri bolnikih z akutnim tveganjem za hipertenzivno krizo je treba oceniti tveganja in koristi zadržanja njihovih antihipertenzivnih zdravil. Preobčutljivostne reakcije, vključno z anafilakso: Pri bolnikih, zdravljenih z zdravilom Gazyvaro, so poročali o anafilaktičnih reakcijah. Pri sumu na preobčutljivostno reakcijo med infundiranjem je treba infundiranje prekiniti in zdravljenje trajno končati. Bolnikov, ki imajo znano, z IgE posredovano preobčutljivost za obinutuzumab, se z njim ne sme zdraviti. Sindrom razpada tumorja: Med uporabo zdravila Gazyvaro so poročali o sindromu razpada tumorja. Bolniki z velikim tveganjem za sindrom razpada tumorja (npr. bolniki z velikim tumorskim bremenom in/ali velikim številom limfocitov v periferni krvi [> 25 x 10^9/l]) in/ali ledvično okvaro (očistek kreatinina < 70 ml/min) morajo prejeti profilakso. To naj sestaljata ustrezna hidracija in dajanje urikostatika (npr. alopurinola) ali primernega alternativnega zdravljenja, kot je uratna oksidaza (npr. rasburikaza), ki se začne 12 do 24 ur pred infundiranjem zdravila Gazyvaro v skladu s standardno prakso. Vse bolnike s tveganjem je treba pozorno spremljati med prvimi dnevi zdravljenja s posebno pozornostjo na ledvično delovanje, vrednosti kalija in sečne kisline. Dodatno je treba upoštevati morebitne dodatne smernice v skladu s standardno prakso. Med zdravljenjem sindroma razpada tumorja je treba odpraviti nepravilnosti elektrolitov, preverjati delovanje ledvic in ravnovesje tekočin ter uporabiti podporno oskrbo, vključno z dializnim zdravljenjem, kot je indicirano. Nevtropenija: Med zdravljenjem z zdravilom Gazyvaro so poročali o hudi in življenje ogrožajoči nevtropeniji, vključno s febrilno nevtropenijo. Bolnike, pri katerih se razvije nevtropenija, je treba skrbno nadzirati z rednimi laboratorijskimi kontrolami krvne slike, dokler ne mine. Če je potrebno zdravljenje, ga je treba uporabiti v skladu z lokalnimi smernicami in razmisliti o uporabi granulocitne kolonije spodbujajočih faktorjev. Morebitne znake sočasne okužbe je treba zdraviti, kot je primerno. Pri hudi ali življenje ogrožajoči nevtropeniji je treba razmisliti o preložitvi odmerka na kasnejši čas. Za bolnike s hudo in dolgotrajno nevtropenijo je zelo priporočljivo dajanje protimikrobne profilakse med zdravljenjem dokler se nevtropenija ne izboljša na stopnjo 1 ali 2. Razmisliti je treba tudi o protivirusni in protiglivični profilaksi. Poročali so tudi o primerih zapoznele nevtropenije ali dolgotrajne nevtropenije. Bolniki z ledvično okvaro imajo večje tveganje za nevtropenijo. Trombocitopenija: Med zdravljenjem z zdravilom Gazyvaro so opažali hudo in življenje ogrožajočo trombocitopenijo, vključno z akutno trombocitopenijo. Bolniki z ledvično okvaro imajo večje tveganje za trombocitopenijo. Pri bolnikih, zdravljenih z zdravilom Gazyvaro, so bile v 1. ciklusu opisane tudi krvavitve s smrtnim izidom. Jasne povezanosti med trombocitopenijo in krvavitvami niso ugotovili. Bolnike je treba natančno spremljati glede trombocitopenije, zlasti v prvem ciklusu; izvajati je treba redne laboratorijske preiskave krvne slike, dokler dogodek ne mine, pri hudi ali življenje ogrožajoči trombocitopeniji pa je treba razmisliti o preložitvi odmerka na kasnejši čas. Transfuzija krvnih pripravkov je v skladu s prakso v ustanovi prepuščena presoji lečečega zdravnika. Upoštevati je treba tudi sočasno uporabo vseh zdravil, ki lahko poslabšajo s trombocitopenijo povezane dogodke, na primer zaviralcev trombocitov in antikoagulantov; to je treba še zlasti upoštevati v prvem ciklusu. Poslabšanje obstoječih srčnih bolezni: Pri bolnikih z obstoječo srčno boleznijo so se med zdravljenjem z zdravilom Gazyvaro pojavile motnje srčnega ritma, angina pektoris, akutni koronarni sindrom, akutni miokardni infarkt in srčno popuščanje. Ti dogodki se lahko pojavijo kot del reakcij, povezanih z infuzijo, in so lahko smrtni. Zato je treba bolnike z anamnezo srčne bolezni natančno nadzirati. Poleg tega je treba pri teh bolnikih hidracijo izvajati previdno, da bi preprečili možno preobremenitev s tekočino. Okužbe: Zdravila Gazyvaro se ne sme uporabiti pri bolnikih z aktivno okužbo, pri bolnikih z anamnezo ponavljajočih se ali kroničnih okužb pa je pri razmisleku o uporabi zdravila Gazyvaro potrebna previdnost. Med zdravljenjem z zdravilom Gazyvaro in po njem se lahko pojavijo resne bakterijske, glivične in nove ali reaktivirane virusne okužbe. Poročali so o okužbah s smrtnim izidom. Bolniki, ki imajo hkrati CIRS > 6 in očistek kreatinina < 70 ml/min, imajo večje tveganje za okužbe, vključno s hudimi okužbami. Reaktivacija hepatitisa B: Bolnikom, zdravljenim s protitelesi proti CD20 (vključno z zdravilom Gazyvaro), se lahko pojavi reaktivacija virusa hepatitisa B, v nekaterih primerih s posledičnim fulminantnim hepatitisom, jetrno odpovedjo in smrtjo. Pred začetkom zdravljenja z zdravilom Gazyvaro je treba pri vseh bolnikih opraviti presejalno testiranje za virus hepatitisa B. To mora obsegati vsaj določitev stanja površinskega antigena hepatitisa B in stanja sredičnega protitelesa proti hepatitisu B. To je mogoče dopolniti z drugimi ustreznimi označevalci v skladu z lokalnimi smernicami. Bolnikov z aktivnim hepatitisom B se ne sme zdraviti z zdravilom Gazyvaro. Bolniki s pozitivnimi serološkimi izvidi za hepatitis B se morajo pred začetkom zdravljenja posvetovati s specialistom za jetrne bolezni; treba jih je nadzorovati in voditi v skladu z lokalnimi zdravstvenimi standardi, da bi preprečili reaktivacijo hepatitisa. Progresivna multifokalna levkoencefalopatija (PML): Pri bolnikih, zdravljenih z zdravilom Gazyvaro, je bila opisana PML. Na diagnozo PML je treba pomisliti pri vsakem bolniku z novonastalimi nevrološkimi spremembami ali spremembi že obstoječih nevroloških stanj. Simptomi PML so nespecifični in se lahko razlikujejo glede na prizadet možganski predel. Pogosti so motorični simptomi s prizadetostjo kortikospinalnega trakta, senzorične nepravilnosti, cerebelarni simptomi in izpadi vidnega polja. Pojavijo se lahko nekateri znaki ali simptomi, ki veljajo za "kortikalne". Ovrednotenje PML vključuje posvet z nevrologom, magnetnoresonančno slikanje možganov in lumbalno punkcijo. Med preiskovanjem suma na PML je treba zdravljenje z zdravilom Gazyvaro prekiniti, če je PML potrjena, pa ga je treba trajno končati. V poštev pride tudi prenehanje ali zmanjšanje morebitne sočasne kemoterapije ali imunosupresivnega zdravljenja. Bolnika je treba napotiti k nevrologu za ovrednotenje in zdravljenje PML. Imunizacija: Varnost imunizacije z živimi ali oslabljenimi virusnimi cepivi po zdravljenju z zdravilom Gazyvaro ni raziskana in cepljenje z živimi virusnimi cepivi med zdravljenjem in do okrevanja celic B ni priporočljivo. *Izpostavljenost obinutuzumabu in utero in cepljenje novorojenčkov z živimi virusnimi cepivi:* Zaradi možnega zmanjšanega števila celic B pri novorojenčkih po izpostavljenosti obinutuzumabu med nosečnostjo je treba pri njih kontrolirati njihovo število. Cepljenja z živimi virusnimi cepivi je treba odložiti, dokler se število celic B pri dojenčku ne popravi. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Študij medsebojnega delovanja niso izvedli. Farmakokinetično medsebojno delovanje: Obinutuzumab ni substrat, zaviralec ali induktor CYP450, encimov uridindifosfat-glukuroniltransferaze (UGT) ali prenašalcev, kakršen je P-glikoprotein. Zato ni pričakovati farmakokinetičnih medsebojnih delovanj z zdravili, ki se presnavljajo s temi encimskimi sistemi. Farmakodinamično medsebojno delovanje: Zaradi imunosupresivnega učinka obinutuzumaba cepljenje z živimi virusnimi cepivi ni priporočljivo med zdravljenjem in do okrevanja celic B. Obinutuzumab v kombinaciji s klorambucilom lahko poveča delež nevtropenije. **Neželeni učinki:** Najpogostejši neželeni učinek pri prejemnikih zdravila Gazyvaro so bile reakcije, povezane z infuzijo, ki so se med prvim ciklusom zdravljenja pojavile pri večini bolnikov. Incidenca z infundiranjem povezanih simptomov se je nato bistveno zmanjšala: s 65 % med infuzijo prvih 1000 mg zdravila Gazyvaro na manj kot 3 % med poznejšimi infuzijami. V ključni študiji se je nevtropenija pojavila pri 41 % bolnikov in trombocitopenija pri 15 % bolnikov. Med drugimi resnimi neželenimi učinki, zabeleženimi med kliničnim razvojem, so bili sindrom razpada tumorja, srčni dogodki in, zelo redko, PML. Ostali neželeni učinki, zabeleženih z večjo incidenco (razlika ≥ 2 %) med prejemniki kombinacije zdravila Gazyvaro in klorambucila ter med prejemniki klorambucila samega ali kombinacije rituksimaba in klorambucila vseh stopenj: *Zelo pogosti:* anemija, diareja in zvišana telesna temperatura. *Pogosti:* okužba sečil, nazofaringitis, herpes labialis, rinitis, faringitis, ploščatocelični karcinom kože, levkopenija, hiperurikemija, atrijska fibrilacija, hipertenzija, kašelj, zaprtost, alopecija, artralgija, bolečina v hrbtu, mišično-skeletna bolečina v prsnem košu, zmanjšanje števila belih krvnih celic, zmanjšanje števila nevtrofilcev in povečanje telesne mase. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdaji dovoljenja za promet je pomembno. Omogoča namreč stalno spremljanje razmerja med koristmi in tveganji zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Univerzitetni klinični center Ljubljana, Interna klinika, Center za zastrupitve, Zaloška cesta 2, SI-1000 Ljubljana, Faks: + 386 (0)1 434 76 46, e-pošta: farmakovigilanca@kclj.si. **Režim izdaje zdravila:** H **Imetnik dovoljenja za promet:** Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija **Verzija: 2.0/15 Informacija pripravljena:** avgust 2015. 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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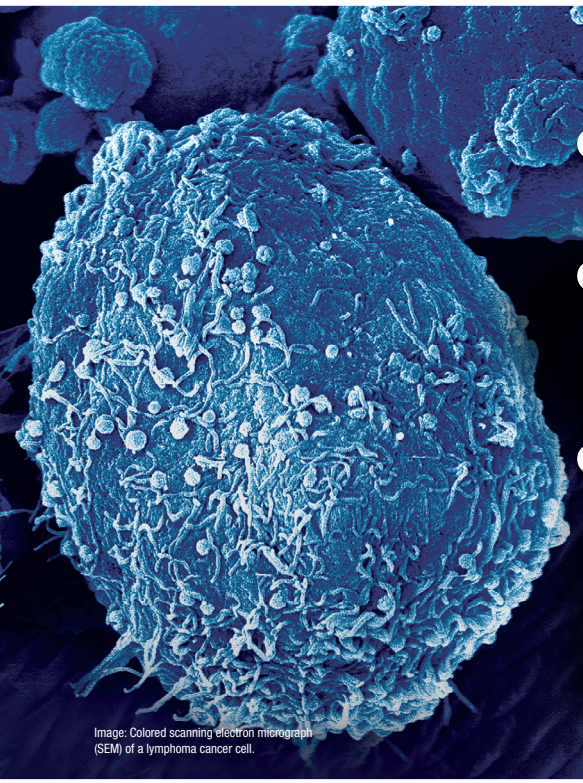

Image: Colored scanning electron micrograph (SEM) of a lymphoma cancer cell.

onko1ogija *cilj*

En cilj: odkrivanje revolucionarnih zdravil za boj proti raku.

onko1ogija *fokus*

En fokus: biti del skupne zaveze za izboljšanje življenja bolnikov z rakom po vsem svetu.

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Skrajšan povzetek glavnih značilnosti zdravila
Adcetris ▼ (brentuksimab vedotin)
Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila (SmPC).

Farmacevtska oblika: 50 mg prašek za koncentrat za raztopino za infundiranje **Terapevtske indikacije:** Zdravljenje odraslih bolnikov s ponovljenim ali z refraktarnim CD30+ Hodgkinovim limfomom (HL) po presaditvi avtologních matičnih celic (ASCT) ali po vsaj dveh predhodnih zdravljenjih, ko ASCT ali večtirna kemoterapija kot obliki zdravljenja ne prideta v poštev. Zdravljenje odraslih bolnikov s ponovljenim ali z refraktarnim sistemskim anaplastičnim velikoceličnim limfomom (sALCL). **Odmerjanje in način uporabe:** Zdravilo Adcetris se mora uporabljati pod nadzorom zdravnika, ki ima izkušnje z uporabo zdravil proti raku. Priporočeni odmerek je 1,8 mg/kg v obliki 30-minutne intravenske infuzije vsake 3 tedne (če je telesna masa bolnika večja od 100 kg, je pri izračunu odmerka treba uporabiti vrednost 100 kg). Zdravljenje se mora nadaljevati do napredovanja bolezni ali pojava nesprejemljivih toksičnih učinkov. Bolniki, pri katerih se bolezen stabilizira ali izboljša, morajo prejeti najmanj 8 ciklov in do največ 16 ciklov. Pred aplikacijo vsakega odmerka tega zdravila je treba preveriti celotno krvno sliko bolnika. Bolnike je treba nadzirati med infundiranjem zdravila in po njem. Za navodila glede rekonstitucije in načina uporabe zdravila glejte poglavje 6.6 v SmPC-ju. **Prilagoditve odmerka:** Če se med zdravljenjem razvije nevtropenija, jo je treba uravnati z odložitvijo odmerka (glejte SmPC). Če se med zdravljenjem pojavi ali poslabša periferna senzorična ali motorična nevropatija, bo odmerek morda treba odložiti ali zmanjšati ali zdravljenje z zdravilom Adcetris prekiniti (glejte SmPC). Okvara ledvic ali jeter: Podatkov o uporabi pri bolnikih z okvaro ledvic ali jeter ni na voljo. Starejši bolniki (≥ 65 let): Podatkov ni na voljo. Pediatrična populacija (< 18 let): Podatkov ni na voljo. V predkliničnih študijah so opazili upadanje priželjca. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katero koli navedeno pomožno snov. Kombinirana uporaba bleomicina in zdravila Adcetris povzroča pljučno toksičnost. **Posebna opozorila in previdnostni ukrepi:** Pri bolnikih, ki so zdravilo Adcetris prejeli po predhodnem zdravljenju z več režimi kemoterapije, so poročali o progresivni multifokalni levkoencefalopatiji (PML). Bolnike je treba skrbno nadzirati glede nastanka novih ali poslabšanja obstoječih nevroloških, kognitivnih ali vedenjskih znakov ali simptomov, ki lahko nakazujejo na PML. V primeru kakršnega koli suma na PML je treba uporabo zdravila Adcetris začasno prekiniti. Če se diagnoza PML potrdi, je treba zdravljenje z zdravilom Adcetris trajno prekiniti. Pri bolnikih, ki so se zdravili z brentuksimabom vedotinom, so poročali o pojavih akutnega pankreatitisa, vključno s smrtnimi izidi. Bolnike je treba skrbno nadzirati glede pojava nove ali poslabšanja že obstoječe bolečine v trebuhu. V primeru potrjene diagnoze akutnega pankreatitisa je treba zdravljenje z brentuksimabom vedotinom trajno ukiniti. Pri bolnikih, ki so se zdravili z brentuksimabom vedotinom, so poročali o primerih toksičnih učinkov na pljuča. Vzročna povezava z brentuksimabom vedotinom sicer ni bila ugotovljena, vendar pa tveganja toksičnosti za pljuča ni mogoče izključiti. V primeru pojava novih ali poslabšanja že obstoječih pljučnih simptomov (npr. kašelj, dispneja) je nemudoma treba izvesti ustrezne diagnostične postopke in bolnika ustrezno zdraviti. Poročali so o resnih okužbah, kot so pljučnica, stafilokokna bakteriemija, sepsa/septični šok (vključno s smrtnimi izidi) in herpes zoster ter o oportunističnih okužbah, kot so pljučnica in oralna kandidoza. Bolnike je treba med zdravljenjem skrbno nadzirati glede pojava možnih resnih in oportunističnih okužb. Poročali so tako o takojšnjih in zakasnelih reakcijah, povezanih z infundiranjem, kot o anafilaktičnih reakcijah. Bolnike je treba med infundiranjem in po njem skrbno nadzirati. Če se pojavi anafilaktična reakcija, je treba dajanje zdravila Adcetris takoj in trajno ukiniti. Če se pojavijo reakcije, povezane z infundiranjem, je treba z infundiranjem prenehati (glejte SmPC). Pri bolnikih s hitro proliferacijskimi tumorji in z veliko tumorsko obremenitvijo obstaja tveganje za sindrom tumorske lize. Te bolnike je treba skrbno nadzirati in zdraviti po najboljši medicinski praksi. Zdravilo Adcetris lahko povzroči periferno nevropatijo, ki je v večini primerov reverzibilna. Bolnike je treba nadzirati glede simptomov nevropatije. Pri bolnikih, pri katerih se pojavi nova ali poslabša obstoječa periferna nevropatija, bo morda treba odmerek zdravila Adcetris odložiti ali zmanjšati ali zdravljenje prekiniti (glejte SmPC). Pri zdravljenju z zdravilom Adcetris se lahko pojavijo anemija, trombocitopenija in nevtropenija 3. ali 4. stopnje. Za prilagoditev odmerka v primeru nevtropenije glejte SmPC. Bolnike je treba skrbno nadzirati glede pojava zvišane telesne temperature. Če se pojavi febrilna nevtropenija, jo je treba zdraviti po najboljši medicinski praksi. Če se pojavi Stevens-Johnsonov sindrom ali toksična epidermalna nekroliza, je treba zdravljenje z zdravilom Adcetris prekiniti in uvesti ustrezno zdravljenje. Poročali so o zvišanih vrednostih ALT in AST. Pri bolnikih, ki prejemajo brentuksimab vedotin, je treba jetrno funkcijo rutinsko spremljati. Pri bolnikih, pri katerih se pojavi hiperglikemija, je treba skrbno spremljati vrednost glukoze v serumu in po potrebi uvesti ustrezno zdravljenje. Zmerna ali huda okvara ledvic in nizke koncentracije albumina v serumu lahko vplivajo na očistek MMAE (glejte SmPC). Zdravilo Adcetris vsebuje največ 2,1 mmol (ali 47 mg) natrija na odmerek. Plodnost, nosečnost in dojenje: Podatkov o uporabi zdravila med nosečnostjo ni. Študije na živalih so pokazale vpliv na sposobnost razmnoževanja. Ženske v rodni dobi morajo med zdravljenjem in še 6 mesecev po njem uporabiti dve obliki učinkovite kontracepcije. Ni podatkov o tem, ali se brentuksimab vedotin ali njegovi presnovki izločajo v materino mleko. Tveganja za dojenega novorojenca/otroka ne moremo izključiti. Plodnost: V predkliničnih študijah je zdravljenje z zdravilom Adcetris povzročilo testikularno toksičnost, lahko pa bi vplivalo tudi na plodnost moških. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Pri sočasni uporabi zdravila Adcetris in močnih zaviralcev CYP3A4 in P-gp, kot je ketokonazol, se lahko poveča pojavnost nevtropenije. Pri sočasni uporabi rifampicina, močnega induktorja CYP3A4, se plazemska izpostavljenost zdravilu Adcetris ni spremenila, vendar pa se je izpostavljenost MMAE zmanjšala. Pri sočasni uporabi midazolama, substrata za CYP3A4, se presnova midazolama ni spremenila, zato se ne pričakuje, da bi se pri sočasni uporabi zdravila Adcetris spremenila izpostavljenost zdravilom, ki se presnavljajo z encimi CYP3A4. **Neželeni učinki:** *Zelo pogosti (> 10 %):* Okužba, nevtropenija, periferna senzorična nevropatija, driska, navzea, bruhanje, alopecija, srbenje, mialgija, utrujenost, zvišana telesna temperatura in reakcije, povezane z infundiranjem. *Pogosti (≥ 1/100 do < 1/10):* Sepsa/septični šok, okužba zgornjih dihal, herpes zoster, pljučnica, anemija, trombocitopenija, hiperglikemija, periferna motorična nevropatija, omotica, demielinizacijska polinevropatija, kašelj, dispneja, zaprtost, zvišanje vrednosti ALT/AST, izpuščaj, artralgija, bolečina v hrbtu in mrzlica. *Občasni (≥ 1/1.000 do < 1/100):* Oralna kandidoza, pljučnica, ki jo povzroča *Pneumocystis jiroveci*, stafilokokna bakteriemija, sindrom tumorske lize in akutni pankreatitis. *Redki (≥ 1/10.000 do 1/1.000):* StevensJohnsonov sindrom in toksična epidermalna nekroliza. *Neznana (pogostnosti iz razpoložljivih podatkov ni mogoče oceniti):* Progresivna multifokalna levkoencefalopatija, febrilna nevtropenija in anafilaktična reakcija. *Resni neželeni učinki zdravila pri bolnikih v študijah 2. faze so bili:* nevtropenija, trombocitopenija, zaprtost, driska, bruhanje, zvišana telesna temperatura, periferna motorična in periferna senzorična nevropatija, hiperglikemija, demielinizacijska polinevropatija, sindrom tumorske lize in Stevens-Johnsonov sindrom. **Posebna navodila za shranjevanje:** Vialo shranjujte v hladilniku (2 °C-8 °C), zaščiteno pred svetlobo. Po rekonstituciji/redčenju je raztopina dokazano kemično in fizikalno stabilna 24 ur pri temperaturi od 2 °C do 8 °C. **Datum revizije besedila:** Januar 2015. Način izdajanja zdravila: Predpisovanje in izdaja zdravila je le na recept. **Številka dovoljenja za promet z zdravilom:** EU/1/12/794/001. **Ime in naslov imetnika dovoljenja za promet z zdravilom:** Takeda Pharma A/S, Dybendal Alle 10, 2630 Taastrup, Danska. **Dodatne informacije so na voljo pri:** Takeda GmbH, Podružnica Slovenija, Dalmatinova ul. 2, Ljubljana, tel: 059 082 480.

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Za podroben profil neželenih učinkov zdravila Adcetris in medsebojno delovanje z drugimi zdravili glejte celoten povzetek glavnih značilnosti zdravila. O katerem koli domnevnem neželenem učinku zdravila poročajte 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.

Samo za strokovno javnost.

SI/BRE/1507/0002, avgust 2015

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Dent RAG, Cole P. In vitro maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95.
Chapman S, Nakielny R. *A guide to radiological procedures*. London: Bailliere Tindall; 1986.
Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by
macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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kapsule
SUTENT®
sunitinibijev malat

Vsak dan šteje

za bolnike z napredovalim karcinomom ledvičnih celic

28. september	15. december	**30. april**	2. avgust
Jesenski festival	Zimske počitnice	**Družinsko srečanje**	Začetek kuharskega tečaja

BISTVENI PODATKI IZ POVZETKA GLAVNIH ZNAČILNOSTI ZDRAVILA

SUTENT 12,5 mg, 25 mg, 37,5 mg, 50 mg trde kapsule
Sestava in oblika zdravila: Ena kapsula vsebuje 12,5 mg, 25 mg, 37,5 mg ali 50 mg sunitiniba (v obliki sunitinibijevega malata). **Indikacije:** Zdravljenje neizrezljivega in/ali metastatskega malignega gastrointestinalnega stromalnega tumorja (GIST) pri odraslih, če zdravljenje z imatinibom zaradi odpornosti ali neprenašanja ni bilo uspešno. Zdravljenje napredovalega/metastatskega karcinoma ledvičnih celic (MRCC) pri odraslih. Zdravljenje neizrezljivih ali metastatskih, dobro diferenciranih nevroendokrinih tumorjev trebušne slinavke (pNET), kadar gre za napredovanje bolezni pri odraslih (izkušnje z zdravilom Sutent kot zdravilom prve izbire so omejene). **Odmerjanje in način uporabe:** Terapijo mora uvesti zdravnik, ki ima izkušnje z uporabo zdravil za zdravljenje rakavih bolezni. *GIST in MRCC:* Priporočeni odmerek je 50 mg peroralno enkrat na dan, 4 tedne zapored; temu sledi 2-tedenski premor (Shema 4/2), tako da celotni ciklus traja 6 tednov. *pNET:* Priporočeni odmerek je 37,5 mg peroralno enkrat na dan, brez načrtovanega premora. *Prilagajanje odmerka*: Odmerek je mogoče prilagajati v povečanjih po 12,5 mg, upoštevaje individualno varnost in prenašanje. Pri GIST in MRCC dnevni odmerek ne sme preseči 75 mg in ne sme biti manjši od 25 mg; pri pNET je največji odmerek 50 mg na dan, z možnimi prekinitvami zdravljenja. Pri sočasni uporabi z močnimi zaviralci ali induktorji CYP3A4 je treba odmerek ustrezno prilagoditi. *Pediatrična populacija:* Uporaba sunitiniba ni priporočljiva. *Starejši bolniki (≥ 65 let):* Med starejšimi in mlajšimi bolniki niso opazili pomembnih razlik v varnosti in učinkovitosti. *Okvara jeter:* Pri bolnikih z jetrno okvaro razreda A in B po Child-Pughu prilagoditev odmerka ni potrebna; pri bolnikih z okvaro razreda C sunitinib ni bil preizkušen, zato njegova uporaba ni priporočljiva. *Okvara ledvic:* Prilagajanje začetnega odmerka ni potrebno, nadaljnje prilagajanje odmerka naj temelji na varnosti in prenašanju pri posameznem bolniku. *Način uporabe:* Zdravilo Sutent se uporablja peroralno, bolnik ga lahko vzame s hrano ali brez nje. Če pozabi vzeti odmerek, ne sme dobiti dodatnega, temveč naj vzame običajni predpisani odmerek naslednji dan. **Kontraindikacije:** Preobčutljivost na zdravilno učinkovino ali katerokoli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** *Bolezni kože in tkiv:* obarvanje kože, gangrenozna pioderma (običajno izgine po prekinitvi zdravljenja), hude kožne reakcije (multiformni eritem (EM), Stevens-Johnsonov sindrom (SJS) in toksična epidermalna nekroliza (TEN)). Če so prisotni znaki EM, SJS ali TEN, je treba zdravljenje prekiniti. *Krvavitve* v prebavilih, dihalih, sečilih, možganih; najpogosteje epistaksa; krvavitve tumorja, včasih s smrtnim izidom. Pri bolnikih, ki se sočasno zdravijo z antikoagulanti, se lahko redno spremlja celotna krvna slika (trombociti), koagulacijski faktorji (PT / INR) in opravi telesni pregled. *Bolezni prebavil:* poleg diareje, navzee/bruhanja, bolečine v trebuhu, dispepsije, stomatitisa/bolečine v ustih in ezofagitisa tudi hudi zapleti (včasih s smrtnim izidom), vključno z gastrointestinalno perforacijo. *Hipertenzija:* pri bolnikih s hudo hipertenzijo, ki je ni mogoče urediti z zdravili, je priporočljivo začasno prenehanje zdravljenja. *Hematološke bolezni:* zmanjšanje števila nevtrofilcev, trombocitov, anemija. *Bolezni srca in ožilja:* srčno-žilni dogodki, vključno s srčnim popuščanjem, kardiomiopatijo, miokardno ishemijo in miokardnim infarktom, v nekaterih primerih s smrtnim izidom; sunitinib povečuje tveganje za pojav kardiomiopatije; previdna uporaba pri bolnikih s tveganjem za te dogodke, ali ki so te dogodke imeli v preteklosti. *Podaljšanje intervala QT:* previdna uporaba pri bolnikih z znano anamnezo podaljšanja intervala QT, tistih, ki jemljejo antiaritmike ali zdravila, ki lahko podaljšajo interval QT, in tistih z relevantno, že obstoječo srčno boleznijo, bradikardijo ali elektrolitskimi motnjami. *Venski in arterijski trombembolični dogodki*; arterijski včasih s smrtnim izidom. *Trombotična mikroangiopatija (TMA)*: TMA, vključno s trombotično trombocitopenično purpuro in hemolitično-uremičnim sindromom, v nekaterih primerih z odpovedjo ledvic ali smrtnim izidom. *Dogodki na dihalih:* dispneja, plevralni izliv, pljučna embolija ali pljučni edem; redki primeri s smrtnim izidom. *Moteno delovanje ščitnice:* bolnike je treba med zdravljenjem rutinsko spremljati glede delovanja ščitnice vsake 3 mesece. *Pankreatitis*, tudi resni primeri s smrtnim izidom. *Hepatotoksičnost*, nekateri primeri s smrtnim izidom. *Holecistitis*, vključno z akalkuloznim in emfizemskim holecistitisom. *Delovanje ledvic:* primeri zmanjšanega delovanja ledvic, odpovedi ledvic in/ali akutne odpovedi ledvic, v nekaterih primerih s smrtnim izidom. *Fistula:* če nastane fistula, je treba zdravljenje s sunitinibom prekiniti. *Oteženo celjenje ran:* pri bolnikih, pri katerih naj bi bil opravljen večji kirurški poseg, je priporočljiva začasna prekinitev zdravljenja s sunitinibom. *Osteonekroza čeljustnic:* pri sočasnem ali zaporednem dajanju zdravila Sutent in intravenskih bisfosfonatov je potrebna previdnost; invazivni zobozdravstveni posegi predstavljajo dodatni dejavnik tveganja. *Preobčutljivost/angioedem*. *Motnje okušanja*. *Konvulzije:* obstajajo poročila, nekatera s smrtnim izidom, o preiskovancih s konvulzijami in radiološkimi znaki sindroma reverzibilne posteriorne levkoencefalopatije. *Sindrom lize tumorja*, v nekaterih primerih s smrtnim izidom. *Okužbe:* hude okužbe z ali brez nevtropenije (okužbe dihal, sečil, kože in sepsa), vključno z nekaterimi s smrtnim izidom; redki primeri nekrotizitajočega fasciitisa, vključno s prizadetostjo presredka, ki so bili včasih smrtni. *Hipoglikemija*: če se pojavi simptomatska hipoglikemija, je treba zdravljenje s sunitinibom začasno prekiniti. Pri sladkornih bolnikih je treba redno preverjati raven glukoze v krvi in, če je treba, prilagoditi odmerek antidiabetika. **Medsebojno delovanje z drugimi zdravili:** (Študije so izvedli le pri odraslih.) Zdravila, ki lahko zvečajo koncentracijo sunitiniba v plazmi (ketokonazol, ritonavir, itrakonazol, eritromicin, klaritromicin ali sok grenivke). Zdravila, ki lahko zmanjšajo koncentracijo sunitiniba v plazmi (deksametazon, fenitoin, karbamazepin, rifampin, fenobarbital, *Hypericum perforatum* oz. šentjanževka). **Plodnost, nosečnost in dojenje:** Zdravila Sutent ne smemo uporabljati med nosečnostjo in tudi ne pri ženskah, ki ne uporabljajo ustrezne kontracepcije, razen če možna korist odtehta možno tveganje za plod. Ženske v rodni dobi naj med zdravljenjem z zdravilom Sutent ne zanosijo. Ženske, ki jemljejo zdravilo Sutent, ne smejo dojiti. Neklinični izsledki kažejo, da lahko zdravljenje s sunitinibom poslabša plodnost samcev in samic. **Vpliv na sposobnost vožnje in upravljanja s stroji:** Sutent lahko povzroči omotico. **Neželeni učinki:** Najbolj resni neželeni učinki (nekateri s smrtnim izidom) so: odpoved ledvic, srčno popuščanje, pljučna embolija, gastrointestinalna perforacija in krvavitve (npr. v dihalih, prebavilih, tumorju, sečilih in možganih). Najpogostejši neželeni učinki (ki so se pojavili pri vsaj 20 % bolnikov v registracijskih preskušanjih) so: zmanjšan tek, motnje okušanja, hipertenzija, utrujenost, prebavne motnje (npr. driska, navzea, stomatitis, dispepsija in bruhanje), sprememba barve kože in sindrom palmarno-plantarne eritrodisestezije. Med najbolj pogostimi neželenimi učinki so tudi hematološke motnje (nevtropenija, trombocitopenija, anemija in levkopenija). Ostali zelo pogosti (≥ 1/10) neželeni učinki so: hipotiroidizem, nespečnost, omotica, glavobol, dispneja, epistaksa, kašelj, bolečina v trebuhu, zaprtje, obarvanje kože, izpuščaj, spremembe barve las, suha koža, bolečine v udih, artralgija, bolečine v hrbtu, vnetje sluznice, edem, pireksija. **Način in režim izdaje:** Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Izjemoma se lahko uporablja pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. **Imetnik dovoljenja za promet:** Pfizer Limited, Ramsgate Road, Sandwich, Kent, CT13 9NJ, Velika Britanija. **Datum zadnje revizije besedila:** 25.06.2015
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SUT-03-15 Samo za strokovno javnost

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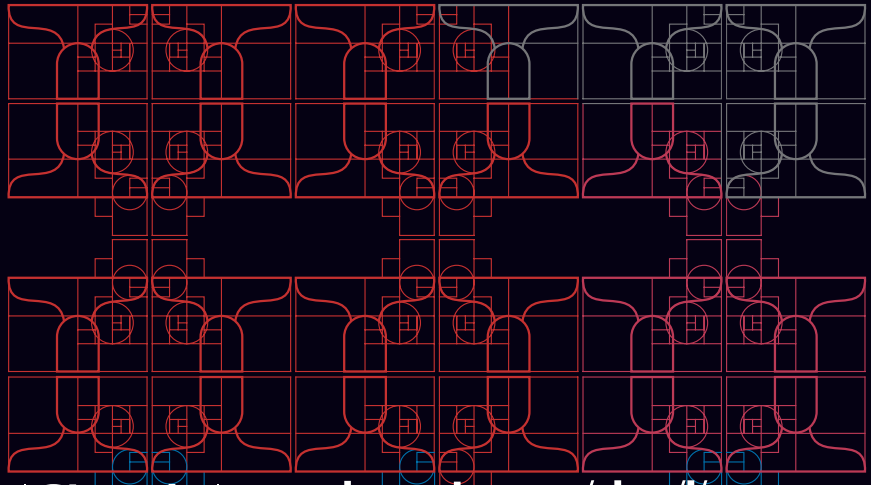

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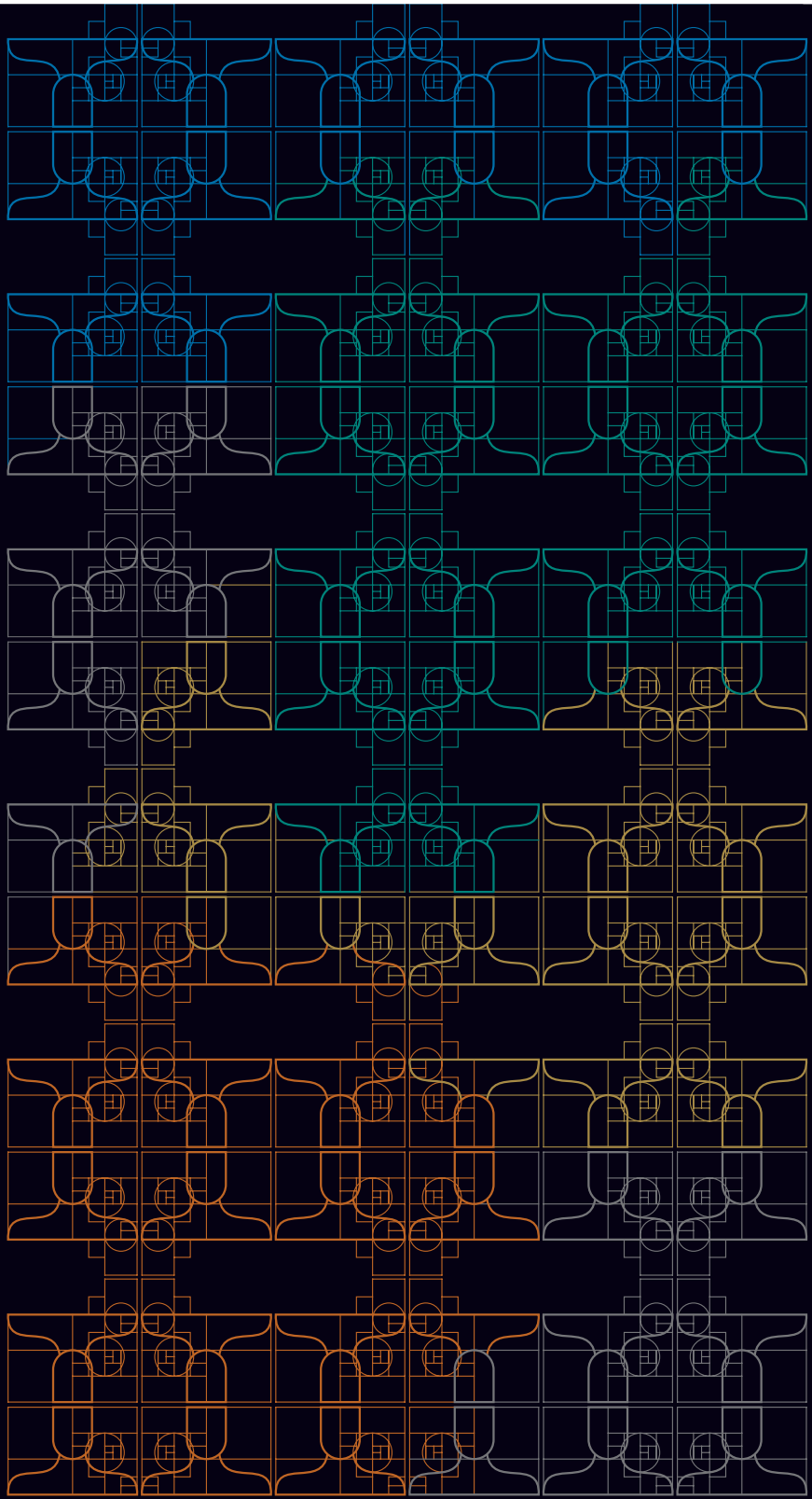

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