RADIOLOGY AND ONCOLOGY

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NEVROENDOKRINI TUMORJI

Somatuline® Autogel® je dolgodelujoči somatostatinski analog, ki si ga bolniki z nevroendokrinimi tumorji lahko injicirajo sami.[1,2]*

Somatuline® autogel®
lanreotid

* Bolniki, ki imajo predpisan vzdrževalni odmerek zdravila Somatuline® Autogel® ter so ustrezno usposobljeni, si lahko zdravilo injicirajo sami ali pa jim ga injicira druga ustrezno usposobljena oseba. Odločitev o tem, če si bo bolnik po ustreznem predhodnem urjenju zdravilo injiciral sam, ali mu ga bo injicirala druga ustrezno usposobljena oseba, mora sprejeti zdravnik.

Reference:
1. Povzetek glavnih značilnosti zdravila Somatuline Autogel
2. Navodila za uporabo zdravila Somatuline Autogel

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Somatuline Autogel 60 mg raztopina za injiciranje v napolnjeni injekcijski brizgi
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(lanreotid)

TERAPEVTSKE INDIKACIJE Dolgotrajno zdravljenje bolnikov z akromegalijo, če se raven cirkulirajočega rastnega hormona (GH) in/ali raven insulinu podobnega rastnega faktorja-I (IGF-I) po kirurškem posegu in/ali obsevanju ne normalizira, ali pri bolnikih, pri katerih kirurški poseg in/ali obsevanje ne prideta v poštev. Lajšanje simptomov, povezanih z akromegalijo. Zdravljenje simptomov, povezanih z nevroendokrinimi tumorji prebavil. Zdravljenje gastroenteropankreatičnih nevroendokrinih tumorjev (GEP-NET) stopnje 1 in podskupine stopnje 2 (indeks Ki-67 do 10 %) srednjega črevesa, trebušne slinavke ali neznanega izvora, če je mesto izvora v zadnjem črevesu izključeno, pri odraslih bolnikih z neoperabilno lokalno napredovalo ali metastatsko boleznijo. **ODMERJANJE IN NAČIN UPORABE** *Akromegalija:* Priporočeni začetni odmerek je od 60 do 120 mg na vsakih 28 dni. Odmerek zdravila je nato treba prilagoditi glede na dosežen odziv pri vsakem bolniku posebej. Če želeni odziv ni dosežen, se odmerek lahko poveča. Če je dosežen popoln nadzor, se odmerek lahko zmanjša. Bolniki, katerih bolezen je z uporabo somatostatinskih analogov dobro nadzorovana, se lahko zdravijo s Somatulinom Autogelom 120 mg na vsakih 42 do 56 dni. Simptome, raven GH in raven IGF-I je treba nadzirati dalje obdobje. *Simptomi, povezani z nevroendokrinimi tumorji prebavil:* Priporočeni začetni odmerek je od 60 do 120 mg na vsakih 28 dni. Odmerek je treba prilagoditi glede na doseženo stopnjo lajšanja simptomov. Bolniki, katerih bolezen je ob uporabi somatostatinskih analogov dobro nadzorovana, se lahko zdravijo s Somatulinom Autogelom 120 mg na vsakih 42 do 56 dni. *Zdravljenje GEP-NET stopnje 1 in podskupine stopnje 2 srednjega črevesa, trebušne slinavke ali neznanega izvora, če je mesto izvora v zadnjem črevesu izključeno, pri odraslih bolnikih z neoperabilno lokalno napredovalo ali metastatsko boleznijo:* Začetni odmerek je 1 injekcija Somatulina Autogela 120 mg na vsakih 28 dni. Zdravljenje mora trajati, kolikor je to potrebno za obvladovanje tumorja. Bolniki z okvaro ledvic in/ali jeter in starostniki: Odmerka ni treba prilagajati. Varnost in učinkovitost Somatulina Autogela pri otrocih in mladostnikih nista bili dokazani. Somatuline Autogel je treba vzeti iz hladilnika 30 minut pred injiciranjem. Aplicirati ga je treba v obliki globoke podkožne injekcije v zgornji zunanji kvadrant zadnjice ali v zgornji zunanji predel stegna. Če si bolnik zdravilo injicira sam, ga mora injicirati v zgornji zunanji predel stegna. Ne glede na mesto injiciranja se kože ne sme stiskati v gubo. Celotno dolžino igle je treba na hitro zabosti navpično v kožo. Zdravilo je treba injicirati izmenično na desno in levo stran. **KONTRAINDIKACIJE** Preobčutljivost na učinkovino, somatostatin ali sorodne peptide ali katerokoli pomožno snov. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI** Lanreotid lahko zmanjša gibljivost žolčnika in povzroči tvorbo žolčnih kamnov, zato je treba bolnike periodično spremljati. Če obstaja sum za zaplete zaradi holelitiaze, je treba prenehati zdravljenje z lanreotidom in uvesti ustrezno zdravljenje. Lanreotid zavira izločanje insulina in glukagona. Ob uvedbi zdravljenja in pri vsaki spremembi odmerka je treba nadzirati vrednosti glukoze v krvi. Morebitno antidiabetično zdravljenje je treba ustrezno prilagoditi. Med zdravljenjem z lanreotidom so pri bolnikih z akromegalijo opazili blago zmanjšanje delovanja ščitnice. Pri bolnikih brez osnovne bolezni srca lahko lanreotid povzroči počasnejše bitje srca, pri čemer pa ni nujno, da je dosežena meja za bradikardijo. Pri bolnikih, ki imajo bolezen srca že pred uvedbo zdravljenja z lanreotidom, se lahko pojavi sinusna bradikardija. *Nosečnost in dojenje:* Uporaba pri nosečnicah le, če je to nujno potrebno. Lanreotid je med dojenjem treba uporabljati previdno. **INTERAKCIJE** Zaradi farmakoloških učinkov lanreotida na prebavila se lahko zmanjša absorpcija drugih sočasno uporabljenih zdravil iz črevesa. Pri sočasni uporabi ciklosporina in lanreotida se lahko zmanjša relativna biološka uporabnost ciklosporina. Morda bo treba prilagoditi odmerek ciklosporina. Pri sočasni uporabi somatostatinskih analogov in bromokriptina se lahko poveča biološka uporabnost bromokriptina. Pri sočasni uporabi zdravil, ki povzročajo bradikardijo, se lahko pojavi aditivni učinek lanreotida na blago zmanjšanje srčnega utripa. Odmerek teh zdravil bo morda treba prilagoditi. Zdravila, ki se v glavnem presnavljajo s CYP3A4 in imajo ozek terapevtski indeks, je treba v kombinaciji z lanreotidom uporabljati previdno. **NEŽELENI UČINKI** Za popolno informacijo o neželenih učinkih, prosimo, preberite celoten povzetek glavnih značilnosti zdravila Somatuline Autogel. *Zelo pogosti (≥ 1/10):* driska, mehko blato, bolečina v trebuhu, holelitiaza. *Pogosti (≥ 1/100 do < 1/10):* hipoglikemija, zmanjšanje apetita, hiperglikemija, sladkorna bolezen, omotica, glavobol, letargija, sinusna bradikardija, navzea, bruhanje, zaprtost, flatulenca, napihnjenost trebuha, neugodje v trebuhu, dispepsija, steatoreja, dilatacija biliarnega trakta, mišično-skeletna bolečina, bolečina v mišicah, alopecija, hipotrihoza, astenija, utrujenost, reakcije na mestu injiciranja (bolečina, bula, zatrdlina, vozlič, srbenje), zvišana vrednost ALT, nenormalna vrednost AST, nenormalna vrednost ALT, zvišana vrednost bilirubina v krvi, zvišana vrednost glukoze v krvi, zvišana vrednost glikiranega hemoglobina, zmanjšanje telesne mase, zmanjšana vrednost pankreatičnih encimov. *Občasni (≥ 1/1.000 do < 1/100):* nespečnost, vročinski oblivi, sprememba barve blata, zvišana vrednost AST, zvišana vrednost alkalne fosfataze v krvi, nenormalna vrednost bilirubina v krvi, zmanjšana vrednost natrija v krvi. Vrsta ovojnine in vsebina: škatla z eno napolnjeno injekcijsko brizgo in eno iglo. Režim izdaje: Rp/Spec Imetnik dovoljenja za promet: Ipsen Pharma, 65 Quai Georges Gorse, 92100 Boulogne Billancourt, Francija
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Radiology and Oncology is a multidisciplinary journal devoted to the publishing original and high quality scientific papers and review articles, pertinent to diagnostic and interventional radiology, computerized tomography, magnetic resonance, ultrasound, nuclear medicine, radiotherapy, clinical and experimental oncology, radiobiology, medical physics and radiation protection. Therefore, the scope of the journal is to cover beside radiology the diagnostic and therapeutic aspects in oncology, which distinguishes it from other journals in the field.

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Socioeconomic inequalities in cancer incidence in Europe: a comprehensive review of population-based epidemiological studies

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Background. Since the end of the previous century, there has not been a comprehensive review of European studies on socioeconomic inequality in cancer incidence. In view of recent advances in data source linkage and analytical methods, we aimed to update the knowledge base on associations between location-specific cancer incidence and individual or area-level measures of socio-economic status (SES) among European adults.

Materials and methods. We systematically searched three databases (*PubMed, Scopus* and *Web of Science*) for articles on cancer incidence and SES. Qualitative synthesis was performed on the 91 included English language studies, published between 2000 and 2019 in Europe, which focused on adults, relied on cancer registry data and reported on relative risk (RR) estimates.

Results. Adults with low SES have increased risk of head and neck, oesophagogastric, liver and gallbladder, pancreatic, lung, kidney, bladder, penile and cervical cancers (highest RRs for lung, head and neck, stomach and cervix). Conversely, high SES is linked with increased risk of thyroid, breast, prostate and skin cancers. Central nervous system and haematological cancers are not associated with SES. The positive gap in testicular cancer has narrowed, while colorectal cancer shows a varying pattern in different countries. Negative associations are generally stronger for men compared to women.

Conclusions. In Europe, cancers in almost all common locations are associated with SES and the inequalities can be explained to a varying degree by known life-style related factors, most notably smoking. Independent effects of many individual and area SES measures which capture different aspects of SES can also be observed.

Key words: socioeconomic status; socioeconomic inequality; cancer incidence; adults; Europe; cancer registry; relative risk

Introduction

Health and disease are not distributed equally and often also not equitably. This has been observed since ancient times by prominent historical figures, such as Hippocrates (or else his contemporaries), who pointed out that higher social standing (power, wealth, freedom, etc.) was reflected in better health[1], German physician Johann Peter Frank, a pioneer in public health who held the view that misery of the common people was the mother of disease[2], and Louis René Villermé, who in 19th century France combined census and mortality data and used this innovative way to show that disease distribution and life expectancy were associated with the distribution of poverty in terms of occupational class[3,4], to name but a few.

When it comes to cancer, some of the earliest studies investigating social inequalities in Europe date back over a century. It was firmly established by then that certain occupations were undoubtedly associated with the development of malignancies – lung cancer in miners and scrotal cancer in chimney sweeps being the most notorious – but links

between social class and cancer were only starting to be explored. There were already observations that social classes differ with respect to cancer rates and mortality. According to some early researches, cancer on the whole was considered a disease of affluence[5], while others found the opposite[6,7], with differences probably being the result of unrefined, developing methodology. Later, when specific cancer types, most commonly gynaecological cancers in women, were investigated, clear differences were seen between classes, such that cervical and uterine cancers were found more frequently among poor while breast cancer was more common among wealthy women.[8]

A century or so after the first studies, in 1997 the International Agency for Research on Cancer (IARC) gathered available evidence from numerous epidemiological studies on the association between socioeconomic status (SES) and cancer morbidity and mortality which were considered to stem from associations between SES and cancer risk factors.[2] A 2019 IARC report responded to the knowledge gap for medium and low-income countries and addressed the importance of political will and know-how to reduce inequalities that are consistently found throughout the world.[9] Since the original IARC report, to our knowledge no detailed review of cancer incidence in adults and SES in Europe has been published. In the intervening time, many new approaches for investigating inequalities have been developed, notably linkage methods that are increasingly used to combine data from many different databases with complete and accurate information on important socioeconomic variables, such as education, occupation and income. At the same time, methods have evolved which use area-based deprivation indices in determining how neighbourhoods influence the risk of cancer among their residents.[9] Finally, cancer inequalities should be viewed as dynamic instead of static, because the magnitude and direction of disparities can change in tandem with socially driven changes in determinants of health and disease.[4]

The aim of our work was thus to comprehensively review studies that have assessed the direction and magnitude of socioeconomic inequalities in location-specific cancer incidence among European adults in the 21st century. We specifically focused on studies that utilised population-based cancer registry data linked to individual or area-level measures of SES. As a result, a large burden of disease that could potentially be attributed to differences in SES in Europe is highlighted.

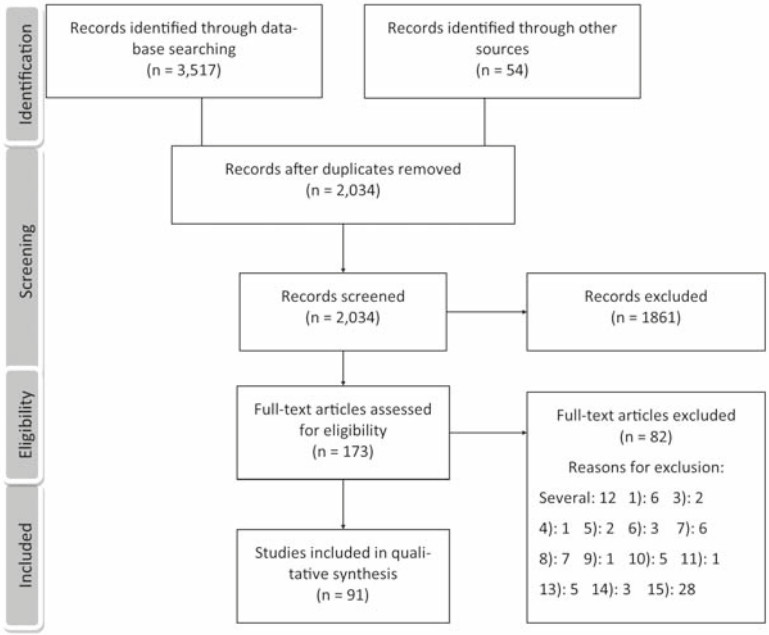

FIGURE 1. The PRISMA diagram detailing the study selection process and results. Reasons for exclusion of full-text articles are indicated by numbers as they feature and are explained in the text under the subheading Search strategy and inclusion/exclusion criteria.

Methodology

Search strategy and inclusion/exclusion criteria

Pursuant to the main aim of our work and following the PRISMA guidelines[10], in July 2019 we systematically searched three databases (*PubMed*, *Scopus* and *Web of Science*) for articles investigating the relationship between cancer incidence in adults in Europe and SES, operationalised either on an individual or area level, that relied (at least in part) on cancer registry/database data. The search strategy was thus constructed by combining six different main search terms with the Boolean operator 'AND' while using 'OR' for individual terms' synonyms. The computer-assisted searches were designed and performed by a research librarian. The main terms were: cancer, incidence, socioeconomic status, cancer registry, adults and Europe (for the full search strategy, refer to Supplementary table 1). European countries were defined according to the United Nations' definition of world regions, while any country that was a member of the European Network of Cancer Registries (ENCR) was additionally included. We searched in titles and abstracts of English-language articles pub-

lished between 2000 and 2019. The search was updated in December 2019.

The PRISMA diagram detailing the study selection process and results is presented in Figure 1. First, duplicates were removed. After that two authors (AM and ST) independently selected articles for further reading based on their title and abstracts. Exclusion criteria at this stage (Screening in Figure 1) were: (1) the article was a review or a meta-analysis that included already identified studies; (2) the abstract did not include a description of methods used to assess the relationship between any SES measure (e.g. education, income, occupational social class, housing or other material determinant of SES, area deprivation) and incidence of any cancer, or did not at least mention that one of the goals/results was assessment of this relationship; (3) the study investigated exclusively all cancers combined; (4) the study population did not include adults; (5) the focus was comparison of two or more larger regions/countries; (6) the focus was on comparing rural and urban regions or else analysis by population density; (7) the study assessed primarily occupational risk or exposure through stratifying participants by type of occupation only (and not by occupational class); (8) the focus was on comparing immigrants and native-born or by ethnicity; (9) the study exclusively analysed incidence by marital status or cohabitation; (10) the study focused on SES-specific risk of advanced disease or primarily on the influence of screening (i.e. analysis of inequalities in detection rather than incidence of cancer was the main research goal); (11) the data on cancer was evidently not from a European country; (12) secondary (and not primary) cancers were analysed; (13) exploration of methodological issues was the main goal; (14) the study did not at all rely on data from cancer registries/databases.

If at least one author considered an article should be read in full, it was included in the list for full-text reading. In the next stage, two authors independently read each article in full to assess whether it should be included in the final selection. If there was uncertainty, a third author's opinion (VZ) was sought. Read articles were screened again using the above criteria, while a further exclusion requirement was also assessed (Eligibility in Figure 1). Namely, (15) if studies did not report results in terms of relative risk (RR) estimate between groups of SES, either as risk ratio, incidence rate ratio, odds ratio, age-standardised incidence rate ratio, standardised incidence ratio, relative index of inequality, standardised rate ratio, hazard ratio or similar. In addition, a snowball approach was used whereby reference lists of articles that were read in full were searched manually for eligible records and conversely, articles that referenced the studies included in the final selection were manually examined. Several English-language cancer registry/database websites were also searched for relevant literature (reported under Identification in Figure 1).

Study data extraction and results synthesis

After the articles for final inclusion were selected, we extracted from studies the following data: first author's full last name and first name initials; article title; studied country/-ies; journal (if available); publication year; study type, period and population; investigated cancer(s) with ICD or ICD-O codes (if available); SES indicator (and its level – individual/area); analysis methods and inequality measures; adjustment/stratification factors; possible study limitations; and main research findings.

Descriptive methods were used to report on the synthesis of research results regarding associations between different measures of SES and many different cancers in Europe. To examine more closely the gap between highest and lowest SES, we compiled cancer location-specific tables with RR estimates for the lowest compared to highest SES category. RR estimates were, when necessary, transformed so that highest SES was always the reference group, except in instances where SIR was calculated with reference to the whole population. The extracted information is provided comprehensively in the Supplementary tables 2–29, stratified by cancer site and by the type of the SES measure applied (individual or area-level).

Results and discussion

Lung cancer

Globally, lung cancer remains the most common cancer in absolute number of new cases[11] and 3rd in Europe where estimated age-standardised rates in men are roughly twice as high compared to women.[12] Smoking is the most important contributing cause of lung cancer and smoking rates vary significantly by SES. A vast amount of information on lung cancer and SES is available from across Europe that overwhelmingly points to increased risk with lower affluence, especially in men.[13-35] With respect to individual level SES (Supplementary table

2), lowest education was associated with a more than threefold risk in certain studies (RR for men and women generally around 1.8 and 1.5, respectively).[13,14,23-25,31-33,36] Very few studies have failed to confirm an increased risk, and only for women.[29,30] Occupational social class, the second most studied indicator of SES, is also a more prominent factor for men.[13,29,35] Comparatively few studies have attempted to evaluate the effect of material components of SES through income, housing tenure or characteristics and car ownership with similar or only slightly greater RR estimates in men compared to women.[13,15,29] Studies relying on area-level SES as a proxy for individual SES (Supplementary table 3) provided RR estimates in the same range.[16-22] Different individual and area factors when mutually adjusted or unadjusted exhibit comparable strength of association. Aside from unemployment, for which observed RRs were occasionally found to exceed 4[13] (a finding which could result also from reverse causality or significant comorbidity that was not adjusted for), generally, point estimates do not exceed RR of 2. Smoking inequality contributes most to lung cancer inequality, as confirmed by studies adjusting for smoking where it accounted for roughly 40–70% of the increased risk in low SES , whereas other lifestyle factors contribute much less.[23,26,33]

Cancers of the upper aerodigestive tract and stomach cancer

Many European studies have shown cancers of the upper aerodigestive tract (UADT) to be strongly associated with lower SES (Supplementary tables 4 and 5).[15-19,27,29,30,32,36-44] As with lung cancer, the association is much stronger for men than women. In Italian[29,30], Lithuanian[32] and multi-country European[42] studies for example, excess incidence of UADT and head and neck cancers among the lowest educated could only be confirmed for men. Similarly, in Germany, area deprivation was associated with elevated risk for oral cavity and upper respiratory tract cancers in men only.[19] The most studied individual-level SES indicator is education, for which overall RR estimates in men range from 1.5 to 3 and are generally even higher than those found for lung cancer. Effect of area deprivation in men in France[16,17,37], Germany[18,19], Spain[38] and Italy[29] was found to be between 1.5 and 2.0, whereas in a Scottish study cancers of the mouth, oropharynx and larynx were each shown to be twice to over 3-times as likely in people from the most deprived compared to the least deprived areas, though they did not stratify by sex.[39] There is convincing evidence that area deprivation has an independent effect on UADT cancer risk, not explained by individual factors.

Research on oesophageal and stomach cancers and SES also points to higher risks with lower individual (Supplementary table 6) and area (Supplementary table 7) SES.[16,17,19,27,32,36,45-47] Yet again, incidence in women seems to be less influenced by SES than in men. In several European countries, men of the lowest social standing or from the most deprived regions had between 1.3 to 3.0-times the risk of developing cancer of the oesophagus, whereas many studies found either less increased[45,46] or could not confirm an increased risk[17,19,32,47] for women, though even in the latter case the effect estimates were always positive, often with a discernible trend across SES categories. Given that different risk factors have been identified for the two major histological types, adenocarcinoma and squamous cell carcinoma (SCC), invaluable information comes from studies that investigated these subtypes and attempted to control for known risk factors in order to clarify to what extent they contribute to inequality. A nationwide case-control study in Sweden[48] found that fruit and vegetable intake as well as Helicobacter pylori infection (a potentially protective factor for oesophageal adenocarcinoma) could not explain any of the SES inequality for either histological subtype. Adjusting for reflux symptoms, body mass index (BMI) and tobacco in adenocarcinoma could explain only part of the SES inequality, whereas tobacco and alcohol in SCC did not contribute to SES inequality. Similarly, in a European multi-centre study[49] smoking, alcohol, BMI, physical activity and dietary intake of total energy as well as fruit, vegetable and meat consumption did not seem to contribute significantly to observed SES inequality in the incidence of oesophageal adenocarcinoma. Therefore, better designed approaches to measure these risk factors with minimised residual confounding as well as further research into as yet unidentified risk/protective factors are needed, especially given significant observed increases in incidence rates of oesophageal adenocarcinoma.[50,51]

Stomach cardia adenocarcinoma has been associated with the same risk factors as oesophageal adenocarcinoma and has also been observed to be on the rise.[52] On the other hand, occurrence of non-cardia adenocarcinoma which is linked to infection with Helicobacter pylori, has been declining.[53] Recent European registry-based studies that looked at the incidence of stomach cancer as

a whole found about 1.5-times (range from 1.1 to about 2) increased risk in lower SES individuals, predominantly men.[16,19,29-32,45,54,55] Rarely, no association could be confirmed for either sex.[17,25,29] There seem to be no major differences in terms of which SES indicator is used, though an Italian study, after adjusting for individuals' education, occupational class and housing characteristics, found no additional effect of area deprivation.[29] A meta-analysis of 11 European case-control and cohort studies estimated that the relative index of educational inequality (which takes into account the trend over categories and category sizes) for stomach cancer was as high as 2.92 (95% CI: 1.37-6.19).[56] When stomach cardia and non-cardia are analysed separately, conflicting conclusions are seen: sometimes associations of similar magnitudes are found for both subsites[46]; are somewhat stronger for non-cardia[47]; or somewhat stronger for cardia.[49,57] The two latter studies were large multi-centric case-control studies that also stratified by histological subtype and found more pronounced educational effects for intestinal compared to diffuse type of gastric adenocarcinoma. Furthermore, they investigated to what extent major risk factors explain SES inequality and both found that surprisingly little of the inequality could be attributed to lifestyle factors such as smoking, alcohol, diet, BMI and physical inactivity, or Helicobacter pylori infection.

Liver, gallbladder and pancreatic cancers

Liver and gallbladder cancers are relatively rare in Europe, while the opposite is the case for pancreatic cancer. All come with a high mortality burden.[58] Relative risks for these three cancers of digestive organs comparing lowest to highest individual and area-level SES are listed in Supplementary tables 8 and 9.

High area-deprivation and low education are linked with up to twofold (usually around 1.5) increased risk of liver and gallbladder cancer.[16,17,19,29,30,36,46,59-61] Considering that many of the causes for these cancers are modifiable (chronic hepatitis B and C infection, alcohol, smoking, non-alcoholic fatty liver disease, obesity and gallstones[62-64]), they are very likely responsible for part of the observed SES inequality, though we can only speculate to what extent since we could not find studies adjusting for these factors.

Smoking, Helicobacter pylori infection and obesity could potentially explain up to half of all incident cases of pancreatic adenocarcinoma, the most prevalent form of this cancer[65], though our review did not reveal a uniform link with SES. On the one hand, low individual SES in Slovenia[27], Denmark[45] and Sweden[36] and area deprivation in the United Kingdom (UK)[59] were linked to increased risk (RRs between 1.1-1.6). In Lithuania[32], Germany[19] and Finland[46], only men with lowest SES had a slightly increased risk. On the other hand, no effects could be seen in France[16,17] or Italy [30] and women in Lithuania with the lowest education actually had a reduced risk (RR 0.92).[32] Within the EPIC cohort, at first no significant effect was seen, although after the results were updated, RR estimates were further from unity and a higher risk in men with primary education or less was found. Confounding by known risk factors was also examined—risk was partly (in men) to almost fully (in women) accounted for by smoking, obesity, diabetes and physical inactivity.[66,67]

Gynaecological and breast cancers

We found that low SES strongly increases the risk of invasive cervical cancer (Supplementary tables 10 and 11). RR estimates found in Europe vary between about 1.2 to 2.5 for the lowest compared to highest educated women.[25,29-33,44,68-70] When area deprivation is used, effect sizes are generally similar and also independent of individual SES.[16-19,21,29,70-72] Particularly prominent seem to be the effects of neighbourhood deprivation[29,70] and individual level material dimension of SES (e.g. income) which seemingly influence risk even more than education.[29,44,68] No contemporary studies investigating *in situ* carcinoma were found, though previously risk for *in situ* carcinoma was also higher with lower SES.[73] Very few authors adjusted for known risk factors, namely those relating to *Human papilloma virus* (HPV) infection, which is necessary for occurrence of cervical cancer, and smoking, which could hasten transformation of precancerous lesions into carcinoma.[74,75] In England, area effect was diminished when teenage conception rates, smoking rates and screening coverage were taken into account.[76] Similarly, in Norway, higher risk among the lower educated was not significant anymore, after controlling for smoking, age at first birth and participation in screening, though the hazard ratio was still close to 2.[33] Importantly, smoking accounted for more than 30% of the inequality, whereas screening and age at first birth contributed only approximately 3 and 6%, respectively. HPV seropositivity might explain the rest of the inequality, but as far as we know, neither this nor any other

study in Europe to date was able to make use of such data. Different histological subtypes have also rarely been studied separately—even though both squamous cell carcinoma and adenocarcinoma require HPV infection to develop, in Finland adenocarcinoma did not show an association with SES which could imply aetiology of the two subtypes varies to a larger degree than previously thought.[69] Cancers of the vulva and vagina are also associated with HPV infection, thus unsurprisingly, a negative association with SES has been observed with RR estimates as high as 2.[44,59]

Some of the highest rates of breast cancer incidence and mortality in the world are found in Europe where in places cumulative life-time risk for women is as high as 30%.[58] Unhealthy lifestyle, hormonal and reproductive factors (menarche, menopause, parity, age at first birth and breastfeeding) increase its risk.[77-80] As shown in Supplementary table 12, most studies found women with higher SES are at an increased risk of developing breast cancer.[14,16,18,19,21,22,26-33,36,81-89] Age-only adjusted RR estimates comparing lowest to highest education are between 0.6 and 0.9, mostly around 0.8. Similar estimates are reported when measuring area-SES (Supplementary table 13). Other measures of SES are used rarely and there seem to be less consistency between them, particularly in those that reflect material SES. Therefore, education through delay in childbearing seemingly explains most of the SES effect.[90,91] Studies that attempted to adjust also for reproductive and life-style factors found RRs either significantly closer to unity, when adjustment was incomplete[82,85,87], or equal risk across SES categories both in pre- and postmenopausal women when adjustment was very careful.[33,83,84,92] Furthermore, no significant educational differences were found among nulliparous women.[92] Inequalities are also stronger for *in situ* compared to invasive breast cancer and remain partially unexplained by known risk factors[30,87,92] strongly suggesting the effect of screening. Overall, inequalities in breast cancer can thus, to a substantial degree, be explained by known risk factors.

Ovarian, fallopian tube and endometrial cancers have rarely been found to be positively associated with SES, most often no association was clearly determinable (Supplementary tables 10 and 11).[16-19,30,32,36,68,93,94] This is unexpected since reproductive/hormonal factors also play a role in tumorigenesis.[95,96] Equally rarely, increased risk of uterine[97,98] and ovarian[36] cancer among low SES groups has also been reported. No strong conclusion could be drawn for these cancers.

Male genital and prostate cancers

Testicular cancer afflicts mostly adolescents and young men.[99] While it was previously thought that most of the risk for its development is determined already *in utero*, it is now evident that postnatal factors also play an important role, perhaps by influencing progression of existing *in situ* testicular carcinomas.[100] Across the world, increased risk of testicular cancer, which is predominantly of germ cell type classified into seminoma and non-seminoma, had often been reported with high SES but the gap has started to narrow in recent decades.[101] Since 2000 in Europe, many countries do not report an association (Supplementary tables 14 and 15); no difference in incidence was thus found in Denmark[102], Slovenia[27], Germany[19,103], France[16,17] or Italy.[30] On the other hand, high area deprivation and household overcrowding in England[59,104,105], low education in Sweden[36] and low occupational social class in Finland[106] were associated with lower risk of seminoma and non-seminoma cancer (RR estimates about 0.7-0.9), though in Finland, the RRs have decreased substantially. In line with findings that HPV infection, poor hygiene, smoking and obesity increase risk for penile cancer[107], we found that most[44,59,108], though not all[106], identified studies also reveal an association between penile cancer and low SES, sometimes stronger for invasive than *in situ* carcinoma.

Representing over 20% of all incident cancer cases (excluding non-melanoma skin cancer), prostate cancer is the most common cancer among European men today.[12] The latest data shows that in most of Europe, incidence rates have stabilised or started decreasing.[109] Some of the potential lifestyle-related factors are smoking, alcohol, obesity, physical inactivity and diet, though no associations have been unequivocally proven.[110,111] However, today it is clear that the burden and its trend is highly influenced by availability of opportunistic screening for prostate cancer by Prostate Specific Antigen (PSA) testing. As shown in many studies (Supplementary tables 16 and 17), lower SES is associated with lower prostate cancer risk, though not everywhere.[19,26,28,112] RRs are between approx. 0.5–0.9, most often around 0.8, again with independent effects of different SES indicators.[14,16-18,22,25,27,29-32,36,71,102,106,113-116] Higher RRs are reported for less compared to more advanced disease[106,113,115]; this points to screening as one of the reasons for the positive gap (affluent men have better access to or are more motivated to undergo opportunistic screening). The gap was increasing

during the first decade of the 21st century[59,115], but has since decreased in certain places[22], perhaps due to changes in clinical use of PSA testing after negative outcomes related to opportunistic screening were becoming increasingly recognised. In a randomised intervention study[113], screening somewhat narrowed the gap for advanced disease when education and income were used as measures of SES, while the gap for renters compared to homeowners widened. Finally, a cohort study in the UK found that adjustment for PSA testing narrowed the gap in risk between least and most deprived only slightly and therefore PSA testing is probably not the only factor behind higher incidence of prostate cancer with increasing SES.[116] Lifestyle factors, which could also explain part of the effect, have not been sufficiently studied yet in this regard.

Urinary tract cancers

Kidney cancer is roughly twice as common in men than in women.[12] Incidence is higher in more developed countries where it is on the rise.[117] Identified risk factors are for the most part life-style related, and are thus influenced by SES[118,119], though within this review studies looking at the association between SES and kidney cancer have provided varied results (Supplementary tables 18 and 19). Nevertheless, it seems that more often than not, lower SES, measured most often as education or area deprivation, is associated with higher incidence in both sexes but slightly more strongly in women.[19,33,36,59,120,121] RRs are most often around 1.2–1.3 and rarely above 1.5. Controlling for risk factors was seldom performed. One study investigated the explanatory power of smoking and alcohol and found that smoking accounted for approximately 30% of the higher risk in low educated women, whereas higher alcohol consumption was apparently protective.[33] In Italy[30] and Lithuania[32], the association was reversed, i.e. risk was increased with higher SES, while in France no association could be found.[16,17] The reason behind the reversed findings is not known, though may be due to advances in diagnostic activities.

Compared to women, bladder cancer rates are as much as five times higher among European men in whom it represents the fourth most commonly diagnosed cancer.[12] Like for kidney cancer, RRs comparing lowest and highest education are moderately elevated in lower educated men and women (Supplementary tables 20 and 21) and range up to 1.5 but are mostly between 1.2–1.3.[16-19,27,30,32,120,121] We could not identify any European study that looked into how known and potential risk factors, primarily smoking and exposure to occupational carcinogens[122], contribute to SES inequality in bladder cancer incidence. Considering smoking is such an important factor, most of the inequality is likely present on its account.

Colorectal cancers

Colorectal cancer is strongly related to lifestyle and is the second most common malignant tumour (excluding non-melanoma) in Europe with respect to the absolute number of cases.[12,123] Incidence used to be higher among affluent Europeans[2,124,125] but a review of studies shown in Supplementary tables 20 and 21[16-20,22,25-28,30-33,46,126-130] has reinforced that in several countries, a reversal towards higher incidence among lower SES groups has occurred. For example, before the 1990s affluent Finnish men and women had an increased risk of colon and rectal cancers[46] but Finland has since seen a gradual narrowing of the educational gap, almost to the point of reversal, especially among men, which is due to relatively larger increases in incidence in lower SES individuals.[127] A similar pattern emerged in Norway.[131] In Denmark at the turn of the century, both colon and rectal cancers were already more common with greater individual disadvantage, particularly material and in men.[126] In Sweden[128] and Italy[30], the lowest educated men and women now have up to about 30% and 15% increased risk for rectal cancer, respectively, with no differences for colon cancer. In the UK [22,129] and Germany [18-20], area deprivation is associated with higher incidence of colorectal cancer as a whole, primarily in men. On the other hand, risk was lower in lower educated men and women in Lithuania for both colon and rectum[31,32], while no clear association could be shown in Ireland[28], the Netherlands [26], France [16,17] and Iceland.[25]

Melanoma and non-melanoma skin cancer

In Europe, skin cancer, including melanoma and non-melanoma (basal cell carcinoma – BCC and squamous cell carcinoma – SCC), has seen some of the fastest growing incidence rates among all cancers. For melanoma, body locations associated with the highest increases are limbs and trunk, which are intermittently exposed to sun radiation.[132]

Many studies in Europe (Supplementary tables 22 and 23) have found a positive association for melanoma and SES.[16-19,21,25,27,29,30,32,33,36,59,133-138] RRs

comparing the lowest to highest educated vary between 0.5–0.9 and are most often between 0.6–0.7. In a stratified analysis by body location, only melanoma of the limbs and trunk could be linked with SES.[134] When area-SES was investigated, RRs were slightly higher (around 0.8). This, along with the fact that in a study after mutual adjustment for individual-SES effect of area-SES could no longer be found[29], points to individual SES as the primary factor for observed differences. Though controlling for risk factors has been scarcely attempted, high intermittent sun exposure among persons with higher SES could explain most of the gap. No study controlled for skin type in Europe, though populations in most countries are homogenous in this respect. Therefore, it is not surprising, for example, that in Norwegian women the number of sunburns accrued and latitude of residence explained most of the excess risk.[33]

Fewer studies were found for non-melanoma skin cancer (Supplementary tables 22 and 23). In Lithuania[31], melanoma and non-melanoma showed equal RRs with respect to education and in Germany[18] non-melanoma cancer showed even stronger positive associations with area deprivation than melanoma though neither distinguished between SCC and BCC. In Denmark, BCC excess risk according to different indicators of high SES was virtually identical to RRs found for melanoma. SCC on the other hand was marginally associated only with higher income.[133,139] Conversely, in Nordic countries SCC was clearly more common in people with the highest education and occupational class[36,140], while in Ireland[141] and Scotland[137], along with BCC, SCC was also positively associated with area deprivation. This could mean that chronic exposure, which is generally considered higher in manual outdoor workers, is actually higher among the affluent, at least in the studied countries, or else they undertake more diligent screening.

Lymphoid and haematopoietic cancers

Haematological cancers, the aetiology of which is unclear, are more frequent in males compared to females.[142] Overall, we could not confirm that these cancers are associated with SES (Supplementary tables 24 and 25).[16,17,27,30,32,36,59,143,144] In Italy[30], risk of Hodgkin lymphoma was non-significantly reduced with RR around 0.8; elsewhere, no associations were found[16,17,32,143] or risk was higher in lower SES groups, such as among men with lowest education and male renters compared to owners in Denmark[144] and in most deprived areas in England with RR of 1.6 for males and 1.4 for females.[59] Overall, non-Hodgkin lymphoma, multiple myeloma and leukaemia also do not seem to be associated with SES, excluding some reports of varied associations. In Germany, for example, risk for all lymphoid and haematopoietic cancers combined was higher in deprived men and women but this categorisation was too crude.[19] Authors of a report from the Haematological Malignancy Research Network in the UK concluded (aside from reporting on lower risk of myeloma in very deprived areas) that there are no SES inequalities for a myriad of disease entities categorised according to the detailed WHO classification.[143]

Central nervous system cancers

Though rare, in adults gliomas and meningiomas are the most common tumours of the central nervous system (CNS).[145] The only well-established modifiable risk factor for CNS tumours is ionising radiation whereas at present there are no conclusive findings regarding exposure to non-ionising radiation (e.g. mobile phone use).[146] Within this review we could not confirm a clear association with SES (Supplementary tables 26 and 27). Given that CNS tumours encompass a variety of types, unsurprisingly, no clear direction of association could be ascertained when all types are analysed together. Thus, with increasing affluence, risk was increased in men in Denmark[147] and Sweden [36] and women in England [59], decreased in men in Italy [30] and in parts of France [17] or else equal in France [16], Germany [19], Lithuania [32] and Norway.[33] Regarding specific types, there are some indications that glioma and acoustic neurinoma are less common with lower affluence while meningioma showed no unequal distribution according to SES.[148-150]

Thyroid cancer

Influenced in part by improved detection of asymptomatic cancers, incidence of thyroid cancer is on the rise.[151] Our review (Supplementary tables 28 and 29) showed that thyroid cancer risk was greater among Lithuanian men and women with higher education (SIR between 0.8-0.9)[32] and in areas with lower deprivation in Germany (SRR between 0.6-0.7).[19] Risk was also increased, although not significantly, in highly educated Norwegian women (HR 0.7).[33] In other countries, researchers could not confirm this link[16,17,59], whereas a study in Sweden found higher risks for lower educated

people.[36] Elevated risk in groups with higher SES could be explained by differential access to diagnostic procedures in favour of high-SES groups. Since there are no studies adjusting for multiple SES measures and risk factors, independent effects of different SES indicators as they relate to differing dimensions of SES (such as material, cognitive etc.) are as yet indeterminable.

Conclusions

In our review we aimed to take stock of national or international studies that have investigated the link between the socioeconomic factors and cancer incidence, focusing specifically on Europe and studies based on cancer registry data published in the 21st century. It was necessary to consider two parts, one dealing with the social status (individual level) and the other with the social environment (neighbourhood level studies). It is evident that very few cancers are not associated with SES: head and neck, oesophagogastric, liver and gallbladder, pancreas, lung, kidney, bladder, penis and cervix are associated with low SES; conversely, high SES is associated with breast, prostate, thyroid and skin cancers. For other investigated locations, no associations were observed or else results are too few or varied to make firm conclusions.

Generally, negative associations are stronger for men than women and can be explained to a very large degree by known life-style related factors, most notably smoking as the single most important modifiable cause of a multitude of different cancers. Interestingly, the studies that mutually adjusted for either several different individual or individual as well as area SES measures have reinforced what has already been known, namely that: i) individual-level SES measures are not simply interchangeable but reflect different aspects of socioeconomic position, from material and cognitive to cultural; and ii) both area and individual SES have independent effects on cancer risk, again highlighting the complexity of the concept of socioeconomic status.

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review

Perioperative radiotherapy versus surgery alone for retroperitoneal sarcomas: a systematic review and meta-analysis

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Background. There is no clear evidence on whether radiotherapy (RT) improves treatment result in patients with retroperitoneal sarcomas (RPS).

Methods. A systematic literature search was performed using PubMed, Scopus and CENTRAL databases. Data were retrieved from published comparatives studies in patients with RPS undergoing surgery alone or RT plus surgery. The primary endpoints were the 5-year OS and the median OS. The secondary endpoints were the recurrence-free survival (RFS) and the R0-resection rate. Continuous outcomes were calculated by means of weighted mean difference (WMD).

Results. Ten out of 374 articles were analyzed. The median OS and the 5-year survival were significantly increased in patients treated with RT and surgery, compared to patients treated with surgery alone ($p < 0.00001$, $p < 0.001$). Median RFS was significantly increased in patients treated with either preoperative ($p < 0.001$) or postoperative ($p = 0.001$) RT compared to patients that underwent surgery alone. Finally, median R0-resection rate was similar between the two groups ($p = 0.56$).

Conclusion. RT along with radical surgery could be the standard of care in at least a subgroup of patients with RPS.

Key words: soft tissue sarcoma; adjuvant radiotherapy; neoadjuvant radiotherapy

Introduction

Retroperitoneal soft tissue sarcomas (RPS) constitute a rare and quite heterogeneous group of mesenchymal neoplasms that are located in the retroperitoneum and count for less than 10–15% of all soft tissue sarcomas (STSs).[1] With an incidence of approximately 0,5–1 case per 100000, these tumors are most often considered sporadic especially in the absence of a genetic syndrome (Li-Fraumeni syndrome, Gardner's syndrome, familial adenomatous polyposis [FAP], Carney-Stratakis syndrome, Hereditary retinoblastoma, etc.).[2] Histological subtypes are the well-differentiated liposarcoma (WDLPS), leiomyosarcoma dedifferentiated liposarcoma (DDLPS), undifferentiated pleomorphic sarcoma, solitary fibrous tumors, malignant peripheral nerve sheath tumors and synovial sarcoma.[3]

Until now surgery with curative intent (R0 resection) remains the gold standard treatment for most patients with resectable disease contributing to long-term disease-free survival (DFS).[4,5] However, complete macroscopic surgical resection

is achieved in about 70% of the patients reflecting the high incidence of local recurrence and disease progression.[6,7] Thus, multimodality treatment involving RT and/or chemotherapy could favor the ability to obtain negative surgical margins with a subsequently better local control of the disease and longer survival.

Radiotherapy to the retroperitoneum is a quite complex procedure and can be administered preoperatively, postoperatively, intraoperatively or even in a combined therapy setting. In the era of newer RT techniques as 3D-CRT and IMRT, the surrounding normal tissues can be protected and acute radiation induced adverse events can be reduced.[5]

While current literature is not clear on whether RT, either preoperatively or postoperatively, reflects on a beneficial result in patients with RPS, we aim to investigate if the combination of perioperative RT and surgical resection benefits the overall survival (OS) and the local control of the disease.

Methods

Search strategy and articles selection

The present meta-analysis was performed according to a protocol, which was agreed by all participating authors, along with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[8] A thorough literature search was performed in PubMed (Medline), Scopus (ELSEVIER), and the Cochrane Central Register of Controlled Studies (CENTRAL) databases (last search: October 25, 2018). The following terms were used in every possible combination: "radiotherapy", "radiation therapy", "surgery", "surgical resection", "retroperitoneal sarcoma". The inclusion criteria were: (i) articles with ≥ 10 patients, (ii) English language, (iii) published from 1990 to 2018, and (iv) human subjects. Two independent investigators (AD, DEM) extracted the available data. Any discrepancies regarding the inclusion and/or exclusion of studies were discussed with the guarantor author (KT) until consensus was reached. Moreover, the kappa coefficient test was used in order to evaluate the level of agreement between the reviewers.

Data extraction

Regarding each study that was included, the extracted data was relative to baseline characteristics (sample size for each group, age, sex). The primary endpoints were the 5-year OS and the median OS. The secondary endpoints were recurrence-free survival and R0 resection rate. Two authors (DEM, FB) performed the data extraction and compared the validity of the data until consensus was reached.

Statistical analysis

The categorical outcomes were evaluated by means of the Odds Ratio (ORs) and the 95% confidence interval (95% CI) were calculated by means of Fixed-Effects or Random-Effects model (Mantel-Haenszel statistical method). OR<1 denoted outcome that was greater in the RT group. Continuous outcomes were calculated by means of weighted mean difference (WMD) with its 95% CI, using Fixed-Effects or Random-Effects (Inverse Variance statistical method) models, appropriately, in order to measure pooled estimates. In cases where WMD < 0, the variables in the RT group were increased. The Cochran Q statistic and the I^2 were calculated in order to assess the between-study heterogeneity.[9] Forest plots were produced regarding the variables that were analyzed.

Quality and publication bias evaluation

The Newcastle-Ottawa Quality Assessment Scale (NOS)[10] was used in order to assess all non-Randomized Controlled Trials (non-RCTs) that were included. The scale ranges from zero to nine stars. The studies that were evaluated with a score equal to or higher than five were considered to have a good level of methodological quality and were finally included. No RCTs were identified and included in the current meta-analysis. Two authors (AD, DEM) rated the included studies independently and a final decision was reached by consensus.

The risk of publication bias was evaluated by the visual inspection of funnel plots. Publication bias could not be further evaluated by means of the Egger's formal statistical test[11] due to the small number of the included studies (less than 10). As a result, the power of the test was significantly compromised.

Results

Article selection and patient baseline characteristics

The flow diagram of the present systematic review and meta-analysis is presented in Figure 1 *(Prisma Flowchart)* and the *Prisma Checklist*. In total, 374 ar-

TABLE 1. Characteristics

Study ID,Year	Journal	Country	Time Period	Type of Study	Patients, n		Female, n (%)		Median Age(Range)		Stars In Ottawa
					SA	RT+S	SA	RT+S	SA	RT+S	
Kelly et al., 2015 [12]	Ann Surg	USA	2003-2011	R	172	32[1]	84 (49%)	17 (53%)	62 (26-92	57 (41-85)	6
Lane et al., 2015 [13]	J Surg Onc	USA	-	R	45	29	23 (51,1%)	16 (55,2%)	60 (52, 68)	57 (51, 61)	5
Nussbaum et al., 2016 [14]	Lancet Oncol	USA	2003-2011	R	3322	563[1], 2196[2]	1713 (51,5%)	250 (44%) 138 (52%)	59,5 (± 14,5)	59,2 (±13,8), 59,5 (±13,9)	6
Pierie et al., 2006 [15]	EJSO	USA	1973-1998	P	21	41[2]	N/A	N/A	N/A	N/A	5
Smith et al., 2014 [16]	Radiother Oncol	Canada	1996-2000	R	104	40[1]	49 (47%)	25 (62%)	N/A	N/A	6
Stoeckle et al., 2001 [7]	Cancer	France	1980-1994	R	55	89[2]	N/A	N/A	N/A	N/A	5
Stucky et al., 2014 [17]	J Surg Onc	USA	1996-2011	R	26	37[1]	9 (35%)	17 (46%)	74	56	6
Toulmonde et al., 2014 [18]	Annals of Oncology	France	1988-2008	R	262	127	-	-	-	-	5
Trovik et al., 2014 [19]	Acta Oncologica	Sweden	1988-2009	R	55	42	22 (58,2%)	15 (33%)	63(15-83)	61(35-82)	6
Zhou et al., 2010 [20]	Arch Surg	USA	1988-2005	R	1175	372[2]	-	-	-	-	5

SA = Surgery Alone; RT+S = radiotherapy+ Surgery); 1 = preoperative radiotherapy; 2 = postoperative radiotherapy

TABLE 2. Summary of the analysis of the categorical and continuous outcomes

Categorical Outcomes	n	OR (95% CI)*	p	Heterogeneity	
				I^2	p
5-year OS total	9	0.69 [0.62, 0.77]	<0.0001	67 %	0.002
5-year OS preoperative RT	5	0.69 [0.56, 0.85]	0.0005	50 %	0.09
5-year OS postoperative RT	4	0.69 [0.61, 0.79]	<0.0001	82 %	0.001
RFS total	6	0.33 [0.24, 0.46]	<0.0001	69 %	0.006
RFS preoperative RT	4	0.19 [0.11, 0.33]	<0.0001	72 %	0.001
RFS postoperative RT	2	0.49 [0.32, 0.75]	0.001	0 %	0.81
R0 resections total	3	0.90 [0.81, 0.99]	0.03	69 %	0.04
R0 resections preoperative RT	2	1.21 [0.65, 2.25]	0.56	82 %	0.02
R0 resections postoperative RT	1	0.89 [0.81, 0.98]	0.02	N/A	-
Continuous outcomes	**n**	**WMD (95% CI)**	**p**	**I^2**	**p**
MOS total	5	-18.94 [-19.14, -18.74]	<0.0001	100 %	< 0.0001
MOS preoperative RT	2	-22.93 [-27.91, -17.96]	<0.0001	30 %	0.23
MOS postoperative RT	3	-18.93 [-19.13, -18.74]	<0.0001	100 %	< 0.0001-

CI = Confidence Intervals; MOS = Median Overall Survival; OR = Odds Ratio; OS = Overall Survival; RFS = Recurrence Free Survival; RT = Radiotherapy; WMD=Weighted Mean Difference

ticles that were originally identified in PubMed, Scopus, and CENTRAL databases and ten articles were finally included in the quantitative synthesis.[7,12-20] The level of agreement between the two reviewers was "very good" (kappa = 0.730; 95% CI: 0.503, 0.957). The study design was retrospective in nine studies[7,12-14,16-20] and prospective in one study.[15] The total baseline characteristics of the included studies are shown in Table 1. The Newcastle-Ottawa Scale (NOS) regarding all included studies and the quality assessment of the RCTs is presented in Table 1. Pooled ORs, I^2 along with *p values* of heterogeneity regarding all outcomes that were measured are summarized in Table 2.

Median overall survival (median-OS)

The median OS was significantly higher in patients treated with preoperative RT followed by surgery compared to surgery alone (WMD: -22.93 [95% CI: -27.91, -17.96]; $p < 0.0001$). The median-OS was also significantly higher in patients treated with surgical resection followed by postoperative RT compared to surgery alone group (WMD: -18.93 [95%

CI: -19.13, -18.74]; $p < 0.0001$). According to the total analysis, the median OS was significantly increased in patients treated with surgical resection and either neoadjuvant or adjuvant radiotherapy compared to surgery alone (WMD: -18.94 [95% CI: -19.14, -18.74]; $p < 0.00001$) (Figure 2).

5-year survival

The median 5-year survival was significantly increased in patients treated with preoperative RT followed by surgery compared to surgery alone (WMD: 0.69 [95% CI: 0.56, 0.85]; $p = 0.005$). The median 5-year survival was also significantly higher in patients treated with surgery followed by postoperative RT compared to surgery alone group (WMD: 0.69 [95% CI: 0.61, 0.79]; $p < 0.0001$). According to the total analysis, the 5-year survival was significantly increased in patients treated with surgery and either neoadjuvant or adjuvant therapy compared to surgery alone (WMD: 0.69 [95% CI: 0.62, 0.77]; $p < 0.0001$) (Figure 3).

Median recurrence-free survival

The median RFS was significantly increased in patients treated with surgical resection and either preoperative (WMD: 0.19 [95% CI: 0.11, 0.33]; $p < 0.0001$) or postoperative (WMD: 0.49 [95% CI: 0.32, 0.75]; p = 0.001) RT compared to surgery alone (Figure 4).

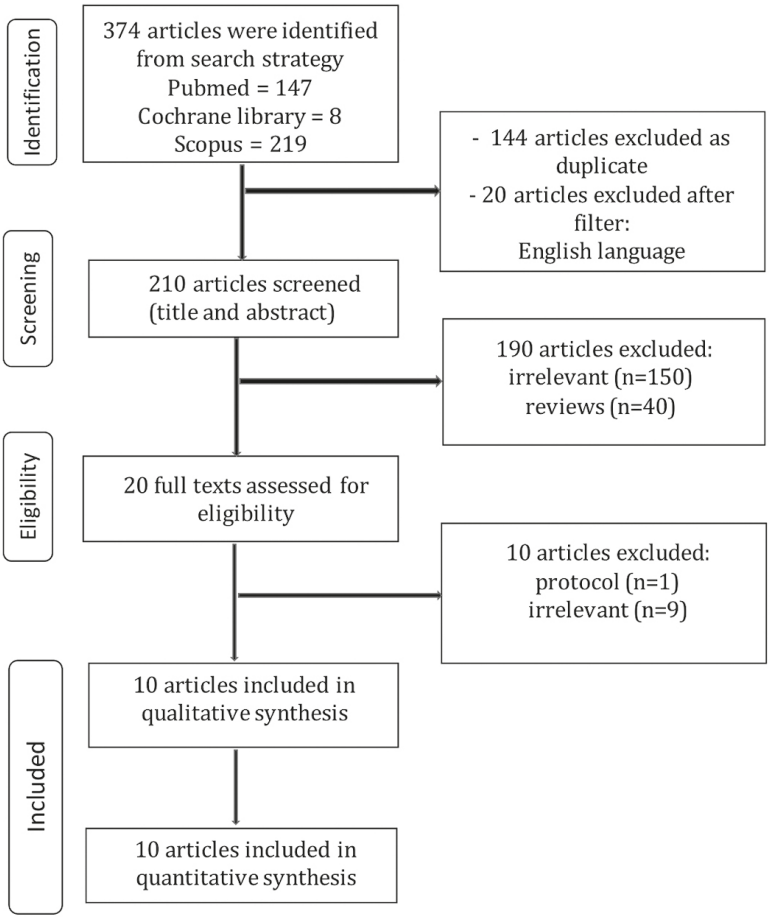

FIGURE 1. Prisma flowchart.

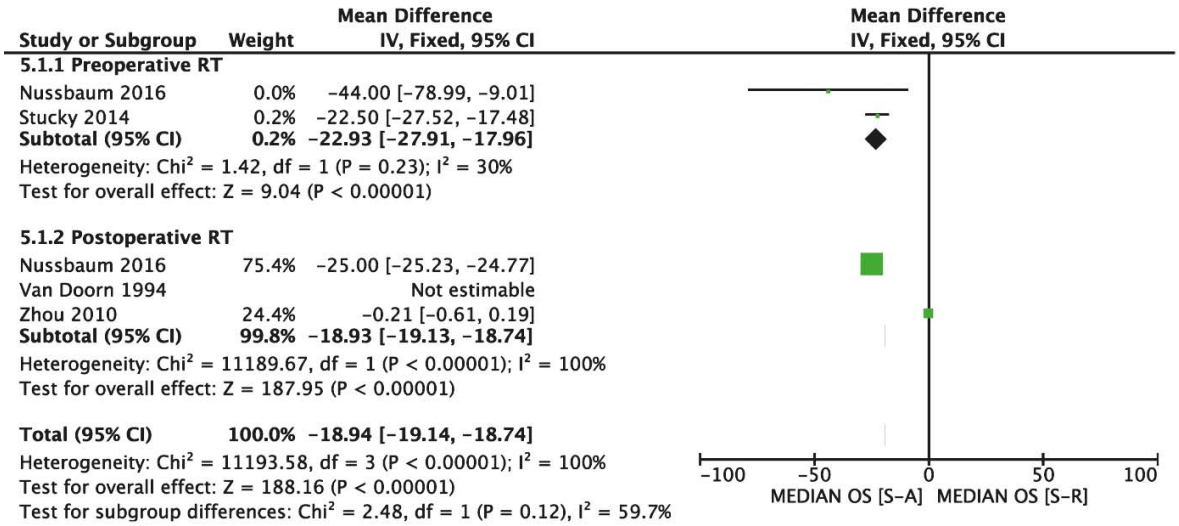

FIGURE 2. Median overall survival.

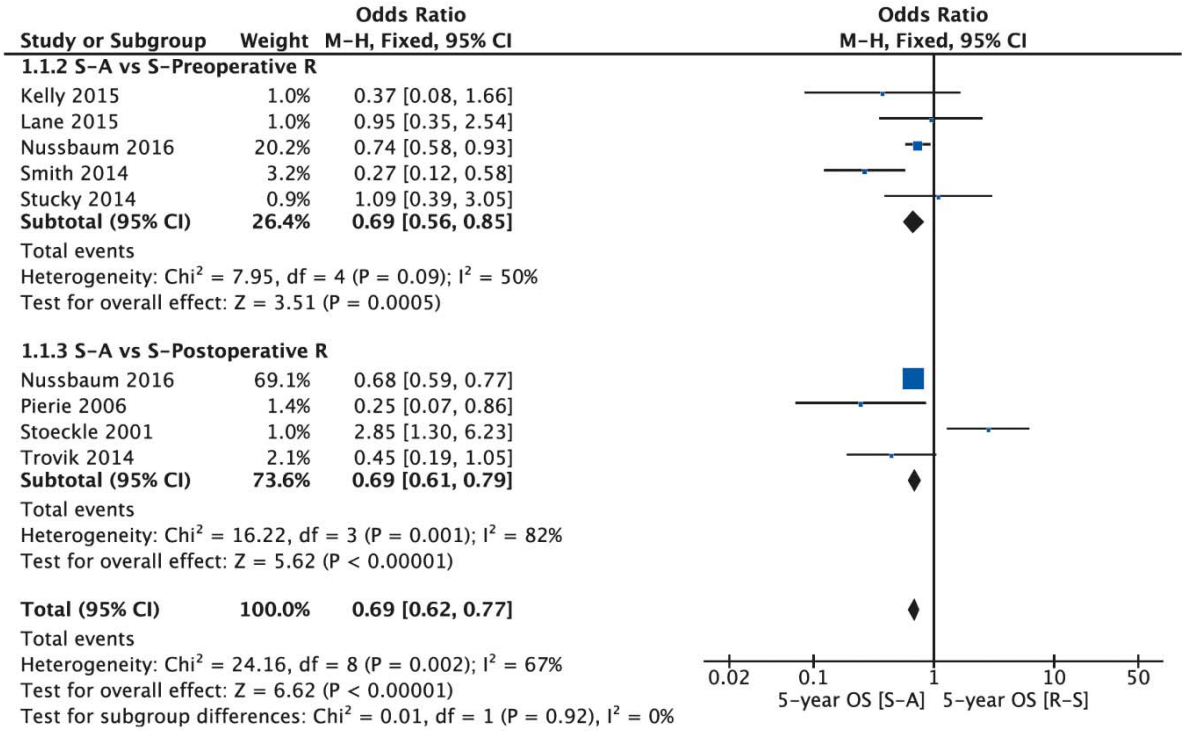

FIGURE 3. 5-year overall survival.

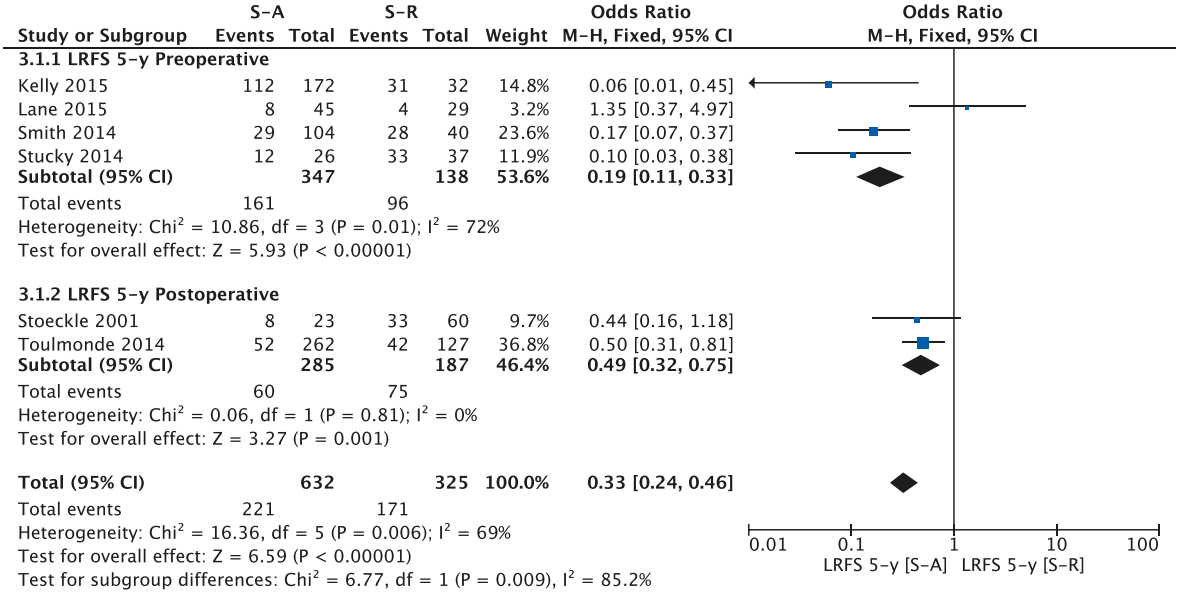

FIGURE 4. Median recurrence-free survival

R0 resection rate

The median R0 resection rate was similar between the groups of neoadjuvant therapy plus surgery compared to surgery alone (WMD: 1.21 [95% CI: 0.65, 2.25]; p = 0.56) (Figure 5).

Publication bias

Funnel plots seemed asymmetrical, with studies being absent from either top or bottom of the graph, thus posing certain publication bias. The small number of included studies was the main

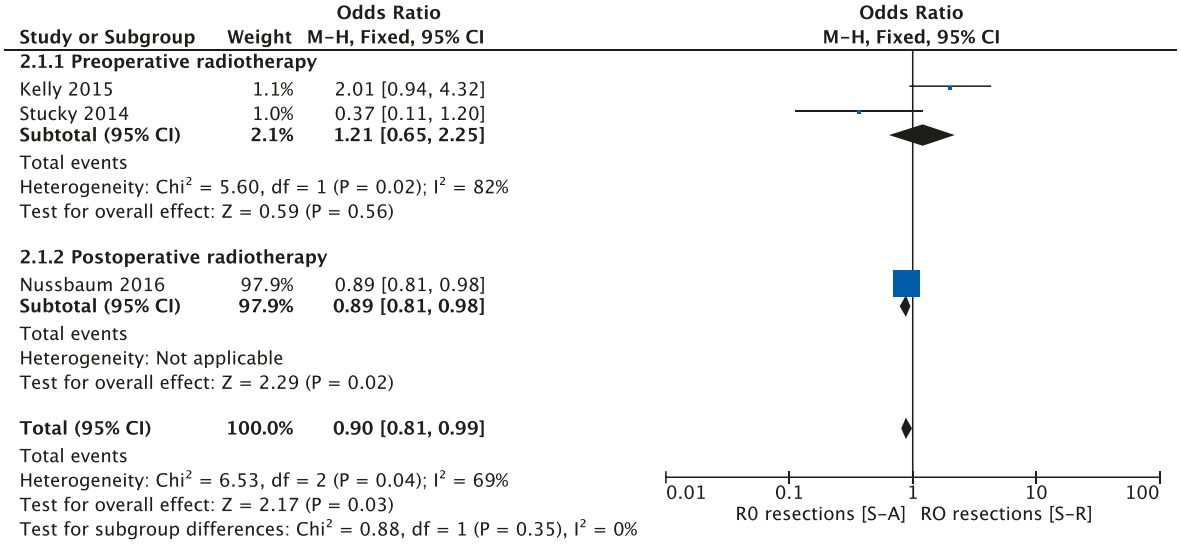

FIGURE 5. R0 resection rate.

reason for the reported asymmetry. Egger test could not be performed because of the inadequate number of studies that were included. Finally, data retrieved is all but in one from retrospective studies and no randomized studies were included.

Discussion

Notwithstanding all the accumulated experience and knowledge regarding the diagnosis and treatment of patients with RPS through the past years, still, those patients' management remains challenging. Current literature evidence is quite insufficient on treatment strategies based mainly on retrospective single-center series, covering small patients' numbers, and treated with various combinations of surgical approaches, with or without adjuvant treatment modalities.

Surgical resection is adequate only when R0 excision of the RPS is feasible. However, due to its late presentation and its tendency to grow in close proximity with vital abdominal structures, in many cases, multivisceral excisions are needed to achieve a good oncologic outcome making it quite difficult to avoid either macroscopic or microscopic residual disease. RPS is often diagnosed in advanced stage, as it is often asymptomatic, and it makes complete excision difficult. Even after aggressive surgical treatment, the median survival of affected patients is 74 months and 5-year all survival rate is 36-58% with recurrence rates often >50%[1,2,4], dictating the need of better local control of the disease.

On this basis, RT could be a logical addition to the patient's management. Only a few studies have tested in a prospective manner the efficacy of RT in patients with RPS either on a neoadjuvant or adjuvant setting. Pierie *et al.*, studied prospectively 103 consecutive patients who were treated for primary RPS and concluded that the most important factor influencing OS and recurrence rate was the complete resection of the disease, and only in patients at high risk of recurrence (*i.e.* high-grade tumors, positive microscopic margins) the addition of RT (IORT plus EBRT) can improve OS and local control of the disease with acceptable level of complications.[15]

Stucky *et al.*, after reviewing 63 consecutive RPS patients concluded that the combination of preoperative radiation plus surgical resection and intraoperative radiation results in excellent local disease control for RPS but not respectively improves overall survival.[17] Moreover, postoperative RT improves the local control of the disease in combination with conservative surgery in patients with negative, marginal or minimally microscopically positive surgical margins. Stoeckle *et al.* stated that adjuvant RT represents the most important prognostic factor for local control of the disease since it is associated with significantly reduced local recurrence rates.[7] High-grade tumors and margin positivity status are at higher risk for local failure and can be considered for intensification of therapy.[15] The combination of surgery, Intraoperative RT (IORT) plus External Beam RT (EBRT) yields favorable local control and survival data.[27] On the

contrary, Pirayeshand *et al.*, re-emphasized the poor outcome of patients with RPS and failed to find any connection between adjuvant RT or chemotherapy and a better outcome either on survival or local control of the disease.[21]

In our metanalysis, it was found that RT delivered either preoperatively or postoperatively, is associated with better median overall and median 5-year survival. In concordance with the results of other retrospective studies, perioperative RT also favors recurrence-free survival compared to surgery alone.[12,13,16] Interestingly, RT did not affect the R0 resection rates as previously reported in the literature.[12] Thus, patients with RPS should be assessed within a multidisciplinary sarcoma tumor board in order to consider RT in their treatment strategy.

Regardless of the timing chosen for RT, physicians should also try to limit the dose of radiation to the surrounding normal tissues. High attention is required in regards to the small bowel, especially in the setting of adjuvant treatment, that may fall into space previously occupied by the removed sarcoma mass and get exposed to high doses of irradiation resulting sometimes in serious complications even in perforation and peritonitis if incidental inclusion of the bowel occurs, especially during IORT.[24] According to current experience, preoperative radiotherapy should be probably preferred. More specifically, the potential advantages of preoperative RT treatment are a) The decrease of residual microscopic local malignant cells. b) Radiosensitivity is higher due to better-oxygenated cells since postsurgical area represents a potentially more radio-resistant hypoxic region). A more radiosensitive target allows the delivery of lower doses of radiation, smaller field sizes and lower toxic adverse events from surrounding organs at risk.[23-25] c) Postoperative adhesions can induce inhomogeneities in the radiotherapeutic treatment plan and suboptimal RT delivery. d) RT can lead to tumor down-sizing / staging. e) RT enables more limited surgery and reduces the amount of normal tissue that needs to be removed. f) RT decreases tumor seeding at the time of surgery. g) In some cases of marginally resectable locally advanced disease, RT can achieve resectability. h) RT may increase R0 resection rate as a result of pseudocapsule that forms around the tumor.[26]

In the modern era, the use of newer and more sophisticated RT techniques as 3D-CRT and IMRT and conformal treatment planning can facilitate surrounding normal organ sparing and avoid acute radiation-adverse events such as enteritis, anorexia, nausea/vomiting and late sequelae as peritonitis.[28] CT simulation and four-dimensional CT (4D-CT) scan for the assessment of the respiratory movement, allow the minimization of the RT dose to the normal tissues, and reduce the incidence of toxicity with excellent local control of the disease.[28-30]

Given the rarity of the disease, proper treatment of RPS must be investigated and determined only after multi-institutional participation in large randomized control trials. STRASS trial is the first, phase III, randomized, multicenter, EORTC study trying to assess whether there is a difference in abdominal recurrence-free survival between RPS patients treated with preoperative RT followed by surgery compared to surgery alone.[22] The results of STRASS trial were presented at the ASCO meeting in 2019 and failed to demonstrate a benefit of preoperative RT for RPS showing no difference in RFS between neoadjuvant RT and surgery vs. surgery alone arms with the exception of liposarcomas in an unplanned subset analysis. There was also, no difference in OS between the two groups. However, in the propensity matched analysis, there was a trend towards improved RFS and LR in the RT arm.

Limitations

Several limitations should be considered before appraising the results of this study. The limitations of this meta-analysis reflect the limitations of the studies included. Nine studies (90%) were retrospective[7,12,13,15-20] and one study (10%) was prospective.[14] No RCT was included. The studies used in this meta-analysis exhibit considerable heterogeneity, limiting the validity of the comparisons between studies and conclusions drawn. Finally, the small number of the included studies poses a publication bias, as it reflects the asymmetry of funnel plots.

Conclusions

After taking into consideration certain limitations, in our metanalysis perioperative RT is associated with improved OS and lower recurrence rates and should be offered selectively, to patients with RPS in the frame of a multidisciplinary team meeting. However, multicentered randomized trials are needed to confirm or revoke these results and assess which patients with RPS could have the greatest clinical and oncological benefit. If the results of these trials confirm the results of our meta-anal-

ysis, which until now comprise the best evidence available, RT along with radical surgery could be the standard of care in at least a subgroup of patients with RPS. This subgroup taking into consideration the STRASS trial's results is probably the LPS subgroup, but this remains to be confirmed in future studies.

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review

Surgical options in treating patients with primary hyperparathyroidism

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Background. Primary hyperparathyroidism is the third most common endocrine disorder for which surgical procedure called parathyroidectomy is the most effective treatment. Since the early 20th century, parathyroid surgery has improved extensively. With the advances in preoperative imaging and with understanding the causes of disease, new and minimally invasive surgical approaches overrode the standard bilateral exploratory operations. Directed parathyroidectomy is currently the standard technique for treatment of primary hyperparathyroidism worldwide.

Conclusions. Surgery is the only definitive treatment of primary hyperparathyroidism. The most appropriate type of surgical procedure depends on the number and localization of the hyperactive parathyroid glands, availability of modern imaging techniques, limitation of each type of procedure and expertise.

Key words: primary hyperparathyroidism; minimally invasive parathyroidectomy; directed parathyroidectomy; endoscopic parathyroidectomy; bilateral neck exploration

Introduction

Primary hyperparathyroidism (PHPT) is a disease of parathyroid glands that results from their overactivity. The estimated incidence of PHPT is approximately two cases per 1,000 persons per year.[1] About 80% of patients have no symptoms; the disease is generally detected during random laboratory blood test. In countries where biochemical test are not commonly used, classical symptoms and signs tend to predominate. Surgical resection is the only potentially curative treatment and remains the leading treatment option for PHPT. If the patient does not meet surgical criteria or refuses surgery, specific pharmacological therapy or close monitoring is recommended.[1]

The evolution of parathyroid surgery

Parathyroid glands were first discovered in Indian rhinoceros by English biologist Sir Richard Owen in 1850.[2,3] Only 30 years later, Ivar Sandstörm from Sweden was the first person to describe the location and blood flow of glands in humans and coined the term *glandulae parathyroidiae*.[1]

It has taken many years for scientists to understand the mechanism of action of parathyroid glands. With the rapid development of thyroid surgery (thyroidectomy) in the early 20th century, common occurrences of tetany were observed.[2] The fact that hypocalcaemia after parathyroid surgery is the definitive cause of tetany was not wholly ac-

cepted for several decades.[3] It was not until 1909 that the first evidence of the relationship between parathyroid glands and the metabolism of calcium was published by MacCallum and Voegtil.[2-4]

Parathyroid surgery as a possible treatment of parathyroid disease was first mentioned in 1915.[5] Through the years, several scientists insisted on the connection between parathyroid disease and bone disease. Schlagenhaufer suggested the removal of a solitary tumour of the parathyroid gland in attempt to cure bone disease (described as *osteitis fibrosa cystica*).[2,3] The professional public was sceptical because of the lack of evidence on the link between bone disease and the tumour. However, it took another 10 years before relationship between parathyroid disease and bone involvement was accepted. The first (successful) parathyroidectomy was performed by Felix Mandl in Vienna in 1925.[5,6] Prior to the surgery, he initially tried to cure his patient (who had cystic bone lesions) with animal parathyroid extracts and transplantation of four fresh parathyroid glands, taken from the street accident victim. After surgical excision of the tumour with preservation of the other three parathyroid glands, the patient's condition improved dramatically. The patient later died from kidney failure.[2,5,6]

After the first successful parathyroidectomy, the surgery became the main treatment of parathyroid disease. Like in any other surgery, however, there are potential risks and complications. In the early 1930s, many surgeons noted the occurrence of severe, life-threatening hypocalcaemia after surgery.[5,6] They (often unsuccessfully) tried to treat hypocalcaemia with a high-calcium and low-phosphate diet or intravenous calcium administration.[7] Churchill and Cope's detailed description of parathyroid surgery facilitated the development of surgical treatment. They employed frozen sections and routinely performed biopsy from normal tissue to rule out hyperplasia.[4] They also wrote recommendations for the course of surgery (based on their experience in 30 observed patients) according to the likelihood of parathyroid hyperplasia or multiple adenomas. With the development of histology and the understanding of function of parathyroid glands, their variations in the number and location, surgical success rate slowly became higher.[7] Near the end of the 20th century; high-tech diagnostic imaging techniques became powerful medical tools that allowed surgeons to operate with minimal invasion to the patient. Since then, the use of minimally invasive surgery has expanded widely and has almost completely replaced the conventional surgical techniques.[5,6]

Anatomy of parathyroid glands

The anatomy of parathyroid glands is highly variable.[7,8] A fundamental understanding of both surgical anatomy and embryology of parathyroid glands is key to successful parathyroid surgery. Nowadays, precise preoperative localization facilitates successful surgical therapy, but a good knowledge of anatomy remains irreplaceable.[7]

Parathyroid glands are yellowish brown endocrine glands, measuring about 6 × 4 × 2 mm and weighing on average 20 to 40 mg.[8,9] The majority of glands are oval, bean shaped or spherical and usually lie on the posterior aspect of the thyroid lateral lobes.[9] Each gland is separated from the thyroid gland by a thin connective tissue capsule. Most people (85%) have four parathyroid glands[7-10]:

- Two superior glands, each of which embryologically arises from the fourth pharyngeal pouch. They lie 1–2 cm above the intersection of the inferior thyroid artery and the recurrent laryngeal nerve at the level of the inferior margin of the cricoid cartilage.
- Two inferior glands, each of which embryologically arises from the third pharyngeal pouch. They are usually (in 70–80% of people) located in the close proximity to the inferior margin of the thyroid gland.

A small number of patients have three or even more than four glands.[8] Rarely, parathyroid glands will be located elsewhere in the neck or in the chest. They are so-called ectopic parathyroid glands.[9] Ectopic superior parathyroid glands can be found para- or retropharyngeal, retrotracheal or in the upper mediastinum, rarely also within the thyroid capsule.[7] The location of the lower parathyroid glands is more variable due to their longer embryologic migration pathway. They can be found anywhere along this embryologic tract: along the thyrothymic ligament, the carotid sheath, in the anterior mediastinum etc.[7-9]

The upper and lower parathyroid glands are supplied by branches of the inferior thyroid artery, which is the major blood supply for lower poles of thyroid.[7,8] To preserve the vascular supply to the parathyroid glands, the ligation of branches of inferior thyroid artery in thyroidectomy should be done with caution as close as possible to the thyroid capsule. Venous drainage is carried out by the inferior, middle, and superior thyroid veins, which drain into the internal jugular vein.[7,8]

TABLE 1. Biological actions of parathyroid hormone (PTH) in the body. PTH increases the serum calcium concentration and lowers the serum phosphate concentration

Organ system	Function of PTH
Kidneys (leading role)	It increases calcium and decreases phosphate reabsorption, stimulates calcitriol production by increasing the synthesis of the enzyme 1-α hydroxylase in proximal tubules.
Skeletal	It raises calcium levels in blood by increasing bone destruction (via osteoblast-mediated activation of osteoclasts) and decreasing the formation of new bone.
Gastrointestinal system	It increases calcium absorption by stimulating the production of 1,25-dihydroxycholecalciferol.
Other (minor role, experimental)	Metabolic effects (reduced glucose tolerance, changes in fat metabolism), effects on the liver, adipose tissue, cardiovascular system, neuromuscular function.

TABLE 2. Clinical presentation of developed primary hyperparathyroidism (PHPT). Symptoms and clinical signs are associated with an elevated serum calcium concentration and/or increased secretion of parathyroid hormone (PTH)

Organ system	Symptoms and clinical signs
General	anorexia, polyuria, polydipsia, weight gain, anaemia
Skeletal	*osteitis fibrosa cystica* (bone pain, decreased bone density or generalized osteoporosis, pathological fractures)
Kidney	kidney stones, renal parenchymal calcifications, nephrocalcinosis, chronic renal impairment
Neuromuscular	proximal muscle weakness, depression, decline in cognitive ability, psychosis
Cardiovascular	arterial hypertension, arrhythmias, left ventricular hypertrophy, vascular wall and myocardial calcification
Gastrointestinal	nausea, vomiting, constipation, ulcer disease, pancreatitis
Rheumatological	gout, pseudogout

Hyperparathyroidism

Hyperparathyroidism is a common endocrine disorder in which one or more parathyroid glands become overactive.[1] They produce and secrete parathyroid hormone (PTH). PTH affects several organ systems through multiple mechanisms. Its main role is regulation of calcium homeostasis but is important as well in vitamin D and phosphate regulation (see Table 1).[1,8,11-14] The most common cause of hyperparathyroidism is excessive and unregulated production of PTH by parathyroid glands, so-called PHPT. More than 80% of PHPT is caused by a single benign parathyroid neoplasm – adenoma.[8] According to epidemiological research data, PHPT is the third most common endocrine disorder.[11,12] It affects 2 in 1,000 people per year in western world and its incidence increases with age, with a peak incidence in the seventh decade of life. The risk of developing PHPT is 4-fold higher in women.[1,8] On the other hand, secondary hyperparathyroidism refers to compensatory physiological response of parathyroid glands to chronic hypocalcaemic state due to other pathological processes (mostly kidney disease).[1,8,12] Unlike PHPT, a disease outside of the parathyroids causes all of the parathyroid glands to become enlarged and hyperactive. Patients with long-standing secondary hyperparathyroidism may develop autonomous parathyroid function due to random mutation in one of the parathyroid glands called tertiary hyperparathyroidism.[12] The clinical picture is usually similar and difficult to distinguish from PHPT. The following paper focuses on the diagnosis and treatment of PHPT.

Clinical presentation and diagnostics

Nowadays, hyperparathyroidism is usually found accidentally during routine biochemical blood tests.[15] Patient history and clinical presentation are often not helpful.[16] Hyperparathyroidism should be considered in asymptomatic patients with elevated serum calcium levels. An atypical form of PHPT with normocalcemia and elevated PTH levels is rare (some authors argue that an atypical PHPT is an early form of the PHPT).[1,7,15] Other possible causes of hypercalcaemia should always be ruled out.[15] Occasionally, non-specific symptoms are found in presumed asymptomatic patients with a more detailed questioning (for example fatigue, depression, neuromuscular symptoms). In the past, the disease was diagnosed in the more advanced phase

TABLE 3. The 2014 Fourth International Guidelines for the Management of Asymptomatic PHPT. Patients need to meet at least one of the following criteria to be advised to have surgery

Measurement	Criteria
Age of patient	< 50 years
Serum calcium concentration (above the upper reference value)	> 0.25 mmol/L (1.0 mg/dL)
Skeletal injury	bone mineral density (DXA): T-score < -2.5 SD*
	spinal fracture (proven by XR, CT, MRI or VFA)
Renal impairment	creatinine clearance < 60 mL/min
	kidney stones or nephrocalcinosis (proven by XR, US or CT)
	24-hour calcium in urine > 10 mmol/L (400 mg/day) or increased risk for kidney stones based on biochemical analysis

* measured on the lumbar spine, hip, femoral neck or distal third of the radius
CT = computed tomography; DXA = dual-energy x-ray absorptiometry; MRI = magnetic resonance imaging; SD = standard deviation; US = ultrasound; VFA = vertebral fracture assessment; XR = x-ray imaging

or so-called classical form, which is less commonly seen today.[16] The developed clinical picture is very diverse due to the broad action of PTH and different calcium concentrations (Table 2).[1,12,14-16]

Laboratory measurement of serum calcium concentrations should be obtained on at least two separate occasions. Hypercalcemia with concomitant elevated PTH levels confirms the diagnosis of PHPT.[1,15] Some patients have atypical form of hyperparathyroidism with elevated PHT levels in the absence of hypercalcemia.[16] This phenotype of PHPT is called normocalcemia with abnormally high PTH. Different secondary causes need to be excluded before diagnosis of atypical PTH is made.[15] At least the serum concentration of phosphate, chlorine, alkaline phosphatase, vitamin D and the patient's acid-base balance must be checked, as well as basic examination of urine and urinary sediment.[1,15,16]

All patients with diagnosis of PHPT (including asymptomatic patients) should undergo a urinary tract ultrasound.[15] Other diagnostic procedures (dual-energy x-ray absorptiometry, bone x-ray, bone biopsy) should be performed only if there is reasonable clinical suspicion.[1,15]

After the diagnosis of PHPT is established, localization studies need to be done to determine if minimally invasive surgical treatment can be performed.[13] The localization studies should not be used to diagnose the disease. Parathyroid glands can be imaged with multiple modalities. The neck ultrasound, CT, scintigraphy or newer hybrid imaging PET/CT and SPECT/CT can be used.[17-20] ^{99m}Tc-sestamibi scan (complemented with neck ultrasound) is still the most commonly used method in many countries.[18] Sestamibi is a small protein labelled with the radio-pharmaceutical technetium-99m. The scan is performed by injecting small amount of radioactive material, which is then absorbed by overactive parathyroid gland. The gamma camera detects radioactive material and shows position of parathyroid glands. Different studies reported the sensitivity of ^{99m}Tc-sestamibi between 77% and 89%, but up to one-third of patients with adenomas could be sestamibi negative.[18]

PET/CT is a newer and promising tool for localization of parathyroid adenomas. Different PET tracers can be used (^{18}F-fluorodeoxyglucose, ^{18}F-fluorocholine (FCH) etc.).[19,21] Many studies are comparing FCH PET/CT with conventional imaging modalities. Behera and Damle reported the incremental role of PET/CT over sestamibi scan in 2016 because of better spacial resolution, possible detection of smaller adenomas and reduced scanning time.[18,20] Retrospective study by Hočevar *et al*. analysed the results of preoperative localization with FCH PET/CT in 151 patients with PHPT.[21] Choline is a precursor molecule for a major component of the cell membrane and is taken up by hyperfunctioning parathyroid cells and neoplastic cells. They concluded that FCH PET/CT is a reliable preoperative localization test prior to directed parathyroidectomy, with operative confirmation of location and therapy success rate above 95%.[21]

Indications and contraindications for surgical treatment

The surgery is indicated in all patients with clinical symptoms of PHPT who agree to be treated with

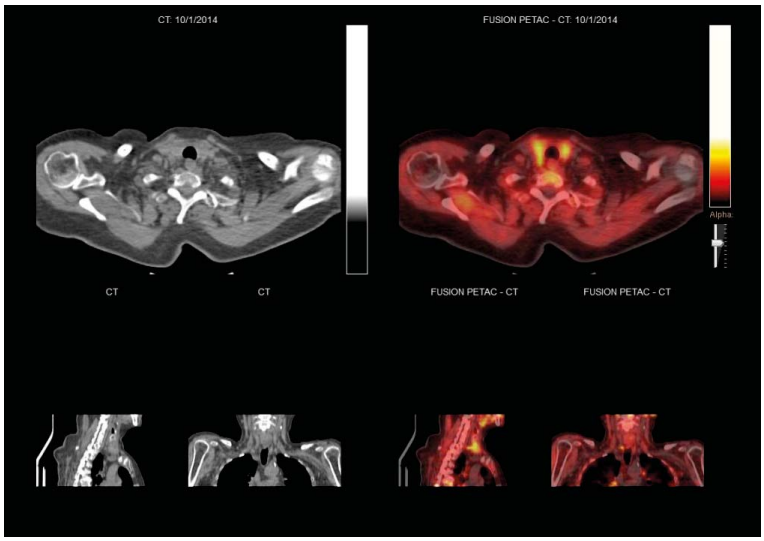

FIGURE 1. ^{18}F-fluorocholine (FCH) PET-CT fusion images of patient with pathologic uptake in the right lower parathyroid gland (solitary adenoma).

surgery.[22] Successful surgical intervention can completely cure the disease.[15] On the other hand, not all authors agree that surgery is beneficial for asymptomatic patients with PHPT. Differences in opinion arise mainly due to poor knowledge of the natural course of the disease, benefits of the surgical procedure and appropriate timing of surgery.[1,16,22] The indications for surgery in asymptomatic patient with PHPT according to the Fourth International Guidelines for the Management of Asymptomatic PHPT are presented in Table 3.[23]

If the patient is a suitable candidate for surgery according to the guidelines, possible contraindications for surgery must be considered.[23,24] Surgery is absolutely contraindicated in patients with familial hypocalciuric hypercalcemia (FHH), because it does not result in cure.[25] FHH is an autosomal dominant disorder that is often confused with PHPT due to similar laboratory findings (normo- or hypercalcemia with elevated PTH). The major feature that distinguishes FHH from PHPT is 24-hour urinary calcium excretion. Urinary calcium concentration below 100 mg in 24-hour urine is diagnostic for FHH.[12,24.25] The relative contraindications are prior vocal cord injury, contralateral laryngeal nerve injury and symptomatic cervical disc herniation (parathyroid surgery is performed in neck hyperextension, which may worsen the condition).[25] The contraindications listed above are common and apply to all types of surgical procedures on the parathyroid glands. Each surgical approach also has specific contraindications, which are mentioned in the descriptions of individual approaches.

Preoperative management of patients

Once the diagnosis of PHPT has been confirmed, determining the best approach for surgery depends on several factors. Before making any decision, the surgeon checks and evaluates the patient's medical history, family medical history, patient's regular treatment, the most recent laboratory results, etc.[24,25] Specific tests (if not already performed) based on clinical symptoms may need to be done to check for the involvement of individual organs (kidney, bone, etc.). The preoperative examination should include at least the following[1,24,25]:

- serum calcium levels and intact parathyroid hormone (iPTH);
- serum value of 25-hydroxy vitamin D;
- serum creatinine concentration;
- 24-hour urinary calcium concentration and creatinine values and
- serum thyroid-stimulating hormone (TSH) and thyroxine levels.

Accurate preoperative localization is crucial for successful surgical outcomes (particularly for minimally invasive parathyroidectomy). It helps to determine the location and number of hyperfunctioning parathyroid glands and it is very important in the case of recurrent neck procedures.[18] In some western Europe countries conventional imaging with ^{99m}Tc-sestamibi was successfully replaced by neck ultrasound and FCH PET-CT imaging (Figure 1).[21] In some cases, additional imaging (e.g. CT, MRI) is required. Invasive procedures (selective venous sampling, selective arteriography) are reserved for patients who have had prior neck surgery and require reoperative surgery[18,23] Due to the frequent concomitant thyroid and parathyroid disease, preventive preoperative analysis of the thyroid gland is performed to avoid increased complications from reoperations.[26]

Types of surgical treatment of primary hyperparathyroidism

The surgeon decides on the type of surgical procedure based on the patient's anamnesis, clinical status and results of preoperative examinations.[24] The patient should be informed of the procedure that is indicated, possible surgical complications

and the overall course of treatment.[25] Today, there are several different surgical approaches that are further described below. With the development of medicine, classical surgical procedures are increasingly being replaced by new approaches that seek to minimize interventions into the human body.[7] There are many different tools/procedures available today to perform surgery as accurately as possible, reduce the number of surgical complications and surgery repetitions, e.g. intraoperative neuromonitoring for identification of the recurrent laryngeal nerve, intraoperative iPTH assay, intraoperative frozen section when malignant tissue is suspected etc.[7,24,25,27]

Bilateral neck exploration

Bilateral neck exploration (also standard, open, conventional parathyroidectomy) is a traditional surgical approach for the treatment of PHPT. Due to the development of less invasive procedures, its application is decreasing.[7] During the operation, the surgeon exposes all four parathyroid glands, therefore precise preoperative localization is not required (but it may be helpful). Parathyroid glands identification can be challenging even for a skilled surgeon due to their unpredictable location.[22,25] According to literature data the success rate of surgery is 95% in experienced hands (the success of the procedure depends directly on the experience of the surgeon).[8,25] In the past, a biopsy of all four parathyroid glands was used to histologically prove pathological parathyroid tissue.[28] Due to the high risk of bleeding and postoperative hypoparathyroidism, it is no longer recommended.[25,28]

Current indications for bilateral parathyroidectomy include[25,28-30]:

- unreliable or inaccessible preoperative imaging;
- preoperative imaging is contraindicated (pregnancy etc.);
- multiple parathyroid lesions;
- ectopic parathyroid glands location (inaccessibility with minimally invasive intervention),
- familial PHPT;
- concurrent thyroid surgery and
- other contraindications for minimally invasive intervention (measurement of iPTH is not available etc.).

The surgical approach has not changed significantly since the first bilateral parathyroidectomy in 1925.[7] Surgery is usually performed under general anaesthesia, local anaesthesia is rarely used (depending on the experience of the surgical and anaesthesia team).[30,31] The patient is lying on the operating room table in a supine position with his upper body at 30 degrees.[25] The surgeon makes a 3 to 5 cm transverse incision (initially the incision was longer) about 2 cm above the collarbone. Subcutaneous fat and platysma are divided and the infrahyoid strap muscles are retracted for optimal exposure of thyroid. Using upward and medial digital retraction of the thyroid gland, the surgeon enters the "parathyroid" space.[7,25] Once the important structures come into view (inferior thyroid artery and recurrent laryngeal nerve), the surgeon continues with the identification of the parathyroid glands.[31] Caution should be used when removing the parathyroid tissue and during connective tissue manipulation, because the rupture of the parathyroid capsule can result in parathyromatosis.[25] The surgeon must identify all four parathyroid glands but remove only the abnormal ones.[31] When in any doubt, frozen section can be done to confirm that the tissue excised is parathyroid in origin. In the event of accidental removal of healthy parathyroid tissue, reimplantation of the tissue should be considered (usually into the brachioradial forearm muscle).[7,25] After successful removal of pathological glands and proper haemostasis, the surgeon approximates the retracted infrahyoid strap muscles and closes the wound with a continuous intradermal suture.[25,28-31]

The most common complication of bilateral neck exploration is recurrent or persistent hyperparathyroidism that results from missed adenoma or parathyromatosis. Rarely, the cause of recurrent hyperparathyroidism is new neoplasia (most commonly in previously unrecognized familial PHPT or multiglandular disease, for example multiple endocrine neoplasia [MEN syndrome]).[25] As well as the success of the operation, the rate of complication depends directly on the experience of the surgeon (to be considered experienced, surgeon should perform at least 50 procedures per year).[25] During the procedure, the superior or recurrent laryngeal nerves on either side can be damaged.[8] Damage can cause long-term hoarseness or voice loss. According to the literature, permanent nerve injury occurs in less than 1% of patients.[8,33] Transient nerve injuries (neuropraxia) are more common and have different recovery times, complete recovery generally occurs within the first six months. Postoperative hypocalcaemia is usually transient and occurs in 25% of patients.[25,33] It is most often due to slow reactivation of healthy parathyroid glands, which have been suppressed

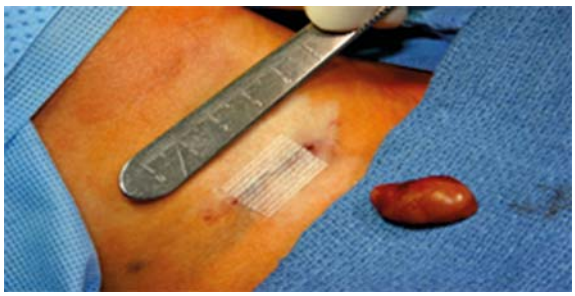

FIGURE 2. Directed parathyroidectomy. The image shows the incision site and the removed parathyroid tissue.

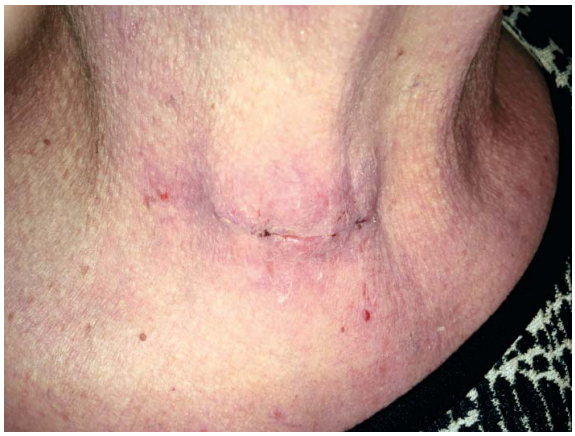

FIGURE 3. Appearance of the incision and surrounding skin 10 days after performing directed parathyroidectomy.

by overactive pathological parathyroid glands. After removal of the adenoma, the remaining suppressed glands will eventually regain their functional capacity.[8,25,32] Patients with low blood calcium levels complain of numbness and tingling in their fingertips and toes, very low concentrations can result in involuntary movements and severe muscle spasms.[25,33] Postoperative bleeding is a rare but dangerous complication, which may lead to respiratory problems and wound infection.[25,33]

Minimally invasive parathyroidectomy

The term minimally invasive parathyroidectomy (also focal, selective parathyroidectomy) has been used since the 1990s to describe newer types of surgeries on parathyroid glands.[34,35] There is no exact definition of the term, but some experts define it as a procedure with minimal dissection (excision of the adenoma without the removal of non-pathological parathyroid glands) and a wound smaller than 2.5 cm.[7] Worldwide, the term currently includes the following procedures: directed parathyroidectomy, endoscopic parathyroidectomy (total endoscopic, video-assisted and robotic parathyroidectomy) and isotope-guided parathyroidectomy.[34] In 2009, the European Society of Endocrine Surgeons (ESES) confirmed that in keeping with prescribed indications and contraindications, minimally invasive parathyroidectomy is a safe and reliable procedure.[35]

Directed parathyroidectomy

With the development and improvement of preoperative imaging diagnostics, parathyroid surgery is focused on minimally invasive surgery.[5,6] Accurate preoperative localization (with neck ultrasound, sestamibi scan and/or FCH PET-CT) is critical to the success of this type of surgery.[17-20] Directed parathyroidectomy is a procedure recommended for patients with solitary adenoma.[7] Worldwide, this type of surgery accounts for 70% of all minimally invasive procedures.[34] The operation is usually performed under general anaesthesia but can be also performed under regional anaesthesia (usually deep cervical block). Regional anaesthesia prevents side effects of general anaesthesia and at the same time allows for intraoperative testing of superior and recurrent laryngeal nerve (by evaluating the patient's speech).[36] Depending on the location of the adenoma, a central or lateral 2 to 4 cm long incision is made at the neck (see Figure 2).[7,13] The surgeon identifies and removes only the pathological parathyroid gland (or solitary adenoma).[25] Other parathyroid glands are not exposed. Since preoperative imaging is not completely reliable, majority of surgeons use iPTH to determine the surgery's success.[13] iPTH levels are determined at anaesthesia induction (general or regional) and 5–10 min after adenoma removal. According to Miami criteria, successful parathyroidectomy was defined as a > 50% decrease of iPTH level compared to the level measured before removal. The half-life of iPTH in plasma is 3–5 min.[37,38] Directed approach reduces the invasiveness of the surgery and shortens the surgery and hospitalization time.[13,32,35] Published research also demonstrates a lower risk of operative complications (postoperative hypocalcaemia, recurrent laryngeal nerve damage) and death, compared to bilateral neck exploration.[7,13,25] The patient recovers faster, with less pain and a better aesthetic result (smaller postoperative scar) (Figure 3).[25,32] The procedure is not appropriate for patients with multiglandular disease, thyroid disease that also requires surgery or a family history of MEN syndrome.[13,25,27,28]

In the last five years, FCH PET-CT has proven to be the most sensitive and specific method in preoperative localization of pathologically changed parathyroid glands.[39] Its reliability to exclude multiglandular disease is so high that iPTH monitoring can be safely abandoned.[40] This shortens the surgery time by 30 minutes, which is very significant given that the average duration of directed parathyroidectomy is less than 20 minutes.

According to a 2007 analysis by Westerdahl *et al*., the long-term results of directed parathyroidectomy with iPTH monitoring are comparable to bilateral neck exploration.[41] Due to the benefits of this procedure primarily in the early postoperative period and the comparable risk of PHPT recurrence, this procedure is (with the patient's consent and the absence of contraindications) indicated as the procedure of choice for removing preoperatively proven solitary adenoma.[41]

Endoscopic parathyroidectomy

Endoscopic parathyroidectomy is a surgical technique using an endoscope.[7] It is divided into two types, depending on the course: total endoscopic and video-assisted parathyroidectomy, which is a combination of endoscopic intervention and open surgical approach.[34] In addition to a directed approach, the endoscope also allows us to perform the entire procedure through small incision wound and with small tissue damage. PHPT is a disease that is otherwise an "ideal disease" for endoscopic surgery, since[34,42-44]:

- in most cases, the cause is a benign tumour,
- the tumour is mostly smaller than 3 cm and
- there is no need for surgical reconstruction after removal of small amount of tissue.

Endoscopic surgery has similar advantages to bilateral parathyroidectomy as other minimally invasive procedures. Compared to directed parathyroidectomy, it is less invasive, it results in less postoperative pain and a better aesthetic result, but surgery is longer, technically more demanding, and requires more expensive surgical equipment.[45] General anaesthesia is always required.[34] Endoscopic intervention is indicated in patients with sporadic PHPT and solitary adenoma, which should be confirmed by preoperative imaging.[43] The procedure is not appropriate in the case of intrathyroid parathyroid adenoma, multiple parathyroid involvement, previous neck surgery, suspected parathyroid cancer, familial PHPT, secondary and tertiary hyperparathyroidism, goitre and in the obese patients.[25,34,43,45]

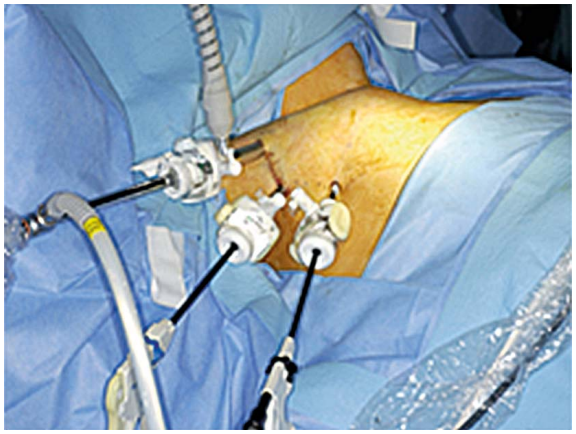

FIGURE 4. Total endoscopic parathyroidectomy.

Total endoscopic parathyroidectomy

Total endoscopic parathyroidectomy was first mentioned by Gagner *et al*. in 1996.[46] Initial technique was carried out entirely under a steady gas flow, introduced through a central trocar. Nowadays, due to the limited exposure of structures, the central approach is used only for the more anteriorly located adenomas.[46] A more frequently used technique is the lateral approach. The intervention begins with the insertion of three working channels for endoscopic instruments (sizes 2, 3 and 10 mm) along the upper anterior margin of the sternocleidomastoid muscle (Figure 4). Carbon dioxide is insufflated to help the surgeon expose the area of work and show up the main structures (lateral border of the thyroid gland, recurrent laryngeal nerve, and both ipsilateral parathyroid glands) with lateral displacement of the infrahyoid muscles and remove the adenoma through a 10 mm trocar.[47]

According to the data, the conversion from endoscopic to traditional 'open' surgery is between 13.4% and 28%.[46,47] The causes are different, most often due to difficult dissection, bleeding or persistence of elevated iPTH after removal of the suspected solitary adenoma.[25,46,47] The success of the endoscopic approach is very favourable in the short term (with the exception of a large number of conversion to the open mode), but the results of long-term studies are not yet available (intermediate results are encouraging).[46,47] Fouyuet *et al*. operated on 200 patients with PHPT, 28% were converted to open parathyroidectomy. After an average 13 months after endoscopic surgery, 197 patients (98%) were reported to be cured.[47] In another prospective study Vidal-Perez *et al*. performed 28 endoscopic lateral parathyroidectomies with no in-

traoperative complications. They reported favourable outcome in 27 of the 28 patients (96%) after an average of 22 months after procedure.[48] The major limitation of the approach represents simultaneous bilateral adenomas, since the approach only allows simultaneous removal of the ipsilateral adenomas.

Video-assisted parathyroidectomy

Video-assisted parathyroidectomy is a combination of endoscopic and open surgery. It was first described in 1999 by Miccoli *et al*.[35,49] The endoscope, with a smaller incision than used in other open procedures, allows the operator to visualize the operative field equally or better. This results in a smaller postoperative scar. The use of two to three times magnification of the operative field makes identification of anatomical structures easier and the risk of damage to the laryngeal recurrent nerve and other important structures is lower. The advantage is seen especially in the case of very posteriorly located parathyroid glands.[25,34,35,50]According to data from Barczynski *et al*. from 2006, patients have less pain after this procedure compared with directed parathyroidectomy. There is reduced need for postoperative analgesia, a shorter incision wound and better cosmetic results, but at the cost of a longer intervention duration and higher intervention costs. The number of video-assisted parathyroidectomy conversions to the open-surgery and the length of hospitalization (compared to directed parathyroidectomy) were not statistically significantly different. There are no data available on long-term outcomes and effectiveness of the intervention.[50]

Video-assisted parathyroidectomy can be performed under local or general anaesthesia.[49,50] The procedure is performed via a central incision located centrally above the collarbone. The endoscope of 5 mm in size is introduced (without a trocar) at an angle of 30 degrees via a lateral incision on the side of the pathological parathyroid gland.[35]

Gas insufflation is not used in comparison with total endoscopic surgery.[50] The endoscope is more mobile, but it requires an additional assistant to manage it. The procedure is performed in the same way as directed parathyroidectomy.[35,50] Some authors also use endoscopes for bilateral exploratory parathyroidectomy in patients with multiglandular disease.[25,31,35]

Radioguided parathyroidectomy

Radioguided parathyroidectomy is a type of minimally invasive surgery, during which the gamma probe is used to guide the surgeon towards the location of the pathological parathyroid gland.[35] As with all minimally invasive procedures, good preoperative imaging plays an important role.[17-20] Good interaction between the entire surgical team and nuclear medicine experts is a prerequisite for this type of surgical intervention. The patient is given an intravenous dose of ^{99m}Tc-sestamibi approximately 2 to 4 hours before the surgery.[35] The uptake of sestamibi into the parathyroid adenoma cells depends on their activity.[20] Intraoperatively, a gamma probe is then used to trace the location with the highest radioactivity (which must be at least 20% higher than background neck activity). The surgeon thus determines the best incision site.[35] However, the use of radioactivity to determine the location has its limitations. The thyroid pathology, which can have increased sestamibi uptake, may be mistaken for an abnormal parathyroid gland. When searching for radioactive ectopic parathyroids in the chest, high concentration of the isotope in the myocardium can be mistakenly detected. If the tissue resected is radioactive and the neck shows equal radioactivity after excision, the patient can be assumed cured.[51] Nonetheless, iPTH assay is used to determine the complete excision of all hyperfunctioning tissue.[21,51]

The method described above is used only by few experienced surgeons.[35] It is mostly used as an alternative method or just as an aid for the surgery. According to Burkey *et al*., the reliability of the gamma probe is only 66% and therefore is not a consistently reliable tool for routine use.[51]

Robotic parathyroidectomy

Robotic-assisted parathyroidectomy is a modern technique and it represents the new generation in the evolution of minimally invasive parathyroidectomy techniques.[52] The beginnings of telerobotic surgical technology go back to the early 21st century when a new established idea of robotic-assisted surgery offered solutions in overcoming the limitations associated with endoscopic techniques (two-dimensional view of the operative filed, limited manipulation with surgical instruments, need for carbon dioxide insufflation and assistants during surgery).[52,53] In 2011, Tolley *et al*. introduced and performed the first robotic-assisted parathyroidec-

tomy without the scar in the neck (by infraclavicular incision), using the da Vinci® surgical system.[53]

As for every other minimal invasive surgical approach, preoperative imaging studies that assist in the localization of lesions have been key elements in patients' selection for targeted robotic-assisted parathyroid surgeries.[17-20,54] Before robotic-assisted parathyroidectomy is preformed, parathyroid adenoma should be properly localized.[54] Tolley *et al*. preformed first robotic-assisted surgery (RAS) for parathyroidectomy in which they made an incision in infraclavicular region on the side of the lesion. Following that, three other small incisions had been performed in the axillary line on the ipsilateral side without using insufflation. Adenoma was successfully removed in all 11 patients that were represented in this pilot study for evaluating robotic parathyroidectomy; although it should be noted that during one of the RAS procedures the surgeon had to switch to the conventional open approach due to unsuitable habitus of the patient. Nevertheless, the robotic procedure took an average of 61 minutes.[53] Many improvements and upgrades have since been introduced into this minimal invasive surgical technique. Nowadays, only one small incision is necessary for RAS.[52] However, due to high prices of robotic systems and other equipment, robotic-assisted surgical technique in parathyroidectomy has been an alternative procedure only in selected medical centres in the USA and Great Britain.[53,54] There are only few known studies about RAS in parathyroidectomy, yet all the known data supports robotic-assisted parathyroidectomy as an equally successful and safe approach for treatment of PHPT as conventional open or endoscopic assisted parathyroidectomy (level 2 and 3 diagnostic evidence).[52] Despite an excellent cosmetic outcome due to small incision and a safe approach to the surgery, high expenses, limited equipment, long duration of the procedure and technical difficulties represent limitations in choosing ideal candidates for RAS.[52-54]

Conclusions

Parathyroid surgery is the only potentially curative treatment of PHPT. Over the last two decades, technological advances and better understanding of the disease have refined surgical techniques. Surgery represents the last step in the overall patient care. Disease recognition, a proper diagnostic process and preoperative preparation of the patient are crucial for choosing the best surgical procedure. The aim of this paper was to discuss newer surgical techniques and their advantages and limitations in comparison to classical methods.

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research article

Sequential intra-arterial infusion of ^{90}Y-resin microspheres and mitomycin C in chemo refractory liver metastatic breast cancer patients: a single centre pilot study

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Background. The aim of the study was to evaluate the safety and feasibility of intra-arterial mitomycin C (MMC) infusion after selective internal radiation therapy (SIRT) using Yttrium-90 (^{90}Y) resin microspheres in liver metastatic breast cancer (LMBC) patients.

Patients and methods. The prospective pilot study included LMBC patients from 2012–2018. Patients first received infusion of ^{90}Y resin microspheres, after 6–8 weeks response to treatment was assessed by MRI, ^{18}F-FDG PET/CT and laboratory tests. After exclusion of progressive disease, MMC infusion was administrated 8 weeks later in different dose cohorts; A: 6 mg in 1 cycle, B: 12 mg in 2 cycles, C: 24 mg in 2 cycles and D: maximum of 72 mg in 6 cycles. In cohort D the response was evaluated after every 2 cycles and continued after exclusion of progressive disease. Adverse events (AE) were reported according to CTCAE version 5.0.

Results. Sixteen patients received ^{90}Y treatment. Four patients were excluded for MMC infusion, because of extra hepatic disease progression (n = 3) and clinical and biochemical instability (n = 1). That resulted in the following number of patient per cohort; A: 2, B: 1, C: 3 and D: 6. In 4 of the 12 patients (all cohort D) the maximum dose of MMC was adjusted due biochemical toxicities (n = 2) and progressive disease (n = 2). One grade 3 AE occurred after ^{90}Y treatment consisting of a gastrointestinal ulcer whereby prolonged hospitalization was needed.

Conclusions. Sequential treatment of intra-arterial infusion of MMC after ^{90}Y SIRT was feasible in 75% of the patients when MMC was administrated in different escalating dose cohorts. However, caution is needed to prevent reflux after ^{90}Y SIRT in LMBC patients.

Key words: liver metastatic breast cancer; chemo resistant; intra-arterial therapy; radioembolization; selective internal radiation therapy; mitomycin C infusion

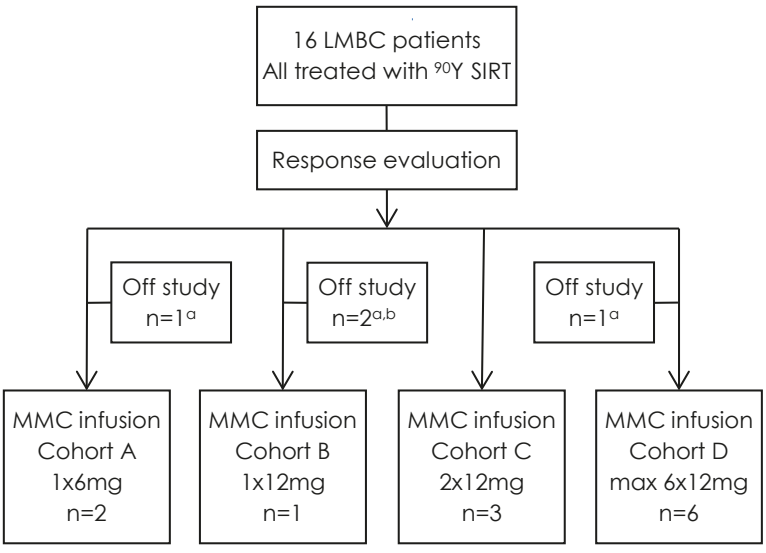
Introduction

Up to 50% of the metastatic breast cancer (mBC) patients eventually develop liver metastases which is associated with a poor overall survival ranging from 1–3 years.[1,2] Liver metastatic breast cancer (LMBC) patients with limited (extra) hepatic disease can obtain a survival benefit from local

treatment options, such as surgery and ablation.[3,4] Unfortunately, most LMBC patients have extended disease whereby systemic treatment by chemotherapy or anti-hormonal therapy, in hormone sensitive breast cancer, is indicated. Systemic treatment with chemotherapy has lengthened overall survival, but haematological, gastrointestinal and neurotoxicity are dose-limiting factors with systemic administration of these agents.[5] Therapies with less systemic toxicity are therefore an attractive treatment option in LMBC patients refractory to systemic chemotherapy.[6]

Several intra-arterial therapies have been applied for LMBC patients, such as radioembolization (Yttrium-90 (^{90}Y) resin microspheres), chemo embolization and chemo infusion, reported as effective and safe treatment options.[7-9] Of these treatments, the best results are reported for intra-arterial radioembolization, with a disease control rate of 72–91% and an overall survival of 6.6–13.6 months.[7,10-12] For over 15 years, LMBC patients are treated by intra-arterial chemo infusion with mitomycin C (MMC) in our institute whereby disease control is obtained in 58% with low adverse events in a very heavily pre-treated cohort.[9]

Since primary breast cancer is sensitive for both systemic chemotherapy and external beam radiation therapy, LMBC patients might also benefit from the combined treatment of chemo infusion and selective internal radiation therapy (SIRT). However, the sequential administration of these intra-arterial therapies *e.g.* SIRT and intra-arterial chemo infusion, has not yet been reported. In this pilot study we prospectively analysed the safety and feasibility of the sequential intra-arterial infusion of ^{90}Y resin microspheres and MMC in patients refractory to conventional systemic chemotherapy.



[a] Due to extra hepatic disease progression
[b] Due the biochemical and clinical toxicities

FIGURE 1. Treatment overview of the 16 patients treated with selective internal radiation therapy with Yttrium-90 containing microspheres (^{90}Y SIRT) and mitomycin C (MMC) infusion in 4 escalating cohorts.

Patients and methods

This prospective phase I pilot study of 16 patients was approved by the ethics committee of our hospital (S 53657). Patients were included from April 2012 until March 2018. All patients signed a written informed consent. Patient selection was performed by a multidisciplinary tumour board consisting of medical, surgical and radiation oncologists together with pathologists, nuclear medicine physicians and (interventional) radiologists.

In Table 1, the inclusion and exclusion criteria for this pilot study are shown. Figure 1 shows the treatment schedule of ^{90}Y SIRT followed by MMC infusion in the different cohorts. The first 3 patients were allocated to cohort A, the second 3 patients to cohort B, the 3 patients thereafter to cohort C and the remaining of the patients in cohort D. Patients first underwent the ^{90}Y treatment and in the absence of progression, the sequential intra-arterial hepatic MMC infusions were administrated 8 weeks later.

Radioembolization with Yttrium-90 (^{90}Y) resin microspheres

All ^{90}Y procedures were performed under local anaesthesia by or under direct supervision of an expert interventional radiologist (GM) and nuclear medicine physician (CD). Previously published work describes the details of the ^{90}Y treatment.[13] Briefly, patients first underwent a detailed baseline angiogram and embolization of enterohepatic arteries with micro-coils according to the expertise of the interventional radiologist. Technetium-99m-macroaggregated albumin (^{99m}TC-MAA) was injected in the target vessels and a planar scintigraphy was performed to determine the lung shunt fraction. Two weeks later patients underwent bi-lobar or uni-lobar injection of ^{90}Y resin microspheres (SIR spheres®, Sirtex Inc, Cosgrove, Australia) under local anaesthesia. The activity of ^{90}Y injected in the patient was calculated based on the body surface area method. In patients where it was possible to use the partition model, the partition model was used instead of the body surface area method. Dependent

TABLE 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Histologically confirmed diagnosis of breast cancer	Bilirubin level > 1.5x upper limit normal
Radiological evidence of liver metastases	Transaminase (AST/ALT) > 2.5x upper limit normal
Liver only or liver predominant with stable extra-hepatic disease	Creatinine > 1.2x upper limit normal
Progressive under (multi-line) systemic chemo or hormonal therapy	Glomerular filtration rate < 60 mL/min/1.73 m^2
Eligible for intra-arterial therapy	Neutrophils < 1000/μL
Age > 18 years	Thrombocytes < 100x10^9/L
Karnofsky performance > 70	Lung shunt fraction > 20%
	Allergy to contrast media
	Active use of oral anticoagulation

ALT = alanine aminotransferase; AST = aspartate aminotransferase

on the site of lesions, the ^{90}Y resin microspheres were injected intra-arterially via a micro catheter to the right and left liver lobe (superselective). A Progreat® micro catheter (Terumo, Europe) or an anti-reflux catheter (Surefire Inc., Westminster, CO, USA) was used for injection of the microspheres. Patients received antiemetics and morphine derivates before ^{90}Y injection when required.

Hepatic intra-arterial chemo infusion with MMC

Six to eight weeks after the ^{90}Y treatment, patients underwent response assessment by magnetic resonance imaging (MRI) of the liver and a whole body [^{18}F]-FDG positron emission tomography/computed tomography scan (^{18}F-FDG PET/CT) together with laboratory tests. If patients showed no untreatable progression of disease on MRI and ^{18}F-FDG PET-CT scan (bases on RECIST criteria 1.1) together with acceptable laboratory results (> 100x10^9 thrombocytes/L), the intra-arterial chemo-infusion of MMC could be administrated. Six to ten weeks after the ^{90}Y procedure, patients received the first infusion of MMC, as previously described by our group.[14] After local anaesthesia, vascular access was obtained by the right common femoral artery and, using a diagnostic 4-French catheter, a micro catheter was placed in the right and left hepatic arteries for injection of MMC in both liver lobes.

Ascending maximal doses of MMC were administrated in different cohorts. Patients were allocated to the MMC cohort before the ^{90}Y procedure. The first cohort (A) received one cycle of 6mg MMC, the second cohort (B) received one cycle of 12mg MMC, the third cohort (C) received 2 cycles of 12mg of MMC and the fourth cohort (D) received the standard regime of MMC infusion consisting of a maximum of 6 cycles of each 12mg of MMC. Before and after the MMC infusion laboratory results were taken to determine whether the dose could be tolerated. The dose of MMC was adjusted when thrombocyte count was < 100x10^9 thrombocytes/L. In the last cohort, the effect of MMC was radiologically evaluated, by CT or MRI, after every two cycles. If these evaluations showed no further intra- or extra-hepatic disease progression, the patient was rescheduled for the next 2 cycles for a maximum of 6 cycles.

Outcomes

The primary outcomes of this study included the feasibility and safety of MMC infusion after ^{90}Y SIRT. The feasibility was defined as the number of patients receiving the MMC infusion after ^{90}Y treatment. The safety was determined by the number of grade 3 and higher adverse events after MMC and ^{90}Y treatment and documented by the Common Terminology Criteria for Adverse Events version 5.0. For the response assessment the modified response evaluation criteria in solid tumours (mRECIST) was used to measure hepatic response by a maximum of two target lesions and categorized into four categories [Complete Response (CR), Partial Response (PR), Stable Disease (SD) and Progressive Disease (PD)].[15]

Statistical analysis

Patient and pre-treatment characteristics were presented as numbers, percentages and median time

with range. Overall survival (OS) was calculated from work-up for ^{90}Y treatment until death or loss to follow up by the Kaplan Meier estimates. All analyses were performed in SPSS (IBM Corp, version 25, Armonk, NY).

Results

Patient characteristics

Table 2 shows the patient characteristics of the 16 treated women. The median time from diagnosis of metastatic breast cancer to start of the study was 28 months (range 7.7–91.0 months). Patients were 26–77 (median 59) years old at the start of the study.

TABLE 2. Patient characteristics

Patient Characteristics	N = 16
Median months from diagnosis metastatic disease until start of study	28 (8–91)
Median age at start study in years (range)	59 (26–77)
Diagnosis of liver metastasis	
Synchronous	2 (12.5%)
Metachronous	14 (87.5%)
Hormone status of liver metastasis (n = 15)	
Estrogen receptor (positive)	8 (53%)
Progesterone receptor (positive)	8 (53%)
HER2Neu receptor (negative)	15 (100%)
Triple negative receptor status	6 (40%)
Tumor burden liver	
< 25%	9 (56%)
25%–50%	6 (38%)
50%–75%	1 (6%)
Prior hepatic treatment	
Surgery/Ablation	1 (6.3%)
Median number of chemotherapy regimens for stage 4 disease	3 (0–8)
Extra-hepatic sites of metastases	
Yes	9 (56%)
No	7 (44%)
Number of additional metastatic sites	
1	5 (31%)
2	2 (13%)
≥ 3	2 (13%)
Location of extrahepatic metastases	
Bone	5 (31%)
Lung	3 (19%)
Non-locoregional nodes	4 (25%)
Brain	2 (13%)

A liver biopsy was taken before start of the study in 15 patients and showed a positive oestrogen receptor in 8 patients, positive progesterone receptor in 8 patients and a negative HER2-neu status in all patients (n = 15). Six patients had a triple negative receptor status. Tumour burden of the liver was < 25% in 9 patients and > 25% in 7 patients. Nine patients had extra-hepatic disease with a median of 1 site (range 0–4) and bone (n = 5) as most common site. Before inclusion, the median number of chemotherapy lines in the metastatic phase was 3 regimes consisting of anthracyclines in 75% and taxanes in 81%.

Treatment

Figure 1 shows a treatment overview of the 16 patients. All 16 patients underwent the ^{90}Y procedure whereby pre-embolization of extra hepatic arteries was performed in 14/16 patients. A median dose of 1.68 GBq (range 1.043–2.140) was inserted in both liver lobes (n = 14) or one liver lobe (n = 2). At response evaluation five patients showed extra-hepatic disease progression, of whom, three patients were excluded for additional MMC infusion and two patients received the sequential MMC infusion, but with additional therapy of Exemestane (Aromasin®) for stabilization of their extra-hepatic disease. One patient was hospitalized after ^{90}Y SIRT for 2.5 weeks because of the side effects of the ^{90}Y treatment and excluded from further treatment.

Median time between ^{90}Y SIRT and first MMC infusion was 2.1 months (range 1.7–12.8 months). In one patient an interval of 12.8 months occurred, because of PD in the liver after ^{90}Y treatment. This patient first received systemic chemotherapy and after progression during this systemic chemotherapy the MMC infusion was administrated. A total of 12 patients were treated in four different cohorts of MMC doses after the ^{90}Y treatment (Figure 1). In cohort D, the maximum of 6 cycles of 12 mg MMC was administrated in 2 patients. In the other four patients the dose was adjusted (n = 2) and discontinued (n = 2) due to hematologic toxicity (thrombocytopenia) and disease progression.

Adverse events

Table 3 shows the adverse events after the ^{90}Y SIRT and MMC infusion. After the ^{90}Y treatment one grade 3 adverse event occurred, consisted of a gastric ulcer treated with proton pump inhibitors and hospitalization. The most common grade 1/2 adverse events after ^{90}Y SIRT were pain 7/16, nausea

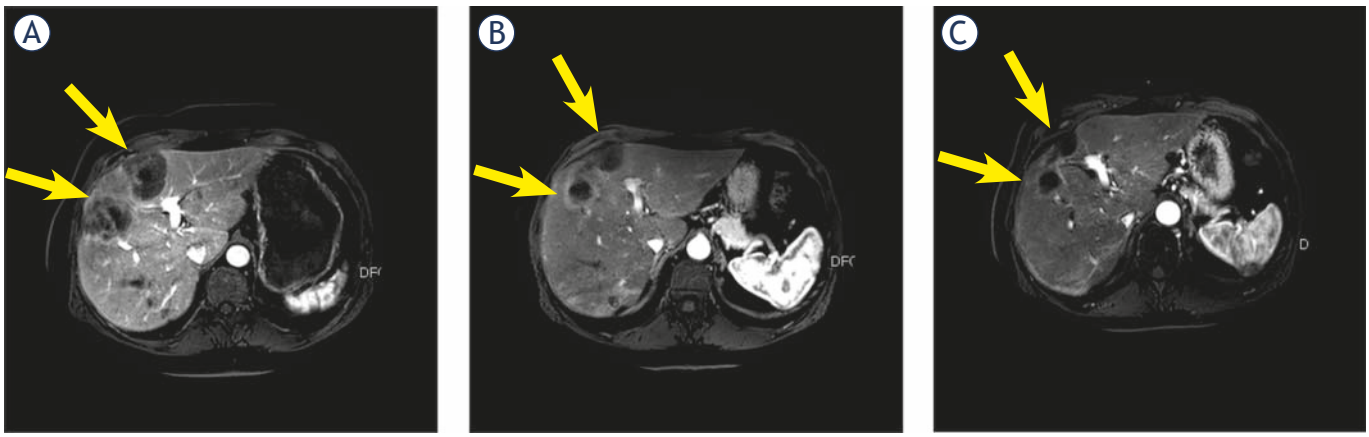


FIGURE 2. Hepatic response on magnetic resonance imaging (MRI) before ^{90}Y treatment **(A)**, after ^{90}Y treatment **(B)** and after 2 cycles of MMC infusion **(C)**. C is a partial response relative to A. Arrows indicate the liver metastases.

5/16 and ulcer 4/16. An elevation in liver function test (aspartate, alanine and gamma-glutamyl transferase) was seen in 8/16 patients.

No grade 3 or higher adverse events occurred after MMC infusion. The most common grade 1/2 adverse event was thrombocytopenia in 11/12 patients and leukopenia in 8/12 patients.

Response, follow up and survival

Response evaluation of the liver after 6 to 8 weeks after ^{90}Y treatment (n = 16) revealed PR in 10 patients and SD in 6 patients (Figure 2). Extra hepatic response evaluation showed PD in 5 patients and SD in 11 patients. Additional hepatic response of

TABLE 3. Number of grade 1 (G1), grade 2 (G2) and grade 3 (G3) adverse events after treatment of selective internal radiation therapy by ^{90}Y labeled microspheres infusion (^{90}Y SIRT) and mitomycin C (MMC) infusion within 30 days. No grade 3 adverse events occurred after MMC infusion

Treatment	^{90}Y SIRT			MMC Cohort A 1x6mg		MMC Cohort B 1x12mg		MMC Cohort C 2x12 mg		MMC Cohort D max 6x12mg	
Adverse events	**G1**	**G2**	**G3**	**G1**	**G2**	**G1**	**G2**	**G1**	**G2**	**G1**	**G2**
Clinical											
Fatigue	2/16	-	-	-	-	-	-	3/3	-	2/6	-
Pain	5/16	2/16	-	-	-	-	-	-	-	1/6	-
Nausea	3/16	2/16	-	-	-	-	-	-	-	1/6	-
Emesis	2/16	2/16	-	-	-	-	-	-	-	1/6	-
Gastrointestinal ulcer	-	4/16	1/16	-	-	-	-	-	-	-	-
Biochemical											
Leukopenia	2/16	-	-	-	-	-	-	2/3	-	4/6	2/6
Thrombocytopenia	3/16	1/16	-	1/2	-	1/1	-	3/3	-	4/6	2/6
Anaemia	-	-	-	-	-	-	-	-	-	4/6	-
Increased aspartate aminotransferase	5/16	1/16	-	-	-	-	-	-	-	2/6	-
Increased alanine aminotransferase	3/16	1/16	-	-	-	-	-	-	-	1/6	-
Increased bilirubin	-	-	-	-	-	-	-	1/3	1/3	-	-
Increased alkaline phosphatase	2/16	1/16	-	-	-	-	-	1/3	-	2/6	-
Decreased eGFR	-	1/16	-	-	-	-	-	-	-	-	-
Increased gamma-glutamyl transferase	4/16	1/16	-	-	-	-	-	1/3	-	4/6	-

eGFR = estimated glomerular filtration rate

MMC infusion after ^{90}Y treatment was PR in 4/12 patient, SD in 5/12 patients and PD in 3/12 patients.

After the study, nine patients were eligible to receive systemic chemotherapy and one patient was eligible for surgery and underwent a segmentectomy. The median OS was 12.6 months (95% CI 10.23–15.0).

Discussion

Intra-arterial therapy is a treatment option in patients with chemo-refractory liver dominant metastatic breast cancer.[6] In our institute MMC infusions are performed for almost 20 years for chemo refractory LMBC whereby a disease control of 58% is obtained with low grade adverse events.[9] In the present study we investigated whether MMC infusion is feasible and safe after ^{90}Y SIRT.

The sequential treatment of ^{90}Y followed by MMC was possible in 75% (12/16) of the enrolled patients. Only low-grade adverse events (grade 1 and 2) were observed after the MMC infusions. In addition, the gradual escalation of the MMC infusions, ranging from 6 mg in 1 cycle to a maximum of 72 mg in 6 cycles, caused no additional adverse events.

During the regular MMC protocol of our institute, patients can receive a maximum of 72 mg in 6 cycles whereby close monitoring is performed to adjust, postpone or stop the MMC infusion after each cycle.[9] In current study, the same protocol was followed in the last cohort, subsequently 4 patients did not receive all 6 MMC cycles. Thus, this pilot shows that the regular protocol of MMC can be followed after prior treatment with ^{90}Y. Patients maintained their performance status after the sequential treatments, and so 9/12 patients were eligible for systemic therapy afterwards and 1/12 even for hepatic surgery. Therefore, this combination treatment can be used as a drug holiday from systemic treatment.

We did observe a high number of gastrointestinal ulcers after ^{90}Y SIRT (n = 5, 31%). All these patients underwent pre-embolization and received a bilobar treatment in the left and right hepatic artery. Despite these precautions, the ulcers occurred probably due to reflux and non-target deposition of the ^{90}Y resin microspheres which is a known risk of ^{90}Y treatment for LMBC.[16] After the introduction of an anti-reflux catheter in the last 6 patients, no more ulcers occurred. According to the literature, several factors are associated with reflux of ^{90}Y resulting in gastrointestinal ulcering, namely prior administration of monoclonal antibodies (*i.e.* bevacuzimab, trastuzumab), the type of ^{90}Y microspheres and the dose of ^{90}Y.[10, 17] In the present study no correlations were found for prior monoclonal antibodies treatment and the dose of ^{90}Y with the development of gastrointestinal ulcers. The development of the gastrointestinal ulcers in the current study was probably caused by several pretreatment and treatment related factors. The use of the anti-reflux catheter showed improvement with no further reported gastrointestinal ulceration. Therefore, caution is needed in future studies.

The median OS of radioembolization in LMBC patients ranges from 6.6–13.6 months, which is in line with the median OS of 12.6 in this study.[10,11,18-20] The median OS was higher compared to earlier studies where patients received only MMC infusion (12.6 versus 7.6 months).[9,14] However, the inclusion criteria for this study were more strict than our routine indications for MMC infusion. Consequently, patients had less extensive local and extra hepatic disease with a better performance status compared to our previous reported cohort of patients treated with MMC infusion only.

The main limitation of this study consists of small patient number in the cohorts and the long inclusion period.

Conclusions

In conclusion, the sequential treatment of intra-arterial infusion of MMC after ^{90}Y therapy is feasible with limited low-grade adverse events after MMC. The regular MMC protocol can be followed with adjustment of MMC dose based on clinical, radiological and biochemical parameters. However, caution is needed to prevent reflux after ^{90}Y SIRT in LMBC patients. Further research is needed to show whether the combination of ^{90}Y and MMC has a benefit over the treatment of MMC or ^{90}Y alone in LMBC patients.

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research article

Scintigraphic load of bone disease evaluated by DASciS software as a survival predictor in metastatic castration-resistant prostate cancer patients candidates to 223RaCl treatment

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Background. Aim of our study was to assess the load of bone disease at starting and during Ra-223 treatment as an overall survival (OS) predictor in metastatic castration-resistant prostate cancer (mCRPC) patients. Bone scan index (BSI) is defined as the percentage of total amount of bone metastasis on whole-body scintigraphic images. We present a specific software (DASciS) developed by an engineering team of "Sapienza" University of Rome for BSI calculation.

Patients and methods. 127 mCRPC patients bone scan images were processed with DASciS software, and BSI was tested as OS predictor.

Results. 546 bone scans were analyzed revealing that the extension of disease is a predictor of OS (0–3% = 28 months of median survival (MoMS]; 3%–5% = 11 MoMS, > 5% = 5 MoMS). BSI has been analyzed as a single parameter for OS, determining an 88% AUC. Moreover, the composition between the BSI and the 3-PS (3-variable prognostic score) determines a remarkable improvement of the AUC (91%), defining these two parameters as the best OS predictors.

Conclusions. This study suggests that OS is inversely correlated with the load of bone disease in mCRPC Ra-223-treated subjects. DASciS software appears a promising tool in identifying mCRPC patients that more likely take advantage from Ra-223 treatment. BSI is proposed as a predictive variable for OS and included to a multidimensional clinical evaluation permits to approach the patients' enrollment in a rational way, allowing to enhance the treatment effectiveness together with cost optimization.

Key words: DASciS software; radium223 dichloride; bone scan index; bone disease; overall survival; mCRPC

Introduction

Bone metastasis is present in 90% of patients with metastatic castration-resistant prostate cancer (mCRPC).[1] Radium-223 dichloride (Ra-223) is an alpha-emitter effective to relief bone metastasis' pain and prolong survival. It is approved as treatment of mCRPC patients with symptomatic bone metastasis and no evidence of visceral metastatic involvement. Since 2018 The European Medicines

Agency (EMA) restricted the use of Ra-223. Today this treatment in reserved for those patients who have already followed two prior systemic treatments for bone-mCRPC or those who are ineligible for other treatments. The EMA also issued a contraindication for use in combination with abiraterone acetate plus prednisone/prednisolone.[2] Ra-223 has a positive impact in limitation of osteoblastic cellular growth in the metastatic and in the bony environment.[3] Ra-223 transfers a high amount of energy (80 keV/μm), in the form of alpha particles with a Linear Energy Transfer (LET) of 27.4 MeV, in a short action range (100 μm). It produces double strand's breakings in tumor cells' DNA with a cytotoxic effect. Thanks to the high LET and the short action range, this treatment has a limited hematological toxicity[4-6] associated with a reduction in pain and an improvement in quality of life.[7,8]

At the moment a validated standard technique to monitor mCRPC patients treated with Ra-223 does not exist, in spite several imaging techniques have been proposed such as PET and MRI.[9,10] Bone scintigraphy is commonly used, thanks to its wide availability and low cost. Moreover, it represents a standard tool recommended for clinical trials designed for CRPC.[11] This imaging technique allows to determinate the skeletal disease burden in these patients, but it is a low accuracy modality to quantify disease or for demonstrating treatment effects because of its low spatial resolution.[12] Indeed, it doesn't specifically identify cancer and it can paradoxically worsen in the face of response (flare phenomenon). Moreover, it frequently shows a slow improvement during active treatments, or it doesn't improve at all. Bone Scan Index (BSI), is defined as the percentage total amount of bone metastasis on whole-body scintigraphic images. It could be a valid tool to assess disease burden in patients with bone metastasis and to evaluate how disease burden changes during treatments. BSI allows to evaluate bone scintigraphy data as a single reproducible quantitative measure, so that it is possible to estimate the bone disease's charge. BSI can be calculated with a specifically developed software, the EXINIBone (BONENAVI software in Japan) and has been validated as an OS predictor in some patient's mCRPC treatment settings.[13-15] However, at the moment EXINIBone is not commercially available in all European countries. In this study we present a newly developed software by an engineering team of "Sapienza" University of Rome for specific BSI calculation (DASciS software).

The aim of our study was to perform a BSI evaluation in a Ra-223 treated patients' cohort, in order to calculate the load of bone disease at starting of treatment and to identify its variations during the treatment as an overall survival (OS) predictor. Another issue addressed in this study was to compare head to head and in association, the OS predictive ability of 3 variable Prognostic Score (3PS), a multidimensional predictive tool proposed by our group in this specific patient setting[16], with BSI, both evaluated at baseline time. The 3-PS (3-variable prognostic score), is a multidimensional clinical evaluation based on: hemoglobin (Hb), Eastern Cooperative Oncology Group (ECOG) performance status (PS) and serum prostate specific antigen (PSA) baseline value. It has been demonstrated that the 3-PS is able to select those mCRPC subjects most suitable to receive the maximum benefit from Ra-223 treatment. It has also been tested as a predictor marker of OS[16] resulting to have a higher accuracy than total alkaline phosphatase (tALP).

Patients and methods

This was an observational, retrospective cohort study performed in 127 mCRPC patients (all patients had biochemical progression of disease under Androgen Deprivation therapy, serum testosterone level < 50 ng/dL, a condition that is considered irreversible even if recently there is evidence of a radio-induced reversion[17], with symptomatic bone metastases receiving Ra-223, enrolled at our Division of Nuclear Medicine. Informed consent was obtained from all individual participants included in the study. It was approved by the local ethical committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Baseline clinical data relevant to the survival analysis were collected, such as age, height and weight, Gleason Score, ECOG PS, number of systemic treatments prior to Ra-223 therapy. Body Mass Index (BMI) was calculated for each patient. Patients' characteristics are summarized in Table 1.

After each therapy's cycle and during the follow-up patient's blood count, PSA and tALP were collected, in order to identify hematological toxicity and to monitor the therapy's effectiveness. 76.6% of patients without visceral metastasis completed the Ra-223 treatment by administering 6 intravenous injections (55 kBq per kg of body weight) every 28 days (Table 1).[18] A ^{99m}Tc-hydroxyethylene diphosphonate (HDP) bone scan was performed before starting the treatment, after 2 or 3 cycles of therapy and after the treatment's end. Follow-

TABLE 1. Baseline patients' characteristics

Baseline variable	Patients (n = 127)	%
Age (years) Mean (range)	73.82 (59–90)	
Heigth (m) Mean (range)	1.71 (1.58–1.95)	
Weight (kg) Mean (range)	78.60 (59–120)	
BMI Mean (range)	26.75 (19.57–39.18)	
Gleason score Mean (range)	6.3 (5–10)	
5	1	0.79
6	3	2.36
7	38	29.92
8	30	23.62
9	28	22.04
10	2	1.59
Unknown	25	19.68
ECOG Performance status Mean (range)	0.86 (0–3)	
0	39	30.7
1	68	53.54
2–3	20	15.75
Extent of skeletal disease		
< 6 metastases	18	14.17
6–20 metastases	46	36.22
> 20 metastases	63	49.61
No. of previous systemic treatments after castration resistance (Docetaxel, Cabazitaxel, Abiraterone, Enzalutamide)		
0	32	25.19
1	42	33.07
2	32	25.19
≥ 3	21	16.53
No. of therapy's cycles administered Mean (range)	5.56 (2–6)	
2	3	2.36
3	3	2.36
4	10	7.87
5	14	11.02
6	97	76.38
No. of systemic treatments after Radium223		
Docetaxel	11	8.6
Cabazitaxel	0	0
Abiraterone	2	1.5
Enzalutamide	6	4.7
Baseline Hb Median (range)	12.14 (7.5–15)	
< 12 g/dl	54	42.51
≥ 12 g/dl	73	57.48
Baseline tALP* Median (range)	300 (34–1750)	
< 226 U/l	79	62.2
≥ 226 U/l	48	87.8
Baseline PLT (10^3/mm^3) Median (range)	249 (74–763)	
Baseline PSA		
< 20 ng/ml	42	33.07
≥ 20 ng/ml	85	66.92

*Cut-off value validated in a previous study[16]

BMI = Body mass index; ECOG = Eastern Cooperative Oncology Group; Hb = hemoglobin; tALP = total alkaline phosphatase; PLT = platelets count; PSA = serum prostate specific antigen

up scintigraphic image study was performed after 3 months, 6 months and 1 year from the end of the treatment. All the images were obtained 2 hours after an injection of 300-740 MBq of HDP.[19] Bone scan images were then processed with a software (DASciS software) that calculated BSI, developed by an engineering team from the Sapienza University.

DASciS software – which stands for Dicom Analyzer Scintigraphy Software – is an automatic tool for bone scan quantitation. Gamma Cameras usually output images in DICOM format. The output file contains the actual images together with all the metadata gathered during the exam. Through DASciS software, we can visually analyze those files, computing the area relative to the ill portions of the patient skeleton. The software performs the computation based on the intensity of the pixels. More specifically, once the operator has selected a pixel on the image that has been recognized as portion of ill skeleton, the software automatically selects all the pixels in the image whose intensity is equal or higher to the picked one. All those pixels are clustered into - potentially - multiple region of interests (ROIs). To avoid false positives - e.g. spot due to benign pathologies - and to refine the clustering process, it is possible to manually remove ROIs that are not interesting and to perform fine tuning over the pixel intensity threshold. Once the operator has successfully analyzed the file, DASciS outputs a file containing the statistics and the relevant metadata of the investigation. More specifically, the statistics include the cumulative percentage of ill regions computed with respect to the total image area of the patient over multiple sessions - i.e. corresponding to different scintigraphy acquisitions. The metadata, instead, contain patient generalities and the date of acquisition. From a more technical point of view, the software has been developed in Java to grant cross-compatibility with all the most used Operating Systems (Windows, MacOS, Linux) and to easily prototype an effective Graphical User Interface (GUI). Image processing is performed using the well-known OpenCV library[20] while DICOM files are handled using the open source PixelMed library. The OpenCV library implements several efficient Computer Vision algorithms, like the ones used in DASciS to perform intensity-based clustering of pixels – based on the well-known approach of Suzuki[21] - and to compute the cluster area – through the Green's theorem.[22]

Summarizing, the DASciS software semi-automatically identifies all the areas of increased fixation of bone targeted radiotracer, that appear as

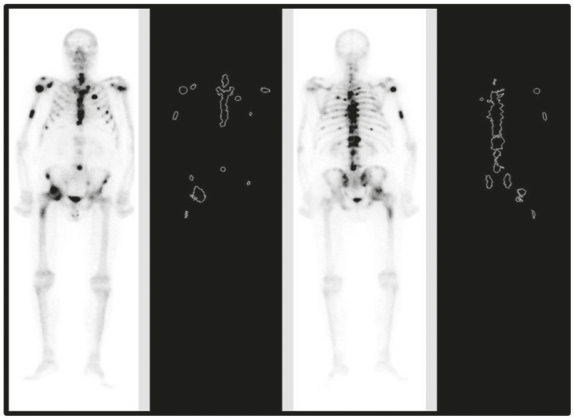

FIGURE 1. Patient of our cohort with bone metastases analyzed with DASciS software. Bone scan index (BSI) value = 5.26%.

spots on the image, only requiring the operator to identify one of these areas. With this method is possible to make a quantitative analysis of the ROIs representing the metastatic bone towards the whole body bone mass, obtaining a percentage of bone metastatic load. Reproducibility of this method was examined by comparing results obtained by three independent, blinded operators, with different degrees of expertise in nuclear medicine techniques. BSI data obtained from images acquired before, during and after the treatment were then analyzed in an OS prediction's perspective. Aiming to evaluate a further correlation with OS, the baseline 3-PS was adopted. This is a predictor of OS, including Hb value, PSA and ECOG PS pretherapy as variables, that in our experience it has proved superior to tALP.[16] In this study, 3-PS was calculated for each patient and compared to BSI data as a predictor of OS.

Statistical analysis

The marginal and stratified survival distributions were estimated through the Kaplan-Meier product-limit estimator. The association between OS and predictors was evaluated by means of Cox regression. At multivariate analysis, for assessment of the independent prognostic performance of BSI, we first performed model selection through forward stepwise based on AUC, and then included all possible confounders. The prognostic performance of BSI alone and in addition to the 3-PS score was evaluated by means of time-dependent ROC curves and related AUC. In order to derive a simple scoring system based on the 3-PS and BSI, we dichotomized BSI and established the optimal number of points by rounding and scaling the log-hazard ratio coefficients in a bivariate model including 3-PS and BSI. To choose the optimal threshold for dichotomization of BSI we evaluated a grid of possible thresholds and maximized the final AUC in order to choose the best one. In order to assess reproducibility among three operators, the intra-class correlation coefficient was used. A significance level of 5% was specified before data analysis. All analyses are conducted in R version 3.4.0.

Results

546 bone scans, collected in the period between October 2013 and September 2018, were analyzed with the DASciS software. During the Ra-223 treatment, for all the 127 patients had a baseline bone scan (Figure 1). 122 of these patients had a second bone scan and 89 of them a third intermediate image. 87 patients had a final bone scan at the end of the treatment. During the follow- up, 60 images were available as a 3 month-after-treatment control, 38 after 6 months from the treatment's end and 23 patients completed the 1-year follow-up. Among 127 patients, 79 died in a period between the starting of the therapy and the year after its end. Exploring the inter-observer reproducibility of the DASciS software evaluation, the intraclass correlation coefficient for BSI was 0.815 (95% CI: 0.776–0.865; $p < 0.001$).

The primary endpoint of this study was to evaluate the association between baseline BSI and OS. OS cumulative incidence is reported in Figure 2. Available data were analyzed, revealing that the baseline percentage of disease is a predictor of OS (Table 2).

This results came from both the univariate analysis (HR: 1.8, 95% CI 1.61–2.02, $p < 0.001$) and the multivariate analysis (HR: 1.79, 95% CI 1.59–2.01, $p < 0.001$) and are also confirmed from the adjusting for all possible confounders measured (HR: 1.82, 95 CI 1.57–2.10, $p < 0.001$). As clearly shown in Table 3

TABLE 2. Expected survival for baseline bone scan index (BSI) intervals

% BSI	Patients number (n = 127)	Median survival (months)	0.95 LowCL (months)	0.95 UpCL (months)
0–3	59	28	19	NA
3–5	33	11	9	12
> 5	35	5	5	7

CL = confidence level

TABLE 3. Univariate and multivariable analysis of overall survival (OS) in relation to baseline variables

Clinical covariates	Univariate models HR (95% CI)	p-value	Multivariable model HR (95% CI)	p-value
BSI	1.80 (1.61–2.02)	< 0.001	1.79 (1.59–2.01)	< 0.001
Age (years)	1.02 (0.99–1.05)	0.184		
BMI	0.94 (0.89–1.00)	0.057		
Gleason Score	0.97 (0.91–1.04)	0.414		
ECOG Performance Status	1.97 (1.48–2.64)	< 0.001	1.74 (1.29–2.36)	< 0.001
N of previous systemic treatments	1.35 (1.12–1.164)	0.002		
Baseline Hb	0.73 (0.64–0.84)	< 0.001		
Baseline PLT / 100	1.51 (1.19–1.92)	< 0.001		
Baseline PSA / 100	1.06 (1.02–1.12)	0.006		
Baseline tALP /100	1.11 (1.06–1.17)	< 0.001		

BMI = body mass index; BSI = bone scan index; CI = Confidence interval; ECOG = Eastern Cooperative Oncology Group; Hb = hemoglobin; HR = hazard ratio; PLT = platelets count; PSA= serum prostate specific antigen; tALP = total alkaline phosphatase

at multivariate analysis only the load of bon disease and EGOG PS resulted statistically related with OS (Table 3).

In our series, only 19 patients received further lines of treatments after Ra-223 (11 received Docetaxel, 2 Abiraterone and 6 Enzalutamide), consequently, our sample does not lend itself to a detailed analysis of the role of the position of the Ra-223 in the sequence of treatment lines on OS.

The baseline BSI was analyzed as a single parameter for OS, determining an 88% AUC. A comparison between BSI and 3-PS was performed, revealing a superiority of BSI in term of OS prediction (the 3-PS AUC was 73%, while the BSI one was 88%). Moreover, the addition of the BSI to the 3-PS determines a remarkable improvement of the AUC (91%). In order to add BSI to the 3-PS we set a cut-off of 3, where patients with a BSI above the cut-off were given 4 points, and zero otherwise. From these data, it can be inferred that, the baseline BSI, used as a single parameter is better than baseline 3-PS in OS prediction. At the same time, using both BSI and 3-PS is the best way to predict the OS.

Scintigraphic data were collected during treatment and follow-up, however the BSI variation trend over time is not significant as a OS predictor (p = 0.36), as shown in Figure 3, where patients BSI values related to the time when they performed bone scans are represented in the boxplot. Index that takes in account BSI and 3-PS appears to be the best OS predictor (Figure 4).

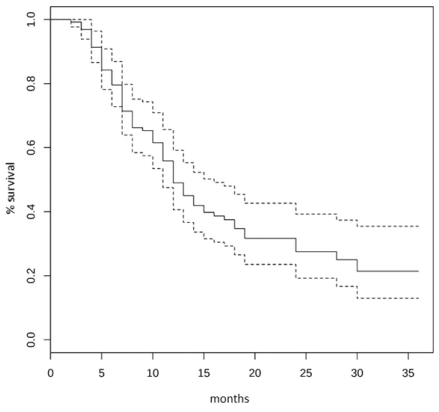

FIGURE 2. Kaplan-Meier curve for overall survival.

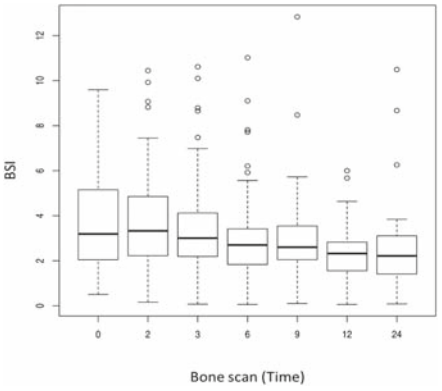

FIGURE 3. Bone scan index (BSI) over time.

0 = baseline bone scan; 2–3 = bone scan after 2 and 3 cycles of Ra-223 therapy; 6 = bone scan after treatment's end; 9 - 12 - 24 = follow-up scintigraphic image study respectively after 3 months, 6 months and 1.5 year from the end of the treatment

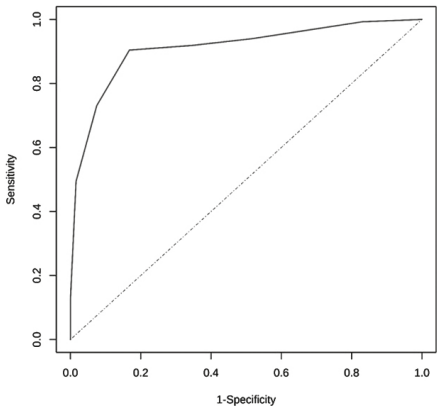

FIGURE 4. ROC curve of overall survival (OS) prediction for a score based on combining bone scan index (BSI) and 3-PS (AUC 91%).

Discussion

Evaluating the overall survival in mCRPC patients treated with Ra-223, several markers were analyzed (ECOG PS, tALP, Hb, PSA, number of previous systemic treatments), however, it was not possible to identify a predictive clinical variable assessing the Ra-223 therapeutic benefit.[23]

PSA can't be considered a valid marker of clinical benefit for this treatment, because Ra-223 acts directly on bone metastases microenvironment, rather than on prostate cancer cells: this can explain why there's a minor decline of this marker during the therapy.[24] On the other hand, this value can sometimes grow despite a good symptomatologic outcome of the treatment: this could be linked to a "flare phenomenon", similar to the one that's find in the administration of other anticancer drugs.[25,26]

tALP is generally considered the most reliable marker in in patients receiving Ra-223. The tALP generally declines at 4 weeks during this therapy. This pharmacodynamic trend was studied in a recent exploratory analysis on LDH, PSA and tALP dynamics.[27] Baseline tALP levels are not correlated to the efficacy of Ra-223, that's why tALP can't be considered as a predictive value in this setting. Data coming from literature have shown that baseline tALP level is prognostic for OS.[28] Anyway, an increased risk of death, time to progression, skeletal- related events, and bone marrow failure are possibly related to pre-treatment tALP levels (≥146 U/L): this admits to hypothesize a predicting role for this marker.[29] In addition to that, from a retrospective analysis of data from the ALSYMPCA trial emerged that patients treated with Ra-223 and with confirmed decline in tALP at week 12 had a significantly longer OS.[30] The 3-PS seems to fulfill this necessity of a predictor marker of OS.[16]

A further implementation of the OS prediction comes from BSI evaluation of bone disease that we propose in our study. Although PET with 18F-sodium fluoride has been shown to be more sensitive in assessing the burden of bone disease in this kind of patients[31], the authors evaluated bone scintigraphy with diphosphonates because it is available for all the Nuclear Medicine Centers, it is significantly cheaper and is the recommended test for enrolling patients in 223-Radium therapy. Interestingly our data are comparable with results available from literature, where, by applying a similar evaluation method, BSI inversely correlated with OS.[12,32] Indeed, the baseline BSI has a better predictive power than 3-PS. However, it is possible to obtain a major effectiveness of the marker by combining it with the 3-PS itself.

According to the EMA recommendations and Pharmacovigilance Risk Assessment Committee (PRAC)[2], the administration of Ra-223 treatment, should be started only after the failure of 2 different therapeutic strategies and in the presence of ≥ 6 bone metastasis. A treatment with Abiraterone acetate plus chemotherapy (Docetaxel, Cabazitaxel) or Enzalutamide should be performed before starting a therapy with Ra-223.[33] In addition to this, is recommended to not start Ra-223 treatment together with Abiraterone administration, because both these drugs act on bone metabolism (ERA 223 – NCT 02043678). Finally, there's an indication for Ra-223 treatment when no other therapeutic strategies are available.[33] At the end of study it can be concluded, in line with O'Sullivan *et al.*[34], that Ra-223 cannot be only reserved for third or later lines of therapy in mCRPC, moreover that is wrong considering the presence of ≥ 6 bone metastasis as an inclusion criterion for its use. This study indeed suggests that OS is inversely correlated with the baseline BSI (the longer OS is expected with the lowest BSI value). Independently from the number of previously administered therapies, from this analysis we can infer that the best OS can be obtained when the load of bone disease is < 5% of the whole skeletal mass. This results show that, it might be more appropriate to consider the percentage of disease burden than concentrating on the number of metastatic lesions drawing up the treatment's directions (Figure 5). A new interesting point of view could be carried by these results, towards the necessity of a re-evaluation of the Ra-223 therapeutic indications. Despite this, the limited number of our sample does not consent us to state definitive conclusions; indeed, a larger number of cases should be considered to confirm these results.

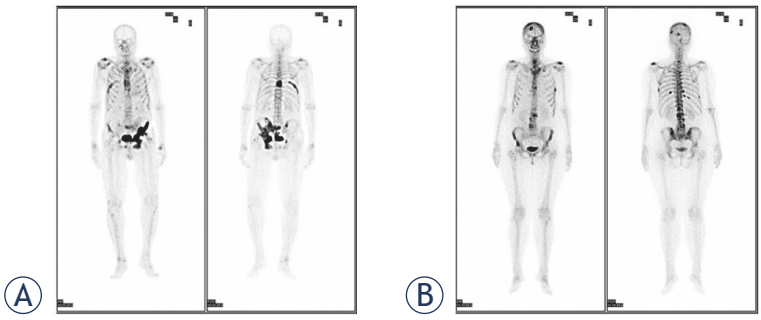

FIGURE 5. 99mTc-HMDP Bone scan, example of evaluation of load of the disease. **(A)** Few but very extensive bone metastases. Bone scan index (BSI) value = 6.13%. **(B)** numerous but small bone metastases. BSI value = 3.4%.

In addition to this, another limitation of this study could come from the follow-up time we considered. In fact we observed patients during a limited period of time, with a maximum survival of 38 months: a longer time frames and larger sample sizes are required to appropriately draw conclusions about overall survival. Anyway, the DASciS software, despite having a theoretical limitation in those lesions with much lower tracer absorption that could be lost, in our experience it does not affect the clinical performance of the software itself, and it appears a promising tool that can help in identifying mCRPC patients that more likely will take advantage of Ra-223 treatment.

From literature data it's known that, in terms of ability to prolong survival, Ra-223 therapy is more effective on patients that are able to receive almost all of the six cycles currently administered according to the treatment scheme.[35] In this context, it's necessary to find a way to stratify the patients at the time of enrollment for this treatment, aiming to select patients that are more likely to benefit from it. In our study, the imaging assessment, in terms of bony disease percentage burden, is proposed as a predictive variable for OS that can be added to a multidimensional clinical evaluation in order to highlight those mCRPC patients that will more probably take advantage from Ra-223 therapy. Approaching the patients' enrollment this way, it might be possible to enhance the treatment effectiveness together with cost optimization.

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research article

Three-dimensional MRI evaluation of the effect of bladder volume on prostate translocation and distortion

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Background. The accuracy of any radiation therapy delivery is limited by target organ translocation and distortion. Bladder filling is one of the recognised factors affecting prostate translocation and distortion. The purpose of our study was to evaluate the effect of bladder volume on prostate translocation and distortion by using detailed three-dimensional prostate delineation on MRI.

Patients and methods. Fifteen healthy male volunteers were recruited in this prospective, institutional review board-approved study. Each volunteer underwent 4 different drinking preparations prior to imaging, with MR images acquired pre- and post-void. MR images were co-registered by using bony landmarks and three-dimensional contouring was performed in order to assess the degree of prostate translocation and distortion. According to changes in bladder or rectum distention, subdivisions were made into bladder and rectal groups. Studies with concomitant change in both bladder and rectal volume were excluded.

Results. Forty studies were included in the bladder volume study group and 8 in the rectal volume study group. The differences in rectal volumes yielded higher levels of translocation ($p < 0.01$) and distortion ($p = 0.02$) than differences in bladder volume. Moderate correlation of prostate translocation with bladder filling was shown ($r = 0.64$, $p < 0.01$). There was no important prostate translocation when bladder volume change was < 2-fold ($p < 0.01$). Moderate correlation of prostate distortion with bladder filling was shown ($r = 0.61$, $p < 0.01$).

Conclusions. Bladder volume has a minimal effect on prostate translocation and effect on prostate distortion is negligible. Prostate translocation may be minimalised if there is < 2-fold increase in the bladder volume.

Key words: prostate translocation; prostate distortion; gland deformation; bladder volume; magnetic resonance imaging; radiation therapy planning

Introduction

Prostate cancer is the commonest male non-cutaneous cancer worldwide, with its incidence continuing to increase due to an ageing population.[1] Current American Urological Association guidelines for localized prostate cancer state that care options offered to patients should include active surveillance, external-beam radiation therapy, radical prostatectomy, brachytherapy and hormone

therapy, with options being modulated according to baseline patient risk group.[2,3] Radiation therapy, either alone or in conjunction with hormonal therapy, is an effective and accepted care option across all risk groups.[3]

In radiation therapy, it is important to perform a secure delivery of high doses with dose minimization to adjacent organs at risk.[4,5] The accuracy of any radiation therapy is however limited by several factors, including set-up error, organ delineation, inter- and intra-fraction organ translocation, and target organ distortion.[6] Rectal distension and, to a lesser extent, bladder filling have been found to be the principal causes of prostate translocation.[4,7-9] Despite this, there is a lack of consensus regarding the optimal degree of bladder filling during radiation therapy, with recommendations encompassing a spectrum of an empty, partially full, comfortably full or full bladder.[4,8,10-13] Some studies have shown that radiation therapy with a full bladder protocol has distinct advantages in relation to dose load to both rectum and bladder.[14,15] However, a proportion of patients will struggle to maintain a full bladder protocol due to advanced age or urinary irritation, thus some authors favour an empty bladder protocol for reasons of patient comfort and the potential for improved volume reproducibility; despite initial concerns, such protocols have been shown to have a low radiation therapy bladder toxicity.[15,16]

According to the literature there is only minimal effect of bladder filling on prostate translocation[8,9,12,14,17,19], however, the methodology of these studies lacks standardization and their quantification methods may lack accuracy. Previous studies have reported on the prostatic mid-point or prostatic boarders as reference points of prostate translocation, but such a methodology may not fully account for prostate distortion and, by implication, changes in the target volume.[10,14,17] Although some studies have incorporated prostate three-dimensional (3D) contouring with computed tomography (CT)[8,12], CT is known to over-estimate prostate volume compared to magnetic resonance imaging (MRI).[18,19] Furthermore, detailed MRI information of prostate translocation and distortion is becoming increasingly important, with radiation therapy delivery systems that integrate MRI scanners for guidance being introduced into the clinic.[20] Thus, the aim of our study was to evaluate the effect of bladder volume on prostate translocation and distortion by using detailed 3D prostate delineation on MRI.

Patients and methods

Study cohort

Fifteen healthy male volunteers were included in this prospective, institutional review board-approved study, with written informed consent obtained in all cases.

Magnetic resonance imaging

MRI examinations were carried out on a 3T MR750 magnet (General Electric Healthcare, Waukesha, WI, USA) using a 32-channel phased-array body coil. The protocol comprised: high-resolution axial T2-weighted (T2w) fast recovery fast spin echo (FRFSE) sequence, TR/TE of 3663/102 ms field-of-view (FOV) 22×22 cm^2, slice thickness/gap 3.0/0.0 mm, in-plane resolution 0.85×0.57 mm, and 3 signal averages; sagittal T2w cube sequence, FOV 22×22 cm^2, slice thickness/gap 2.0/0.0 mm, in-plane resolution 0.43×0.43 mm. MRI was performed on 4 consecutive days, with participants completing the following different preparations prior to imaging, with MR images acquired pre and post-void:

Preparation 1. Pass urine, no drinking 2 hours prior to scanning
Preparation 2. Pass urine, no drinking 1 hour prior to scanning
Preparation 3. Pass urine and drink 250 mL water 1 hour prior to scanning
Preparation 4. Pass urine and drink 500 mL water 1 hour prior to scanning

Computerised image analysis

The overall workflow diagram designed for MR image processing is depicted in Figure 1. Figure 1A represents the first phase of our procedure for the quantitative evaluation of the prostate translocation and distortion. For each study, the co-registration of the two MRI sequences was manually performed using ITK-SNAP in consensus with a board-certified uro-radiologist with 8-years' experience in reporting prostate MRI studies – ITK-SNAP is a well-known medical image analysis framework based on the C++ Insight Toolkit (ITK) library. The registration was carried out in the sagittal plane according to bony landmarks (*i.e.*, pelvic bones and lumbar spine) aiming to preserve the effects of soft-tissue deformation and movement.[21,22] The obtained affine transformation matrix (*i.e.*, rigid-body transformations along with the scaling

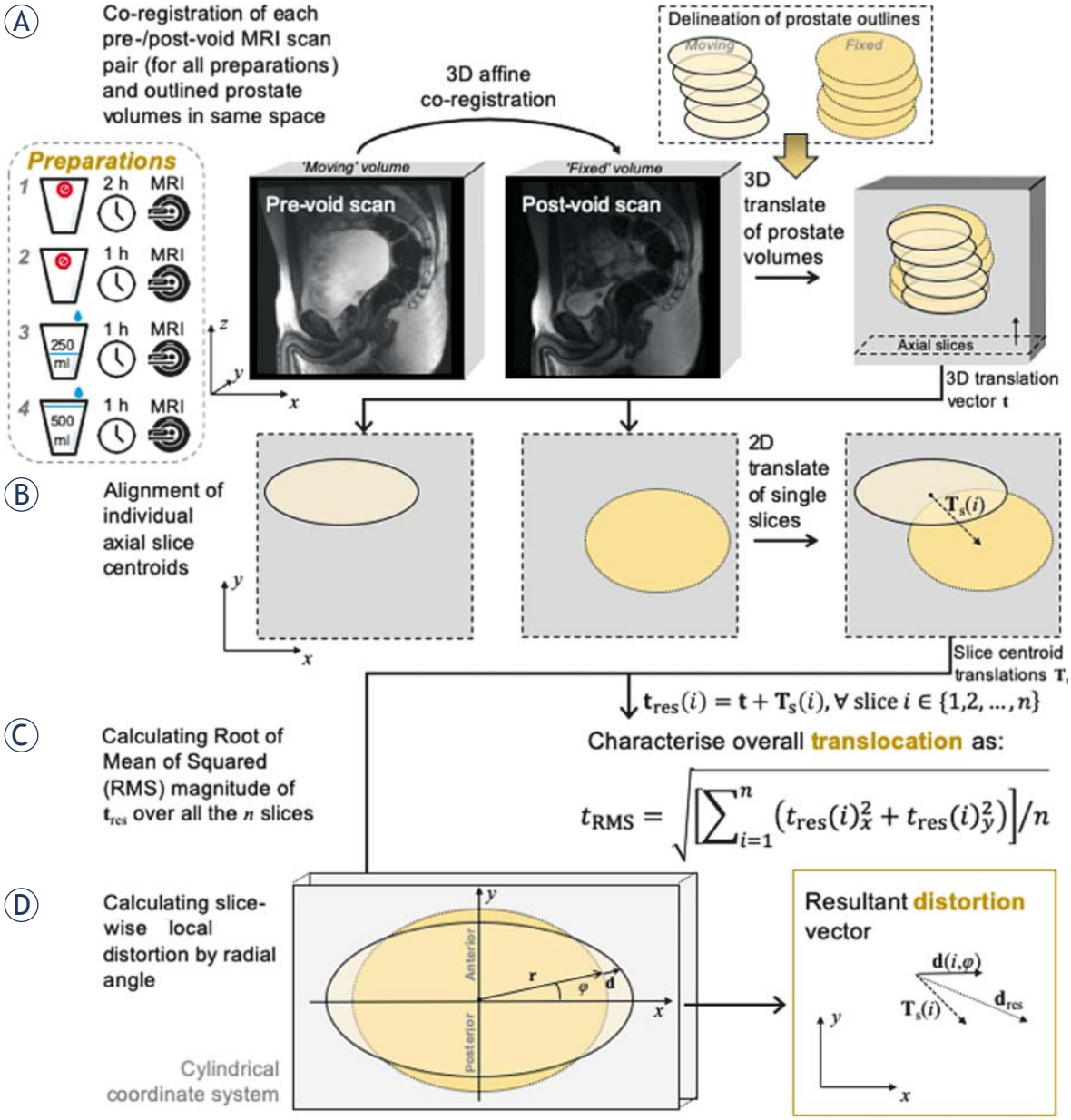

FIGURE 1. Overall scheme of the performed MR image analysis tasks. **(A)** 3D affine co-registration of each pre-void scan ('moving' volume) against the post-void scan ('fixed' volume). This operation is executed for all the four preparations described in the leftmost box. Subsequent manual delineation of the prostate on the two scans by using the axial reformatting. 3D rigid-body (translation alone **t**) volume alignment between the centres-of-mass of the two prostate glands under investigation. **(B)** For each slice, the volume sections are aligned so that their centroids are coincident (information stored in the 'tree' of slice centroid translations $\mathbf{T}_s$). **(C)** Calculation of the RMS of the resultant translocation vector $\mathbf{t}_{res}$. **(D)** Computation of the resultant distortion vector $\mathbf{d}_{res}$, by considering also the subdivision of the axial plane into the anterior and posterior half-planes.

to take into account different FOVs) was then applied by means of advanced normalisation tools (ANTs).[23,24] For each pre-/post-void MRI scan pair for each of the four preparations, the pre-void scan (*i.e.*, 'moving' volume) was co-registered in this way against the corresponding post-void scan (*i.e.*, 'fixed' volume). Each co-registered image was then reformatted in the axial plane to allow for a more accurate and clinically relevant prostate delineation. The prostate was then manually delineated, by means of an in-house software tool, from the most inferior to the most superior location where the prostatic tissue could be clearly identified, excluding the seminal vesicles, according to the independently acquired high-resolution T2w FRFSE axial images.

For more detailed analyses, the prostate was subdivided into apex, mid-gland, and base sectors by dividing the whole prostate gland into thirds and into the anterior and posterior gland for assessing distortion directions in the axial plane. More details about the computational and physical concepts underlying our analysis are provided in Supplementary Material. Briefly, by exploiting the computational framework for prostate deformation assessment proposed by Gill *et al.*, the two prostate outlines under investigation are aligned to their centres, the slice delineations of the 'moving' volume are then translated onto the 'fixed' reference system (Figure 1B).[25] The 'resultant translocation' t_{res} is then computed to characterise the global translocation of the prostate (Figure 1C). The Root Mean Square (RMS) value of the magnitude of the resultant translocation vector is calculated by averaging over all the slices. Finally, Figure 1d shows the 'resultant distortion' d_{res} for evaluating the combined effects of both translational and local distortions; three examples of distortion maps, along with the corresponding fixed and moving volumes, are displayed in Figure 2.

Bladder volume and rectal distention assessment

Bladder volumes were calculated by using whole volume segmentation on sagittal T2w cube sequence using an in-house tool developed in Matlab (Math Works, Natick, MA, USA).[26] Relative bladder volume difference was defined as absolute volume difference divided by post-void bladder volume. Rectal distention was derived using maximum sagittal and axial dimensions (anal canal to peritoneal reflection), and subjectively scored following a previously reported 5-point Likert scale: 1 = no stool/gas, 2 = minimal, 3 = small amount, 4 = moderate, 5 = large amount of stool/gas.[27]

Group design

Bladder and rectal volumes are potential confounders that may alter prostate position, thus a division into two groups was performed according to any change or otherwise in rectal and/or bladder volume. Important change in rectal distention was based on the work of Villiers *et al.*, where no effect on prostate translocation was demonstrated when rectal volume was <56 mL, equivalent to our baseline score of 2/5.[10,27] Thus, significant rectal distension was defined when an increase or decrease in scoring by ≥ 1 point was observed, however changes in rectal volume for a baseline score of < 2 were disregarded.[10,27] Significant bladder distension change was defined if there was ≥ 2 fold change of bladder volume. The inclusion into the bladder-change group required no significant change in rectal distension and, likewise, inclusion criterion into the rectal-change group mandated no significant bladder volume change. Studies with concomitant change in both bladder and rectal volume were excluded. The translocation cut off values of 3 mm and 5 mm were defined according to the values in the literature, as a tight planning of target volume (PTV) margin is needed for hypofractionation regimens in order to increase target dose whilst minimizing dose to the surrounding tissues.[28,29] Typically, a 3 mm to 5 mm PTV margin is recommended clinically to limit the dosimetric consequences of both intrafraction and interfraction motion.[28,29]

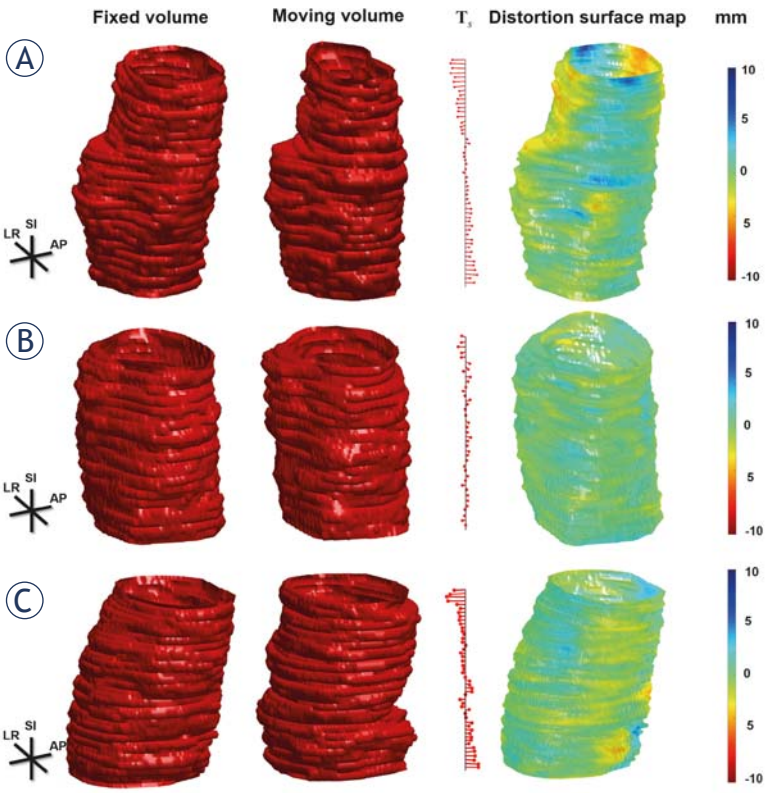

FIGURE 2. Example distortion maps of three MRI studies. **(A)** showing significant prostate translocation with significant base distortion in a study from the rectal group, **(B)** showing negligible prostate translocation and distortion in a study from the bladder group, and **(C)** showing significant prostate translocation but negligible prostate distortion in a study from the bladder group. The fixed and moving volumes are depicted in the first and second columns, respectively. In order to show the slice section difference as well as the local translation, the 'tree' of slice centroid translations $\mathbf{T}_s$ and the distortion surface map (along with the corresponding colour map expressed in mm) are shown in the third and fourth (fifth) columns, respectively.

TABLE 1. Inter-group translocation comparison for whole gland and prostatic sectors

	Bladder group Mean ± SD (mm)	Rectal group Mean ± SD (mm)	*p*-value
Whole gland	2.46 ± 1.73	4.44 ± 1.74	<0.01
Apex	1.86 ± 1.39	3.96 ± 1.92	<0.01
Mid-gland	2.31 ± 1.83	4.46 ± 1.88	<0.01
Base	2.98 ± 2.05	4.70 ± 1.85	0.03

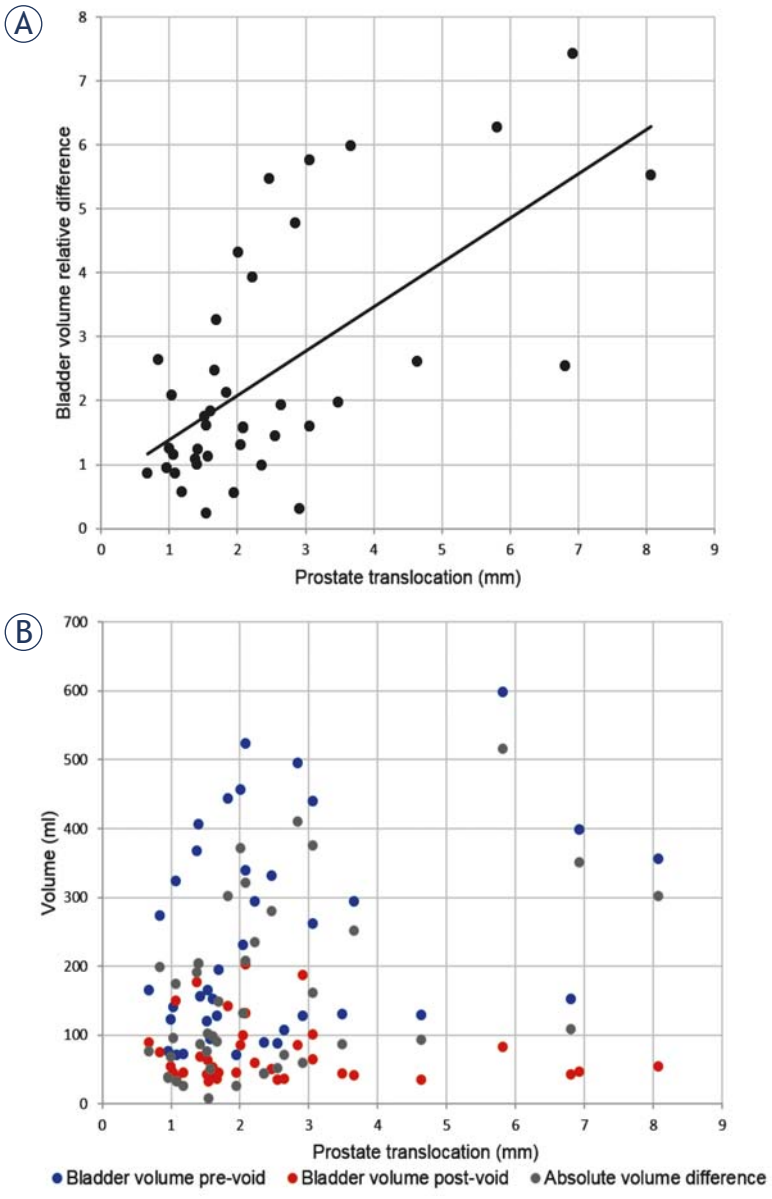

FIGURE 3. Prostate translocation plotted with relative bladder volume difference ($r = 0.64$, $p < 0.01$) **(A)**. Prostate translocation plotted with bladder volumes (pre-void, post-void, absolute difference) **(B)**.

Intra-observer repeatability

All prostate contouring was conducted by a single observer. After the primary prostate contouring, in order to assess intra-observer repeatability, a subset of 10 studies were randomly selected, and the prostate was re-contoured in a blinded fashion by the same observer at a separate sitting. We applied the same computerized image analysis methods devised for quantitatively evaluating the bladder volume effect on prostate translocation and distortion. In particular, the two prostate gland delineations, performed on the same MRI study by the same radiologist, were employed in place of the prostate volumes delineated on the pre-/post-void MRI scan pairs (concerning the four preparations investigated).

Statistics

Unpaired Student's *t*-test was used to compare continuous variables between two groups. Analysis of variance (ANOVA) was used to compare translocation and distortion in between prostatic sectors. *Post hoc* comparisons were adjusted for multiplicity using Bonferroni correction. Pearson's correlation coefficient (r) was calculated to evaluate correlation. Significance was set at $p < 0.05$. Statistical analysis was performed with SPSS v.17.0 (SPSS Inc., Chicago, IL, USA).

Results

Fifteen volunteers (mean age 35.9 years, median 34, range 27–53) completed the study. The average prostate volume was 39.1 ± 10.2 mL (range: 32.1–56.7). In one volunteer, the MRI protocol was incomplete, and in 19 studies significant rectal distension change was identified. Thus, a total of 40 scans were included into the bladder volume study group. For the 19 studies with significant rectal distension change, 8 did not have significant bladder volume change and formed the rectal volume study group.

Prostate translocation - Bladder group

The mean pre-void bladder volume was 237.3 ± 150.2 mL (range: 40.9–598.1), and mean average post-void volume 74.1 ± 46.4 mL (range: 32.9–203.4). The absolute difference in bladder volume change was 163.1 ± 126.1 mL (range: 8.0–515.9). The median value for rectal distension was 3 (range:

TABLE 2. Prostate distortion expressed as mean and distortion values of the 90th percentile

	Bladder group		Rectal group		*p*-value
	Mean ± SD (mm)	90th percentile ± SD (mm)	Mean ± SD (mm)	90th percentile ± SD (mm)	
Whole gland	1.40 ± 0.36	2.55 ± 0.62	1.71 ± 0.33	3.20 ± 0.56	0.02
Apex	1.42 ± 0.53	2.44 ±0.80	1.46 ± 0.31	2.53 ± 0.43	0.80
Mid-gland	1.19 ± 0.27	2.19 ± 0.50	1.61 ± 0.41	2.90 ± 0.67	<0.01
Base	1.61 ± 0.46	2.84 ± 0.80	2.01 ± 0.5	3.73 ± 0.83	0.02
Whole gland, anterior	1.41 ± 0.35	2.56 ± 0.60	1.59 ± 0.30	2.91 ± 0.50	0.14
Whole gland, posterior	1.40 ± 0.37	2.54 ± 0.65	1.82 ± 0.35	3.36 ± 0.61	<0.01

1–5). The mean average absolute change in rectal volume was 5.6 ± 6.2 mL (range: 0.0–30.2).

Whole prostate translocation of ≥ 5 mm was observed in 4/40 (10.0%) patients and ≥ 3 mm in 9/40 (22.5%) patients. Prostatic sector subdivision showed statistically significant differences in translocation between the base and apex (Table 1). Base translocation of ≥ 5 mm was observed in 5/40 (12.5%) patients and of ≥ 3 mm in 15/40 (37.5%) patients. Mid-base translocation of ≥ 5 mm was observed in 4/40 (10.0%) patients and ≥ 3 mm in 9/40 (22.5%) patients. Apex translocation of ≥ 5 mm was observed in 3/40 (7.5%) patients and of ≥ 3 mm in 4/40 (10.0%) patients. Figure 3A depicts prostate translocation plotted against bladder volume (pre-void, post-void, absolute difference). There was a significant difference when subdivision was made according to relative bladder volume difference. The group with ≥ 2-fold increase in bladder volume (N = 17) showed higher translocation values than the group with < 2-fold increase (N = 23) in bladder volume at 3.47 ± 2.21 mm *versus* 1.72 ± 0.65 mm, respectively (p < 0.01). The directions of prostatic translocation are shown in Figure 4. When plotting the prostate translocation against relative bladder volume difference, there was a moderate positive correlation (r = 0.64, p < 0.01) (Figure 3B), driven by prostate translocation in the antero-posterior (AP) direction, which was the only direction showing a significant difference.

Prostate translocation - Rectal group

Within the rectal group, the mean pre-void bladder volume was 84.4 ± 11.9 mL (range: 67.7– 99.0), and post-void bladder volume was 55.3 ± 9.9 mL (range: 38.8–70.9). The absolute difference in bladder volume change was 29.1 ± 14.7 mL (range: 8.2–48.0). On the pre-void study, the median value for rectal distension was 3 (range: 1–5) and on the post-void study the median value was 4 (range: 3–5). The average absolute difference in rectal change was 36.3 ± 19.7 mL (range: 14.5–77.5). Whole prostate translocation of ≥ 5 mm was observed in 2/8 (25.0%) patients and ≥ 3 mm in 7/8 (87.5%) of patients. There was no significant difference in translocation between the prostatic sectors (Table 1, Figure 4). Base translocation of ≥ 5 mm was observed in 4/8 (50.0%) patients and of ≥ 3 mm in 6/8 (75.0%) patients. Mid-gland translocation of ≥ 5 mm was observed in 3/8 (37.5%) patients and of ≥ 3 mm in 6/8 (75.0%) patients. Apex translocation

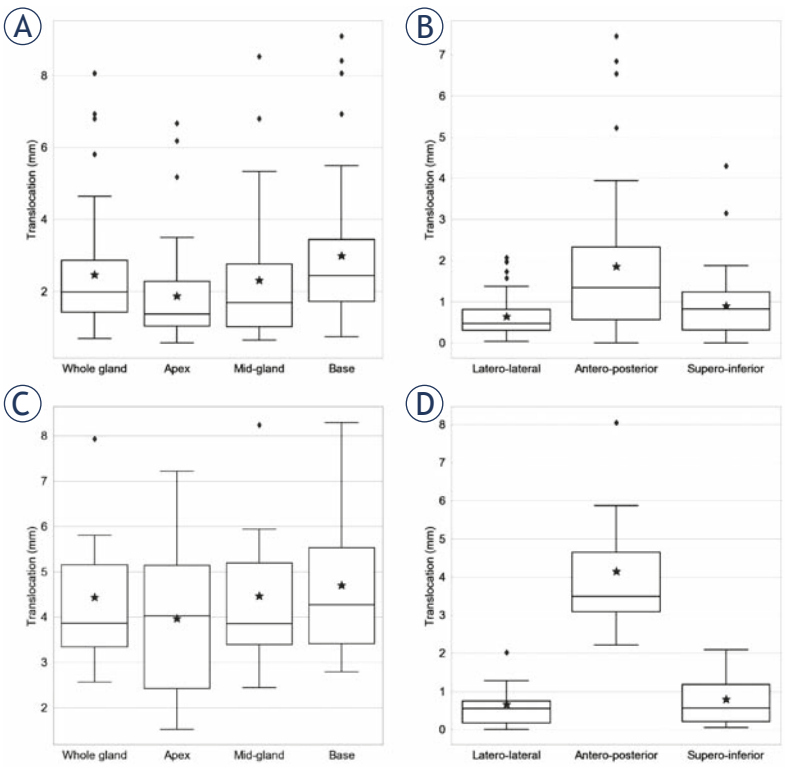

FIGURE 4. Translocation in the bladder group according to the prostatic sectors **(A)** and directions of translocation **(B)**. Translocation in the rectum group according to the prostatic sectors **(C)** and directions of translocation **(D)**. Each boxplot shows a black solid line and a grey star marker that denote the median and mean values, respectively.

of ≥ 5 mm was observed in 3/8 (37.5%) patients and of ≥ 3 mm in 6/8 (75.0%) patients. No correlation was found when plotting rectal volume against prostate translocation. Studies within the rectal group demonstrated significantly higher degrees of whole gland prostate distortion compared to the bladder group (Table 1).

Prostate distortion

Important differences were observed between the groups, with the rectal group showing higher levels of prostate distortion (Table 2). Similarly, important differences were observed between the groups in the degree of posterior whole gland distortion (Table 2). In the bladder group the maximum prostate distortions for whole gland, base, mid-gland and apex were 9.0 mm, 9.0 mm, 9.0 mm and 8.3 mm, respectively. In the rectal group the maximum prostate distortions for whole gland, base, mid-gland and apex were 10.1 mm, 9.5 mm, 10.1 mm and 6.6 mm, respectively. When plotting the whole gland distortion, there was a moderate positive correlation for relative bladder volume difference ($r = 0.61$, $p < 0.01$), but not for the rectum volume difference.

Intra-observer reproducibility

High reproducibility was observed with only minimal discrepancies. The reliability measurements for the translocation direction were 0.08 ± 0.07 mm (range: 0.00–0.27) in the latero-lateral (LL) direction; 0.11 ± 0.07 mm (range: 0.00–0.27) in the AP direction and 0.4 ± 0.03 mm (range: 0.00–0.10) in the supero-inferior (SI) direction. Whole gland RMS translocation reproducibility and distortion reproducibility are noted in Supplementary Table.

Discussion

The results of our study suggest that bladder volume has only a minimal effect on prostate translocation and its effect on prostate distortion is negligible. Furthermore, it appears that prostate translocation may be minimised if there is a <2-fold increase in the bladder volume. Previous studies evaluated the effect of bladder volume on prostate translocation and distortion, typically utilizing CT for assessment.[7-9] CT is known to over-estimate prostate volume by up to 35% compared to MRI, with MRI providing better differentiation of prostatic anatomy, particularly the posterior border, prostate apex and base-seminal vesicle interface.[18,19] In our study, detailed MRI prostate delineation on axial slices with sub-millimetre in-plane resolution was performed to more accurately quantify the degree of prostate translocation and distortion secondary to changes in bladder or rectal volumes, using a previously devised computational framework.[25]

Bladder volume has generally been reported to have a weak association with prostate translocation.[7,8,10] In our study moderate correlation of prostate translocation with bladder filling was shown in the AP direction. Villeirs *et al.* described a considerable increase in prostate translocation in the superior-inferior direction with bladder volumes above 300 mL.[10] The results of our study show that inter-fractional fluctuations of bladder volume should ideally be kept to a minimum, since there is a negligible prostate translocation with <2-fold changes in bladder volume. Thus, a "comfortably full" bladder is a reasonable aim, given the low probability of inducing a subsequent ≥ 2-fold increase.[10] Furthermore, this is supported by a recent study that investigated two preparation protocols, finding no difference in bladder volume when enforcing a strict bladder-filling protocol or when giving an instruction to maintain a comfortably full bladder.[13] Of note, the authors also concluded that expectations of maintaining a strictly controlled bladder volume at a repeat sitting scan causes patient discomfort and can have a negative impact on the treatment.[13]

Several studies have reported that rectal distension can significantly impact prostate translocation, whilst the impact of bladder filling appears more negligible.[7-10] This is in accordance with our study, given that the differences in rectal volumes yielded higher levels of translocation than differences in the bladder volume.[7-10] In both groups the amplitudes of prostate translocation were similar to previously published studies, and with a similar pattern of displacement observed, with the most prominent direction of translocation being in the AP direction.[7-10] In both groups the base of the prostate was shown to have a larger amplitude of translocation than the apex, presumably due to apex being relatively fixed in position due to the surrounding pelvic floor musculature.[10,30] In the bladder group, only 10% of examinations resulted in a translocation ≥ 5 mm, with a maximum translation of 8 mm. This falls within the range of planning target volume margins (5–8 mm) when using daily cross-sectional imaging, and based on soft-tissue registration or use of implanted fiducial markers.[4,31]

Previous studies have evaluated distortion in relation to radiotherapy treatment planning.[32-35] Our study focused on differences in bladder volume and the effect of this on whole gland distortion. We observed similar mean and maximum values of prostate distortion compared to previous studies.[32-35] When comparing study groups, the results show that rectal volume differences impact prostate distortion to a higher degree than bladder volume differences. Furthermore, this group difference was most pronounced in the posterior prostate. Nichol *et al.* previously investigated the effect of bladder and rectal fillings on prostate distortion; however, they did not prove any association concluding that this was due to their use of bowel and bladder regimens.[35] In our study we were also unable to show an association with rectal volume, possibly due to the small number of examinations within the rectal sub-study group, however, a moderate positive correlation with prostate distortion was observed in the bladder group.

A strength of our study is the robust methodology used, with sub-millimetre 3D whole gland delineation, which should be considered a gold standard. Only two studies utilized three-dimensional contouring in the process of prostate translocation evaluation, even though CT was used as the imaging modality.[8,12] Despite whole gland delineation being time consuming, future Machine Learning methods might be exploited for automated prostate segmentation, reducing the operator-dependence and the outlining time in manual segmentation procedures, making this method more feasible in both research and clinical settings.[36]

Our study has some limitations. The study population was composed of healthy volunteers, which may not be representative of a patient population, which typically would be older, with larger prostatic volume and the potential for outflow obstruction. It should also be noted that the study design allowed for relatively extreme differences in bladder volume from full (pre-void) to almost empty (post-void); such conditions would be unusual during relatively short clinical intra-fractional periods. Thus, the relatively minimal effect shown by bladder volumes in our study offers further reassurance from a clinical standpoint. Lastly, the number of cases within the rectal group was small due to the evaluation of rectal volume effect on prostate being only a secondary aim of the study. The effect of rectum volume appears to be greater than the effect of bladder volume on prostate translocation, however further work with more controlled methodology is needed to establish the effect.

In conclusion, bladder volume has only a minimal effect on prostate translocation and effect on prostate distortion is negligible. Prostate translocation may be minimalised if a < 2-fold increase in the bladder volume is maintained for the study duration.

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research article

Safety margin assessment after microwave ablation of liver tumors: inter- and intrareader variability

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Background. The aim of the study was to evaluate the inter- and intrareader variability of the safety margin assessment after microwave ablation of liver tumors using post-procedure computed tomography (CT) images as well as to determine the sensitivity and specificity of identification remnant tumor tissue.

Patients and methods. A retrospective analysis of 58 patients who underwent microwave ablation (MWA) of primary or secondary liver malignancies (46 hepatocellular carcinoma, 9 metastases of a colorectal cancer and 3 metastases of pancreatic cancer) between September 2017 and June 2019 was conducted. Three readers estimated the minimal safety margin in millimeters using side-by-side comparison of the 1-day pre-ablation CT and 1-day post-ablation CT and judged whether ablation was complete or incomplete. One reader estimated the safety margin again after 6 weeks. Magnetic resonance imaging (MRI) after 6 weeks was the gold standard.

Results. The intraclass correlation coefficient (ICC) for estimation of the minimal safety margin of all three readers was 0.357 (95%-confidence interval 0.194–0.522). The ICC for repeated assessment (reader 1) was 0.774 (95%-confidence interval 0.645–0.860). Sensitivity and specificity of the detection of complete tumor ablation, defined as no remnant tumor tissue in 6 weeks follow-up MRI, were 93%/82%/82% and 33%/17%/83%, respectively.

Conclusions. In clinical practice, the safety margin after liver tumor ablation is often assessed using side-by-side comparison of CT images. In the study, we were able to show, that this technique has a poor reliability (ICC 0.357). From our point of view, this proves the necessity of new technical procedures for the assessment of the safety distance.

Key words: radiology, interventional; liver neoplasms; safety margin; interindividual variability; intraindividual variability; tumor ablation

Introduction

Thermal ablation methods, such as microwave ablation (MWA), have established themselves in recent years as a suitable therapy for various malignancies.[1] The execution of a tumor ablation involves many challenges. One crucial factor for a successful ablation and the prevention of residual tumor tissue or the onset of a tumor recurrence is maintaining a sufficient safety margin. There is currently no clear consensus on what a sufficient safety distance is.[2] Most authors recommend a safety margin between 5-10 mm.[1,3-7] The precondition for the determination of a suitable safety distance, however, would initially be a proper measurement method. So far, this has been proved another major challenge. Several studies have investigated postinterventional methods and measurement techniques, such as computed tomography (CT) or magnetic resonance imaging (MRI), to be able to make a valid decision about a complete ablation.[8-10] Other authors try to improve the intraprocedural

tumor detection and the assessment of the ablation margin as a recently published study used a FDG PET/CT guided ablation for the intraprocedural determination of the safety distance and achieved good results.[11] Many authors favour the MRI for ablation margin control.[10,12] However, in most cases tumor ablation is performed under CT guidance and the safety margin is assessed in the CT images, at least in the periinterventional setting. For best treatment, the decision whether ablation is complete or not should be made as immediately as possible. Therefore, in most cases, native or contrast-enhanced CT scans are performed, and the extent of the ablation is decided by side-by-side comparison of the pre- and post-interventional images or by simple and fast measuring techniques during the intervention like measurement with a simple distance measurement tool. Unfortunately, we do not have reliable data on the consistency and reproducibility of these subjective estimations of a sufficient safety distance in a real-world setting. For this reason, in this study we investigated the inter- and intrareader variability of the safety margin assessment after microwave ablation of liver malignancies.

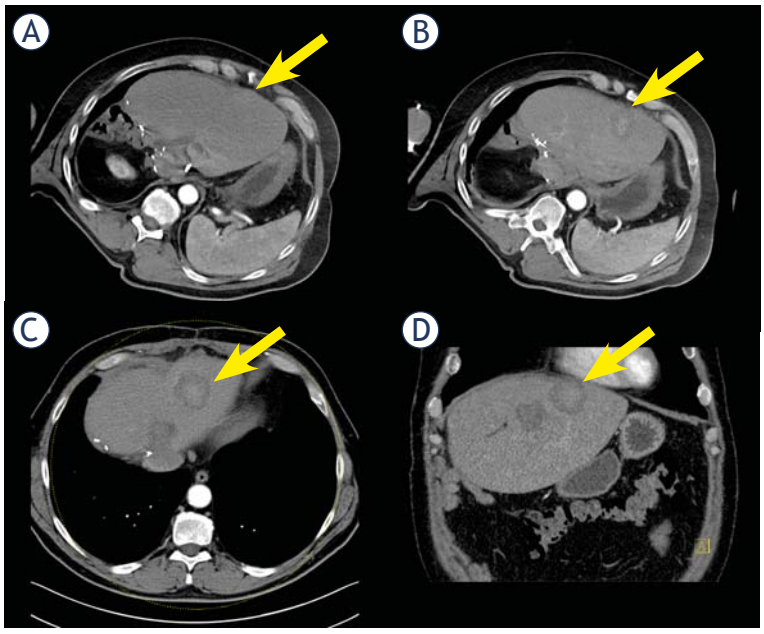

FIGURE 1. (A) Pre-interventional arterial phase in which the tumor is almost invisible. This not only complicates ablation but also post-interventional detection of residual tumor tissue. **(B)** The result immediately postinterventionally with a corresponding ablation defect. **(C)** Axial and **(D)** coronal show the situation one day postinterventionally. Due to the different breathing position, the tumor was more peripheral the day before, whereas on the following day healthy liver tissue around the ablation defect is visible. Measuring the safety distance is particularly difficult in these cases.

Patients and methods

Study design and participant selection

The local ethics committee approved this retrospective study. Written informed consent was obtained from all patients. A total of 58 patients were included in this study, who were treated with microwave ablation between September 2017 and June 2019. Tumor entities were hepatocellular carcinoma (HCC) and metastases of colorectal and pancreatic cancer. Exclusion criteria were the patient's refusal to participate in the study and other tumors than those mentioned above. All patients received a CT-scan one day before ablation and on the first postinterventional day (Figure 1). Subsequently, all patients were independently assessed by three interventional radiologists regarding the safety margin between tumor and healthy liver tissue using side-by-side measurement. No special evaluation software was used to simulate the procedure in everyday practice as accurately as possible. The orientation was based on reference points that could be reproduced exactly, *e.g.* prominent vessel outlets, foreign material or calcifications. Of course, the different breathing positions had to be taken into consideration as well. One of the three readers re-evaluated the patients after six weeks to detect possible intraindividual variability. The six weeks period of time between the two readings should ensure, that the reader could not remember the patients and avoid a bias. The minimum safety distance, the maximum safety distance and whether the ablation was considered complete or incomplete, *i.e.* technical efficacy, were estimated. The 6 weeks follow-up MRI was regarded as the gold standard for technical efficacy.

Statistics

Intraclass correlation (ICC) estimates and their 95% confident intervals were calculated using R irr statistical package version 3.5.1 based on a mean-rating (k = 3), absolute-agreement, 2-way mixed-effects model. The intraclass correlation coefficient (ICC) was calculated for the estimation of the minimal safety margin. ICC values less than 0.5 are considered indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability. Bland-Altman analyses were used to assess agreement in the side-by-side measurements between the two blinded readings (minimal safety margin) by the same radiologist and between the

TABLE 1. Baseline characteristics

Number of patients	N = 58
Age	
mean (years)	62.84 (10.85)
range (years)	36–83
Gender	
male (%)	53 (91)
Ablation method (%)	
microwave ablation	58 (100)
Liver segments	
I	3 (5)
II	4 (7)
III	6 (10)
IVa	7 (12)
IVb	3 (5)
V	7 (12)
VI	4 (7)
VII	12 (21)
VIII	9 (16)
Tumor entity	
Hepatocellular carcinoma	46 (79)
Metastasis colorectal cancer	9 (16)
Metastasis pancreatic cancer	3 (5)

readings (minimal safety margin) by the three independent radiologists.

Results

Patient and tumor characteristics

58 patients were included and evaluated. The mean age was 62.84 (10.85) years. 53 patients (91%) were male. All 58 patients were treated with MWA. Most tumors (n = 12) were located in liver segment VII, followed by segments VIII (n = 9) and IV a and V with n = 7 each. The minority of tumors were found in segments I and IV b with n = 3 each. The baseline data are shown in Table 1.

Inter- and intrareader variability

The intraclass correlation coefficient (ICC) for estimation of the interindividual variability of the assessment of the minimal safety margin for all three readers was 0.357 (95%-confidence interval 0.194–0.522), indicating a poor reliability. The ICC for estimation of the variability of two repeated estimations of reader 1 was 0.774 (95%-confidence interval 0.645–0.860), indicating a good reliability for repeated measurements.

Bland–Altman plots were calculated to show intra- and interindividual variability (Figure 2). A systematic error was not detectable. The standard deviation in the intrareader-result was smaller compared to the interindividual evaluations. Nevertheless, deviations of more than 5 mm can be detected in

TABLE 2. Contingency table of all the three independent readings compared with the six weeks follow-up MRI as gold standard

	Incomplete (6 weeks MRI)	Complete (6 weeks MRI)
Reader 1		
Incomplete	2	3
Complete	4	41
Reader 2		
Incomplete	1	8
Complete	5	36
Reader 3		
Incomplete	5	8
Complete	1	36

TABLE 3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the three independent readings (R 1, 2, 3)) compared with the six weeks follow-up MRI as gold standard

	R 1	R 2	R 3
Sensitivity	93%	82 %	82 %
Specificity	33%	17 %	83 %
PPV	91 %	88 %	97 %
NPV	40 %	10 %	39%

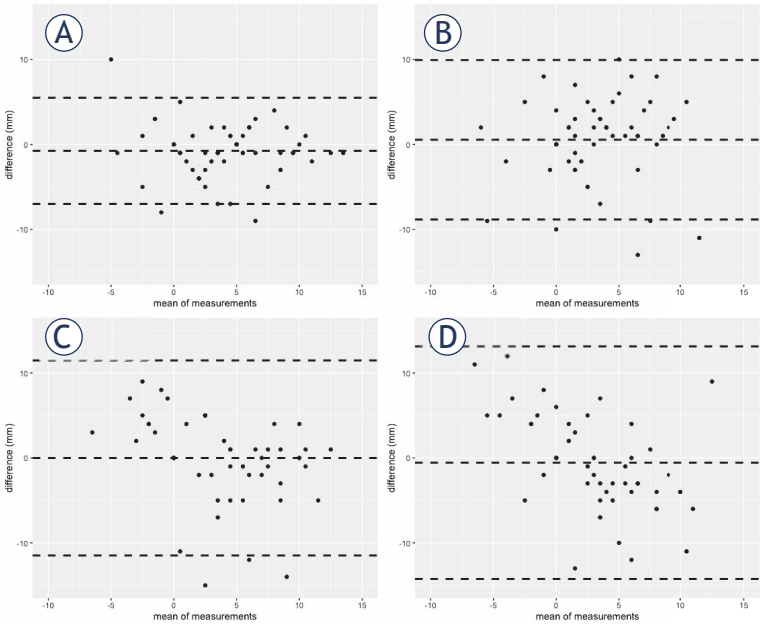

FIGURE 2. Bland-Altman plots: intra- **(A)** and inter-reader **(B)** = reader 1 *vs.* reader 2, **(C)** = reader 2 *vs.* reader 3, **(D)** = reader 1 *vs.* 3) agreement for minimum safety margin measurements. The middle line shows the mean percentage difference in measurements and the dashed lines above and below show the 95% reference range. Measurements within the 95% reference range can be considered as intrinsic measurement errors (or variations) that are associated with the given measurement tools and imaging techniques. Therefore, a narrower reference range indicates a lower measurement error/variation.

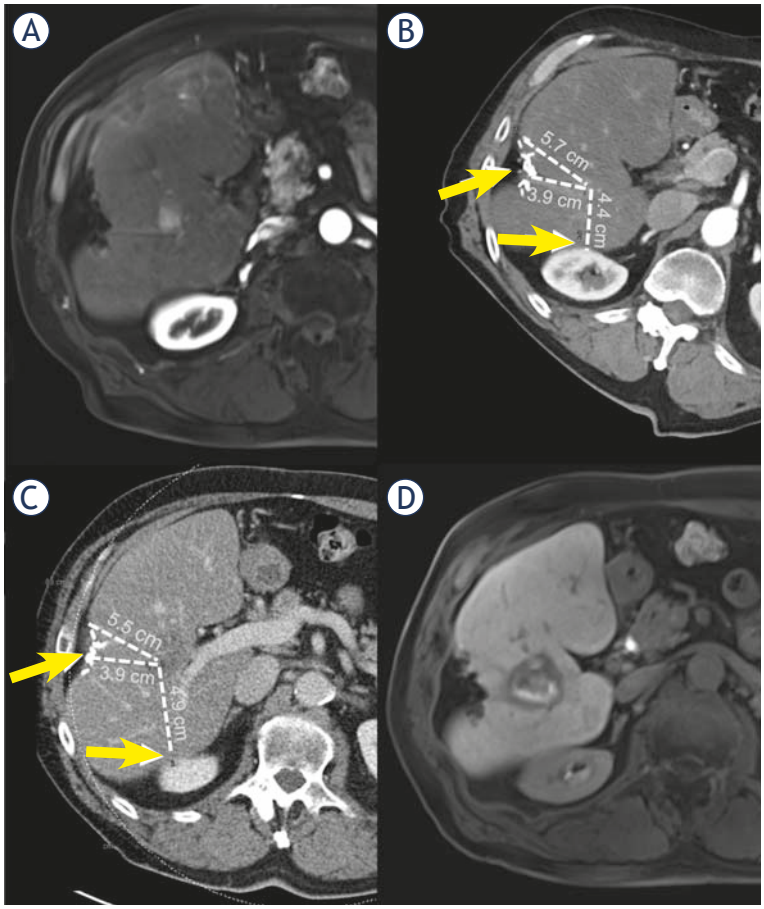

FIGURE 3: The HCC in segment VIII **(A)** was pre-interventionally localized using landmarks such as metal clips (arrow) and anatomical landmarks like the kidney (arrow) **(B)**. Post-interventionally, the same landmarks are used and the target area is localized by distance measurements (dashed lines) from different angles **(C)**. The MRI follow-up after 6 weeks confirmed complete ablation **(D)**.

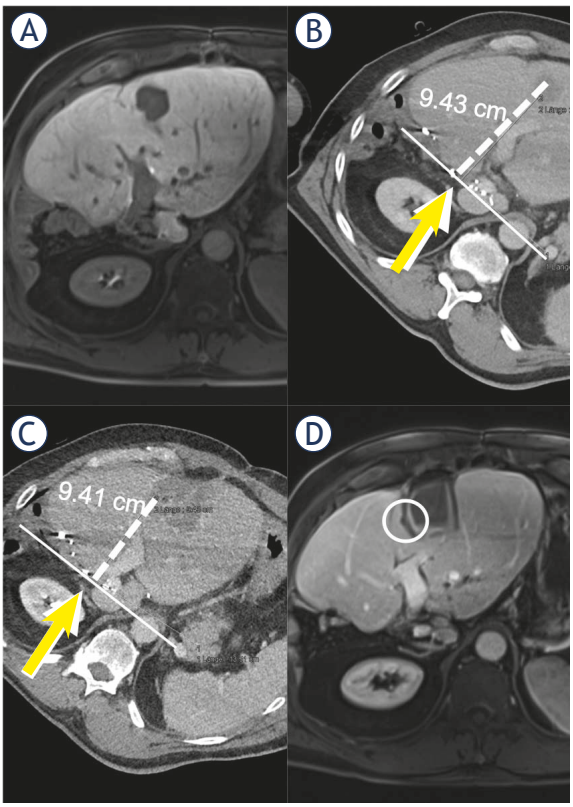

FIGURE 4: Metastasis was best seen in the portal venous phase **(B)**. In this case, clips after hemihepatectomy serve as orientation. A line is drawn (solid line) and the distance (dashed line) is measured by means of a clip at an angle of 90 degrees. The same fixed points are used postinterventionally. This already shows only a small safety distance in the peripheral area. In the 6 weeks follow-up MRI residual tumor tissue (circle) was detected.

some measurements. The differences of the safety margins measured by the two readers are clearly larger in comparison to the deviations between both measurements performed by one reader.

Assessment of complete ablation

The readers achieved a sensitivity and specificity of 93%/82%/82% and 33%/17%/83%, respectively. The positive predictive value (PPV) was 91%/88%/97%. The negative predictive value (NPV) was 40%/10%/39%. The results are shown in Table 2 and 3.

Discussion

There is agreement that a safety distance is necessary after ablation of a liver tumor to prevent local tumor recurrence. When defining the optimal safety distance, there are already different approaches and no general definition. Most authors favour a minimum distance of 5 mm (1,3–7).

We agree with this in principle. In our opinion, however, the measurement methods are rarely described or questioned. Therefore, our approach was to question the measurement of the safety distance in the daily routine (Figure 3 and 4).

This confirmed our impression that measurement with the standard tools provided by the CT software can lead to difficulties in measurement and thus to considerable intraindividual differences. Although the reading was performed by three experienced interventional radiologists, the inter-reader variability was poor.

One reason could be the localization of the tumor. Subcapsular tumors represent a special measuring challenge. The same applies for tumors in the

immediate vicinity of other organs or vessels that are also difficult to measure.

Another aspect that can lead to considerable differences in the evaluation of the distance is the choice of the reconstruction planes and the layer thickness. Zhao *et al.* claims to achieve best results with 1D or 3D 2.5 mm slices compared to 2D. From our point of view, an evaluation of the ablation zone in three planes is absolutely but also leads to a higher interreader variability.

A contentious aspect is always the experience of the interventionalist. Therefore, in our study the reading was carried out by an experienced radiologist (5 years experience), a specialist radiologist (7 years experience) and the head of the Centre for Interventional Oncological Radiology. The aim was to rule out diagnostic errors due to inexperience. Nevertheless, there were considerable differences between all three readers, which called the measuring method into question.

In our opinion, the fact that the intraindividual differences were smaller shows that there is no systematic error. The measurement results are interindividually different but not random. In our opinion, this indicates that our study results are reliable and meaningful.

New measurement methods or software for tumor segmentation are already being investigated in some studies.[5,8,9,12-16] The results were promising and improved the assessment of ablation success. Our study was able to show that conventional measurement methods are inaccurate and can lead to large interindividual differences. We therefore support the development of new measurement methods to achieve more reliable measurement results.

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research article

Percutaneous mechanical thrombectomy in patients with high-risk pulmonary embolism and contraindications for thrombolytic therapy

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Background. High-risk pulmonary embolism is associated with a high early mortality rate. We report our experience with percutaneous mechanical thrombectomy in patients with high-risk pulmonary embolism and contraindications for thrombolytic therapy.

Patients and methods. This was a retrospective analysis of consecutive patients with high-risk pulmonary embolism and contraindications to thrombolytic therapy. They were treated with percutaneous mechanical thrombectomy which included thrombectomy and additional thrombus aspiration when needed. Clinical parameters and survival to discharge were measured.

Results. From November 2005 to September 2015 we treated 25 patients with a mean age of 62.6 ± 12.7 years, 64% were men. Mean simplified Pulmonary Embolism Severity Index was 2.9. Mean maximum lactate levels were 7.8 ± 6.6 mmol/L, vasopressors were used in 77%, and 59% needed mechanical ventilation. Mechanical treatment included thrombus fragmentation complemented with aspiration (56%) and aspiration using Aspirex®S catheter (44%). Local (5 patients; 20%) and systemic (3 patients; 12%) thrombolytics were used as a salvage therapy. We observed nonsignificant improvements in systemic blood pressure (100 ± 41 mm Hg *vs* 119 ± 34; p = 0.100) and heart frequency (99 ± 35 min^{-1} *vs* 87 ± 31 min^{-1}; p = 0.326) before and after treatment, respectively. Peak systolic tricuspid pressure gradient was significantly lower after treatment (57 ± 14 mm Hg *vs* 31 ± 3 mm Hg; p = 0.018). Overall the procedure was technically successful in 20 patients (80%) and 17 patients (68%) survived to hospital discharge.

Conclusions. In patients with high-risk pulmonary embolism who cannot receive thrombolytic therapy, percutaneous mechanical thrombectomy is a promising alternative to reduce pulmonary artery pressure.

Key words: high-risk pulmonary embolism; treatment; percutaneous mechanical thrombectomy

Introduction

Pulmonary embolism (PE) remains a significant cause of cardiovascular morbidity and mortality worldwide, with overall in-hospital mortality rates ranging from 25% for patients with cardiogenic shock to 65% for those requiring cardiopulmonary resuscitation.[1,2]

Current guidelines suggest classification of pulmonary embolism severity and the risk of early (in-hospital or 30 day) death into high-, intermediate- and low-risk.[3] Patients with PE presenting with shock or hypotension are at high risk of in-hospital death; high-risk pulmonary embolism (HRPE).[3] Most deaths occur within the first few days after diagnosis mostly due to acute right ventricular failure.[3,4]

Treatment of HRPE is based on hemodynamic and respiratory support, unfractionated heparin infusion, and reperfusion therapy with systemic thrombolytic agents (class IB), surgical pulmonary embolectomy (class IC) or percutaneous catheter-directed (mechanical) thrombectomy (PMT) (class IIaC).[3] Experimental evidence also suggests that extracorporeal cardiopulmonary support can be an effective treatment especially as a bridge to surgical pulmonary embolectomy.[5–7] PMT improves pulmonary flow with embolus/thrombus modification and may be particularly useful if contraindications to fibrinolysis are present and surgical embolectomy is not feasible or available. PMT has been shown effective in patients with HRPE.[3] However, no large or solid data regarding the efficacy and safety of PMT treatment of pulmonary embolism are available.

The objective of this retrospective study is to evaluate the immediate haemodynamic effects of PMT in patients with HRPE and contraindications for thrombolytic therapy. Our secondary objective was to compare technical sucess and in-hospital mortality in patients who did and did not receive adjunctive thrombolytic therapy.

Patients and methods

Patients

A retrospective review was performed of 25 consecutive patients with high-risk pulmonary embolism over a 10-year period (from November 2005 to September 2015) who had been referred to our department for pulmonary digital subtraction angiography (DSA) and potential catheter intervention. The protocol was approved by the Slovenian National Ethics Committee (Number 0120-124/2018/4) which waived the need for informed consent. The criteria for study inclusion were patients with HRPE and contraindications to thrombolytic therapy. The clinical definition of HRPE was established in the presence of cardiogenic shock or hypotension, the latter defined as systemic systolic blood pressure (sSBP) < 90 mm Hg, or a pressure drop ≥ 40 mm Hg for > 15 min not caused by arrhythmia, hypovolemia, or sepsis. The diagnosis was made by computed tomography angiography in 24 of 25 cases. In addition, transthoracic echocardiography was performed in 11 patients. The study included 25 patients (62.6 ± 12.7 years; 16 men, 9 women). The youngest patient was 32 and the oldest 81 years old. The most common presenting symptom was dyspnoea (n = 14; 56%) followed by syncope (n = 8; 32%), chest pain (n = 7; 28%), cardiac arrest (4; 16%) and cough (n = 2; 8%). All patients had contraindications for thrombolytic therapy, including 12 with recent major surgery, 4 with ongoing or recent bleeding, 3 with neoplastic disease, 3 with traumatic injuries, two with suspected bleeding and one with recurrent pulmonary embolisms despite thrombolytic therapy. Despite contraindications a salvage thrombolytic therapy was used in 8 of 25 (32%) patients. 3 (12%) patients received systemic fibrinolytic agents during resuscitation in addition to PMT. In the rest 5 (20%) patients local thrombolysis was introduced after PMT according to operator preferences as on top salvage therapy.

Procedures

Our standard protocol for percutaneous mechanical thrombectomy includes the use of a long sheath for both percutaneous embolectomy with thrombectomy devices and thrombus fragmentation complemented with manual thrombus aspiration with an aspiration catheter. After local anaesthesia, a 6 French (F) short introducer sheath (Cordis Corp., Miami, FL, USA) is placed in the right or left common femoral vein and access to the pulmonary arteries is obtained with a 5 F pigtail catheter (Cordis Corp., Miami, FL, USA) advanced over 0.035-inch guide wires (Cordis Corp., Miami, FL, USA). Through the pigtail catheter in the pulmonary artery, a 260 cm guidewire (Amplatz Super Stiff, Boston Scientific Corporation, Natick, MA) is placed in the peripheral pulmonary artery. The 6 F sheath is exchanged over the guidewire for an adapted 90 cm long 8 F or 12 F introducer sheath (Cook, Bloomington, USA). The introducer sheath is placed in either the right or the left main pulmonary artery. The 0.035-inch 180 cm angled hydrophilic guidewire (Terumo Glidewire, Somerset, USA) is passed through the thrombus and left in a peripheral part of the pulmonary artery. With the guidewire remaining in the peripheral pulmonary artery, the 5 F or 6 F pigtail catheter is inserted. The 6 F catheter with a 10 mm pigtail wrap is used in the central portion of the pulmonary artery, whereas the 5 F catheter with a 6 mm pigtail wrap is used in the peripheral part of the pulmonary artery. The distal end of the pigtail catheter is placed distaly to the thrombus, then the catheter is spun quickly so that the distal curve serves as a rotor blade to fragment the thrombus. The catheter is rotated manually around the axis of the stationary guidewire. After pigtail thrombus fragmentation,

TABLE 1. Effects of mechanical percutaneous mechanical thrombectomy (PMT)

Clinical characteristic (n=25)	Before PMT	After PMT	P value
Heart rate, min^{-1}	100.2 ± 34.5	86.6 ± 31.3	0.326
Arterial systolic pressure, mm Hg	91.6 ± 40.0	121.9 ± 34.3	0.100
Peak systolic tricuspid pressure gradient, mm Hg	55.4 ± 13.3	29.8 ± 5.2	0.018

Mean ± standard deviation is shown.

we sometimes performed additional manual clot aspiration with large-lumen percutaneous transluminal coronary angioplasty guide catheter (8-Fr Guider-Softip; Boston Scientific; Scimed or Brite tip; Cordis; Johnson and Johnson, Florida, USA). Strong manual aspiration is created through a regular Luer-lock 50 ml syringe plunger while slowly withdrawing the catheter through the introducer long sheath. To evaluate the distal embolization resulting from catheter thrombectomy, pulmonary angiography is performed several times during these procedures (flow 5 ml/sec; 10 ml of contrast media). In cases, without thrombus fragmentation, we used Aspirex®S 8 F and 11 F catheter device (Straub Medical AG, Wangs, Switzerland). Aspirex®S is introduced through a 12 F introducer sheath (Cook, Bloomington, USA) over an exchange guide wire and then advanced to the occlusion site. The catheter is gently withdrawn and pulled back during aspiration. The heart rate and blood pressure were monitored during the whole procedures. Thrombectomy was discontinued as soon as systemic arterial pressure increased. Technical success was defined as an angiographic improvement of pulmonary flow. At the beginning of each procedure, all patients received 5,000 international units of unfractionated heparin intravenously. After the procedure patients were treated with UHF, guided by aPTT until haemodynamic and respiratory stabilisation and until kidney function was stable. After that LMWH or oral anticoagulant was started. Thrombolytic threatment was provided using alteplase as systemic or local therapy. Systemic therapy included a total of 100 mg of alteplase as a 10-50 mg slow bolus (1–2 min) and the rest as 2 hour intravenous infusion. Local therapy was individualized and was administered as alteplase infused over pulmonary artery sheath at the rate of 1 mg/h for up to 15 hours.

Follow-up

The clinical status, systemic blood pressures, bleeding, and transthoracic echocardiography of all patients were recorded after the procedure and before they were discharged. All patients received warfarin for at least 6 months. Survival to hospital discharge was monitored. Significant bleeding was defined as BARC 3 and 5.[8]

Statistical analysis

All quantitative data were expressed as mean ± standard deviation (SD). Dichotomous variables are expressed as percentages. $p < 0.05$ was considered significant. Means were compared using the Paired-Samples T-Test.

Results

25 patients met the inclusion criteria for the study. The majority of patients were in obstructive shock at presentation with mean maximum lactate levels of 7,8 ± 6,6 mmol/L, vasopressors were used in 77% and 59% needed mechanical ventilation, veno-arterial extracorporeal membrane oxygenation was used in one case. Mean simplified Pulmonary Embolism Severity Index (sPESI) was 2.9. Fourteen (56%) patients underwent thrombus fragmentation using a pigtail catheter which was complemented with manual thrombus aspiration in 7 patients. The Aspirex®S percutaneous thrombectomy device was used in 11 (44%) patients. In one patient, treated with Aspirex®S, additional manual aspiration of the peripheral thrombosis was performed for even better pulmonary perfusion. In one patient surgical embolectomy with right atrium thrombus removal was performed three days before PMT. Seventeen patients (68%) were treated with PMT only, 3 (12%) patients received systemic fibrinolytic agents during resuscitation in addition to PMT and in 5 (20%) patients local thrombolysis was introduced after PMT according to operator preferences as on top therapy.

We observed nonsignificant improvements in arterial systolic blood pressure (92 ± 40 mm Hg *vs* 119 ± 34; $p = 0.100$) and heart rate (99 ± 35 min^{-1} *vs* 87 ± 31 min^{-1}; $p = 0.326$) before and after treatment, respectively. Peak systolic tricuspid pressure gradient was significantly lower after treatment (57 ± 14 mm Hg to 31 ± 3 mm Hg; $p = 0.018$) (Table 1). There was no diference in arterial systolic blood pressure, heart rate or peak systolic tricuspid pressure gradient pre and post procedure between patients treated with PMT only and those with additional thrombolysis.

TABLE 2. Characteristics of the study group

Clinical characteristic	All patients (n =25)	PMT without lysis (n = 17)	Systemic or local lysis + PMT (n = 8)	p-value
Age, years	62.6 ± 12.7	66.1 ± 11.2	55.4 ± 13.5	0.05
Breathing frequency before, min^{-1}	21.6 ± 10.1	24.4 ± 8.6	16.8 ± 12.0	0.25
sPESI score	2.9 ± 0.7	3.1 ± 0.7	2.6 ± 0.5	0.11
Max. lactate, mmol/L	7.8 ± 6.6	5.5 ± 7.1	10.7 ± 4.6	0.10
Max troponin I, µg/L	5.6 ± 10.8	2.4 ± 3.4	10.6 ± 16.3	0.09
Technical success, %	80	82	75	0.67
Survival to hospital discharge, %	68	76	50	0.19
Cardiogenic schock, %	67	54	88	0.11
Mechanical ventilation, %	61	47	88	0.06
Vasopressors or inotropes, %	77	71	88	0.18
Bleeding, %	16	12	25	0.40
Transfusion, %	24	12	50	0.04
Aspirex, %	44	53	25	0.19

Mean ± standard deviation is shown if not stated otherwise; NS = not significant (p > 0.05); PMT = percutaneous mechanical thrombectomy; sPESI = Simplified Pulmonary Embolism Severity Index

Patients treated with a combination of PMT and thrombolysis were younger (55.4 ± 13.5 *vs* 66.1 ± 11.2 years respectively; p = 0.05) than patients treated with PMT alone. There was, however, no difference in maximum lactate, troponin I level, sPESI score, mechanical ventilation, cardiogenic schock, vasopressors/inotrope use, bleeding, transfusion or Aspirex use between patients of the two groups (Table 2). There were more transfusions in patients with thrombolysis (12% *vs* 50%; p = 0.04).

Overall PMT was technically successful in 20 patients (80%) and 17 patients (68%) survived to hospital discharge. Technical success and survival to hospital discharge were not significantly different between patients with PMT only and those with PMT plus thrombolysis (82% *vs* 75% and 76% *vs* 50%, respectively) (Table 2).

Aside from one BARC 3b puncture site bleeding there were no major procedural complications. Minor complications developed in 6 of 25 patients (24%): transient bradycardia during the catheterization in five patients and groin hematoma in one patient. An inferior vena cava filter (Bard, Crawley, UK) was inserted in nine patients (36 %).

Discussion

Our single center retrospective study demonstrated the efficacy and safety of PMT in patients with HRPE. HRPE remains an important clinical problem with a high mortality rate. Systemic thrombolytic therapy is currently indicated on top of heparin anticoagulation for acute HRPE accompanied by hemodynamic instability.[3] But systemic thrombolysis carries a significant risk of bleeding which is approximately 13% of major bleeding and 1.8% of intracranial or fatal haemorrhage, particularly, when pre-disposing conditions or comorbidities existed.[3]

If absolute contraindications to thrombolysis are present and if performed in an experienced center in HRPE surgical embolectomy, in addition to anticoagulation, is often used.[3,9–11] Preoperative thrombolysis increases the risk of bleeding, but it is not an absolute contraindication to surgical embolectomy.[12] In the case of early embolectomy before the hemodynamic collapse, perioperative mortality rates of 6% or less have been reported.[13,14] Pulmonary embolectomy is technically a relatively simple operation, but only a few tertiary care centers offer emergency surgical embolectomy with round-the-clock availability. Additionally in the sickest of patients in severe shock or cardiac arrest time needed to perform surgery may change the prognosis of the patient. In these unstable patients with HRPE PMT may be an effective and safe treatment for improvement of pulmonary flow with thrombus modification.[15]

The goal of interventional treatment is the improvement of pulmonary flow with the removal or distal embolization of obstructing thrombi from

the main pulmonary arteries to facilitate right ventricle recovery. Acute increase of pulmonaty artery pressure leeds to right ventricle failure which is a predictor of mortality.[16,17] We showed that PMT can reduce pulmonary artery pressure. In patients threatened by right ventricular failure, even a small hemodynamic improvement may be life-saving and extend the critical time frame for further recanalization. Moreover, the increased total surface area of the fragments may accelerate the efficacy of spontaneous intrinsic lytic activity.[18]

Although we only included patients with contraindications for systemic thrombolysis some of the patients received local thrombolysis and some even systemic thrombolysis as a salvage therapy. Not many clinical data is available about PMT. In a review on interventional treatment that included 35 non-randomized studies 67% of patients received additional thrombolysis.[3] Our data proved the effectiveness of PMT also in combination with salvage thrombolytic therapy. The effectiveness of the PMT alone was similar to combined therapy of PMT and thrombolysis. Conversely, if further pharmacologic thrombolysis is desired following primary PMT, an extended thrombolytic infusion can still be performed.[19,20] Even more, after fragmentation, a greater surface area of thrombus can be exposed to the thrombolytic; consequently, less drug and perhaps less time is needed to achieve thrombolysis. However, although we failed to show any difference hospital survival in patients with or without thrombolytic therapy, there is a trend to increased mortality in patients who received thrombolysis. This is probably due to worse clinical condition of patients who received thrombolysis (trends towards higher maximal lactate and troponin, more mechanical ventilation, vasopressor and inotrope use and more cardiogenic schock). Interestingly the sPESI score does not show the difference between the groups which could reflect the fact that it was calculated at addmision and patients condition often deteriorated afterwards. We also failed to show a difference in bleeding between patients with or without thrombolytic therapy. This is probably due to small sample size. However, puncture of a femoral vein (even with 12 F sheath) does not seam to bring a high additional risk since we only had one procedure related bleeding event. Although there was no difference in bleeding we observed more transfusions in patients with thrombolysis. This was due to non-bleeding related transfusions (anemia or suspected bleeding).

Veno-arterial extracorporeal membrane oxygenation could be used to rescue patients when thrombolytic treatment fails or as temporary hemodynamic support prior to surgical[21] or catheter-based embolectomy.[22]

Our study has limitations. First, it is a retrospective, single-center study. Second, patients were treated according to the available strategy, not systematic indications. This includes individualized thrombolytic treatment. Also due to a very short timeframe available in these critically ill patients not all the patients had echocardiography done before the PMT and thus peak systolic tricuspid pressure gradient difference is of limited value. Due to retrospective nature of the study and a long period of inclusion some data are missing. Third, we did not perform a protocol-based follow-up based on long-term echocardiography and imaging to detect chronic thromboembolic pulmonary hypertension development.

In conclusion, in patients with high-risk pulmonary embolism who cannot receive thrombolytic therapy, percutaneous mechanical thrombectomy is a promising alternative to reduce pulmonary artery pressure.

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research article

Electrochemotherapy in treatment of canine oral malignant melanoma and factors influencing treatment outcome

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Background. Oral malignant melanoma is the most common, but aggressive oral cancer in dogs with poor prognosis. Electrochemotherapy (ECT) has therapeutic potential in such tumors as effective local treatment. Therefore, the aim of this prospective clinical study was to evaluate treatment effectiveness of ECT in as first line treatment for canine oral malignant melanoma, and search for factors influencing treatment outcome.

Methods. Sixty-seven canines with primary oral malignant melanoma, non-candidates for first-line therapy, were enrolled. All dogs received ECT and follow-up exams for the span of two years.

Results. Based on RECIST criteria, the objective response rate was 100%, 89.5%, 57.7%, and 36.4%, in stage I, II, III and IV, respectively. Only patients in stage I, II and III with partial or complete response improved their quality of life. The median time to progression was 11, 7, 4 and 4 months, and median survival time after the treatment was 16.5, 9.0, 7.5 and 4.5 months, for patients in stage I, II, III and IV, respectively. Significantly better was local response in stage I and II disease ($p = 0.0013$), without the bone involvement ($p = 0.043$)

Conclusions. Electrochemotherapy is effective local treatment of oral canine malignant melanoma when no alternative treatment is available. Better response is expected in stage I and II patients with tumors without bone involvement.

Key words: cancer; dog; electroporation; electrochemotherapy

Introduction

Oral malignant melanoma represents 6% of neoplasms in canines, being the most common oral cancer among that species. It is more aggressive and has a poorer prognosis than any other melanoma.[1,2] The mean age at diagnosis is 11.6 years.[3]

Most common breeds affected by melanoma are Cocker Spaniel, German Shepherd, Pointer, Weimaraner, Golden Retriever, Labrador Retriever, Poodle, Chow-Chow and Boxer. It usually infiltrates locally, with metastatic progression in more than 80% of the cases. Typically, there is a better outcome and prognosis with early diagnosis and/or rostral location. On the other hand, worse prognosis is associated with late diagnosis, caudal location, presence of satellite lesions, and when dysphagia and dyspnea are present.[4]

It is known that lack of treatment is associated with poor survival times (average of 65 days)[2],

while best survival times are achieved with clean margins at surgical excision plus radiotherapy. Therefore, the latter is considered the first-line treatment for this neoplasm, with a recurrence of distant metastasis that can arise 4 to 8 months after the end of it. Given the fact that most of the tumors in advanced stages are already involving bony structures, complete surgical resection is often very aggressive and has poor prognosis. Adjuvant chemotherapy does not increase response rates or adds any survival benefit to the patients.[5,6]

Electrochemotherapy (ECT) is a therapeutic modality that has been gaining ground in Oncology since the Standard Operating Procedures were published in 2006.[7] ECT consists in the application of an electric field to a tumor in order to increase the uptake of bleomycin that was previously administered (locally or intravenously) at a very low dose[8,9]; alternatively, cisplatin can be used locally with equally good results.[10] ECT is primarily indicated for cutaneous and subcutaneous tumors of any histology. After the electric field is applied, a cell membrane permeabilization is produced by a physical phenomenon known as electroporation, affecting all tumor cells, regardless of the histological tissue.[11] A meta-analysis of ECT clinical studies in human oncology showed that the overall objective response (OR) rate varies from 62.6% to 82.2% depending also on which route was used to administer the drug.[12] Great efforts are being made to extend ECT to non-cutaneous locations such as liver[13], brain[14], and bones.[15] Moreover, an endoscopic electrode was developed for treating the colon.[16] The considerable experience and deeper understanding gained since ECT inception, lead to the publication of new and more flexible Standard Operating Procedures.[17] In veterinary medicine, ECT is a well-established therapy with multiple indications and proven efficacy.[18,19] It has been reported very good results for treating mast-cell tumors (OR 62.3-100%)[20–22], sarcomas (OR 90%)[23], perianal tumors (OR 94%)[24], nasal duct tumors (OR 91%)[24,25], among others, with minimum to absent side effects.

In vivo, ECT is remarkably more effective in immunocompetent subjects.[26] Different mechanisms for the induced local immune response have been demonstrated in preclinical studies. Some of them have also been observed in human patients. However, this local immune response does not induce an abscopal effect, and intense efforts are being made in order to achieve it.[27,28]

The clinical experience with melanoma immunotherapies seems promising, with increasing evidence that combined approaches may be required to ensure durable responses in the majority of the patients.[29]

In this prospective clinical study, we present results obtained from 67 canine patients affected by oral malignant melanomas that were treated with ECT and later followed-up for up to two years. We also discuss ECT benefits in comparison to classic therapeutic approaches and search for factors influencing treatment outcome.

Methods

In this longitudinal prospective study, sixty-seven (67) canine (*Canis familiaris*) patients with oral malignant melanoma and not candidates for first-line therapy were enrolled. Primary goals consisted on evaluating local response and survival times. In addition, we also decided to evaluate quality of life, time to progression and disease-free survival times. Treatments were performed from 10/2014 through 12/2017 at Centro de Especialidades Medico Veterinarias, Buenos Aires, Argentina.

Inclusion criteria:

- Patients with oral malignant melanoma at any stage.
- Patients able to stand general anesthesia.

Exclusion criteria:

- Pregnant females
- Patients are candidates for surgery and owner agrees to perform treatment.

Patient evaluation

Participants' diagnosis was confirmed by histopathology (surgical biopsies). Immunohistochemistry was performed in selected cases for amelanotic melanoma or when diagnosis was not definitive.

Patients were staged according to the WHO staging system for canine oral melanoma (Table 1).[30] They underwent a complete physical examination, complete blood count (CBC), and serum biochemi-

TABLE 1. WHO criteria for staging canine oral melanoma

Stage	Tumor diameter	Lymph node involvement	Metastasis
I	< 2 cm	No	No
II	2 - 4 cm	No	No
III	> or = 4 cm	No	No
	Any	Yes	No
IV	Any	Yes or No	Yes

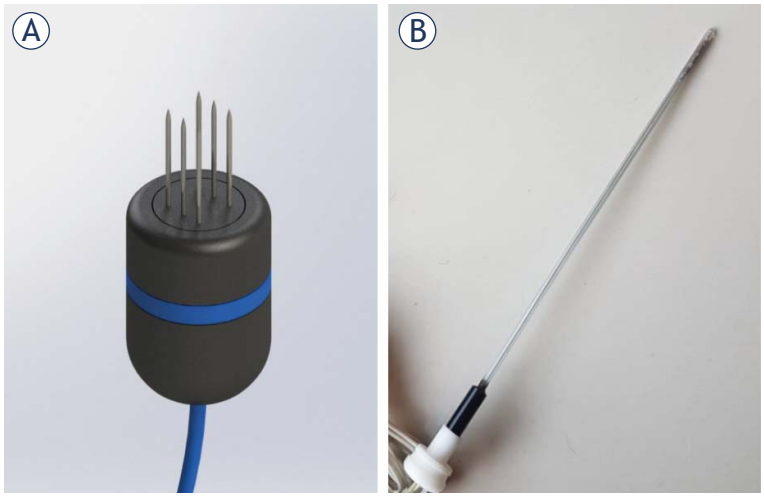

FIGURE 1. Electrodes used to treat patients. In **(A)** a render of the six-needle electrode used. It consists of two rows of needles separated 4 mm from each other. In **(B)** a picture of the Single Needle Electrode for nasal duct treatment.

cal analysis. The size of the tumor was measured using a caliper or by CT scan for masses involving deeper structures such as retro-orbital region, nasopharynx or nasal cavity. In addition, to confirm or rule out bone involvement when plain radiographs were not fully diagnostics.

Evaluation for metastatic disease was performed via 3-view thoracic radiographs, abdominal ultrasound and fine-needle aspirate of regional lymph nodes. CT scan was performed when radiographs were not diagnostics. Data gathered in all patients included: age, weight, sex, breed, stage at time of diagnosis, histologic subtype, presence or not of bone infiltration, location of the tumor within the mouth (was considered caudal when compromising the caudal third of the hard palate, soft palate, oropharynx, angle of the mandible, ramus of the mandible or the tonsillar region. Otherwise, it was considered to be rostral), local response, quality of life, presence/absence of metastasis, number of ECT sessions, new metastasis after treatment, time to progression, disease-free survival times (when CR was obtained), overall survival times and cause of death.

ECT procedure

ECT and surgical resection of enlarged regional lymph nodes were performed under general anesthesia.

Chosen anesthesia protocol was proved to provide adequate comfort throughout the procedure, and consisted on premedication with IM (intramuscular) administration of xylazine (Xylazine 100®, Richmond, Buenos Aires, Argentina) 0.5 mg/kg and tramadol (Tramadol®, John Martin, Buenos Aires, Argentina) 2 mg/kg. Induction was performed with IV (intravenous) administration of propofol (Propofol Gemepe®, Gemepe, Buenos Aires, Argentina) 2-3 mg/kg. For maintenance, isoflurane (Zuflax®, Richmond, Buenos Aires, Argentina) 2–3% and intravenous fentanyl (Fentanyl Gemepe®, Gemepe, Buenos Aires, Argentina) 2 mcg/kg were used. Amoxicillin with clavulanic acid (Clavamox® Zoetis, Buenos Aires Argentina) 15 mg/kg/bid and meloxicam (Meloxivet®, John Martin, Buenos Aires, Argentina) 0.2 mg/kg/SID were administered orally for prophylaxis and analgesia after the treatment according to the needs of each patient.

ECT was initiated by using IV (intravenous) bleomycin (Blocamicina®, Gador, Buenos Aires, Argentina) at a dose of 15 000 IU/m^2 of body surface. Eight minutes later, in order to allow drug distribution, electric pulses were delivered (following Standard Operating Procedures)[17] with a BTX ECM 830 (Harvard Apparatus, Holliston, MA, USA) unit. Each train of pulses consisted of eight (8) square wave monopolar pulses of 400V (1000V/cm) 100 μs long at 10 Hz. The number of trains applied varied according to the tumor size, aiming to cover the whole tumor volume plus safety margins beyond it. In all cases, a 6-needle electrode was used (Figure 1A.), but for nasal duct invasion, the Single Needle Electrode® was indicated (Figure 1B.).[25] This electrode uses the same pulse parameters, but thirty-two (32) instead of eight (8) pulses are delivered on each train. Recommendations to report results in electrochemotherapy studies from Campana *et al.* were followed.[31]

Patient follow-up

Patients were scheduled for checkups at 14, 30 and 60 days after treatment. If no further sessions were needed, follow-ups were required every 3 to 4 months until the end of the study. On each visit, a complete clinical examination and thoracic radiographs were performed to determine if lymph node enlargement and/or development of lung metastasis respectively were noticed; as they represent the most common sites for metastatic dissemination.[6]

Treatment response evaluation

According to the RECIST criteria, the response was determined at 30 days and confirmed at 60 days

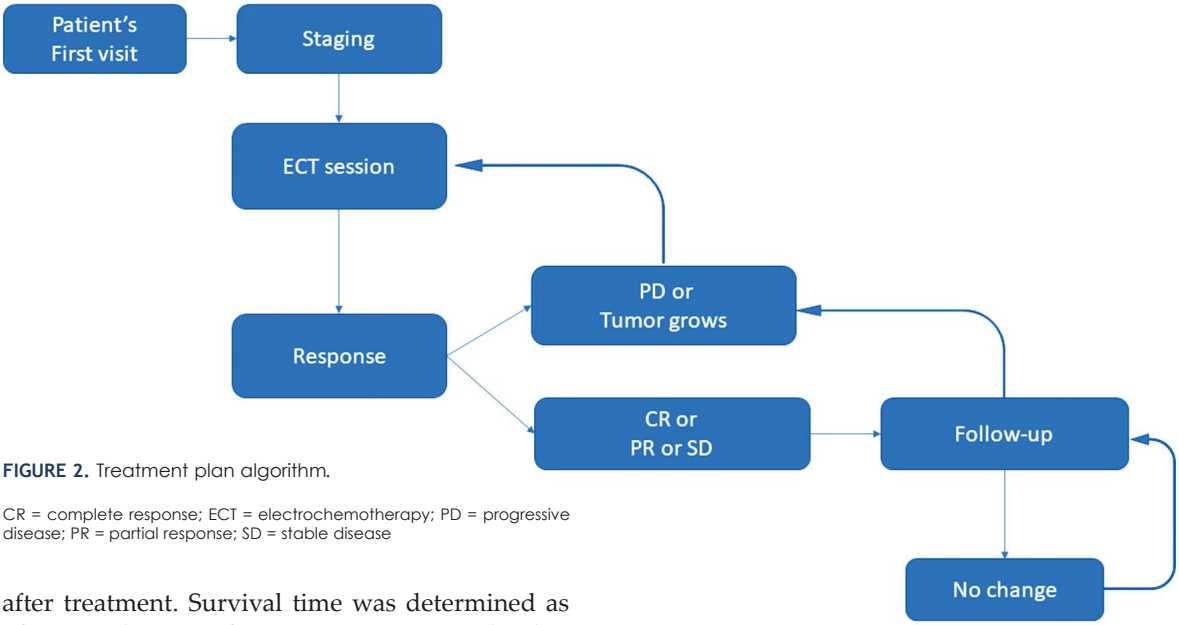

FIGURE 2. Treatment plan algorithm.

CR = complete response; ECT = electrochemotherapy; PD = progressive disease; PR = partial response; SD = stable disease

after treatment. Survival time was determined as of time in between first ECT treatment and either end of the study or death of the patient. Time to progression was determined to be from the day of the ECT session that achieved a confirmed response through the day of an either local or distant relapse. Disease-free survival time was calculated only among patients that were able to achieve a CR. It was determined as time between a CR was achieved through the day where a relapse, local or distant, was evidenced.

Quality of life

The quality of life was assessed before the first ECT treatment and by each follow up exam using a questionnaire inspired in the observations of Lynch *et al.*[32] It was filled by the owner (provided in additional material). It consisted of three (3) clinical and behavioral questions, with values between 0 and 3. Final score was obtained by the sum of all questions, with lower scores representing better results (score of 0) and higher scores worse quality of life (score of 9). In order to consider if quality of life was either improved or worsened, a difference of at least two (2) units was required when comparing both scores (day of the treatment *vs*. each follow-up).

Data obtained by the treating oncologist was also included in the patient's medical record.

Criteria for ECT retreatment and additional therapy

If tumor growth was detected in any given follow-up visit, then a new session was scheduled. Likewise, if the achieved response was a progressive disease (PD), a new session was performed. Any enlarged regional lymph node noticed by the time of a follow-up (with metastatic spread determined by fine needle aspirate) was surgically removed (Figure 2).

Statistical analysis

Statistics were performed using MedCalc® version 19.1. Fisher exact test was used to determine the significant association between local response and different factors studied. Multivariate logistic regression analysis was performed with the factors that showed significant association with local response.

Kaplan-Meier curves were calculated, and their significance determined by the log-rank test, for different factors affecting survivorship and time to progression. Cox multiple regression analysis using forward regression method was performed with statistically significant factors for survival.

Ethics approval and consent to participate

All regulations from the Consejo Profesional de Médicos Veterinarios were followed. Informed consents were signed by the owners. This work was approved by the IACUC of the School of Veterinary Sciences, University of Buenos Aires, Argentina. Protocol number: 2018/31.

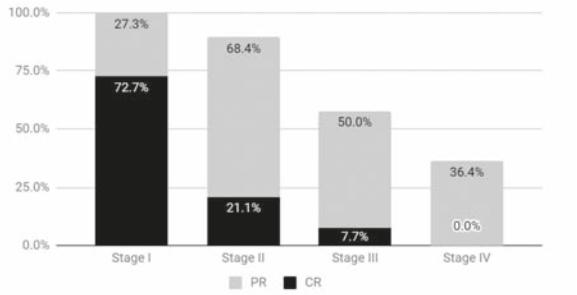

FIGURE 3. The bars show the sum of partial and complete response percentages obtained on each stage. For stage I: complete responses (CR) 72.7% (8) partial responses (PR) 27.3% (3) stable diseases (SD) 0% progressive diseases (PD) 0%, stage II: CR 21.1%(4) PR 68.4% (13) SD 0% PD 10.5% (2), stage III: CR 7.7% (2) PR 50% (13) SD 26.9% (7) PD 15.4% (4) and stage IV: CR 0% PR 36.4% (4) SD 36.4% (4) PD 27.3% (3).

Results

Demographics of the treated patients is in Table 2. The patients were followed up for two years. Two (2) out of the sixty-seven (67) treated patients remained alive by the end of the study. Sixty-one percent of the patients (41) required a single session to achieve a final response, 30% (20) required two, 7% (5) required three and 1% (1) required four procedures. Time between sessions varied in each case depending on tumoral regrowth, and usually is between one and two months.

Local response

After a median of 1.5 treatment sessions (regardless of the stage), the overall objective response (OR) rate was 70.1% (47); with 20.9% (14) complete responses (CR), 49.3% (33) partial responses (PR), 16.4% (11) stable diseases (SD) and 13.4% (9) progressive diseases (PD). Local response in each stage is presented in Figure 3.

Logistic regression analysis showed that early stages of disease (stages I and II) were associated with significantly higher objective response rates than late stages (III and IV), 93.3% *vs*. 51.4%, respectively (HR: 0.08, 95% CI: 0.02–0.36, p = 0.0013) (Table 3).

Time to progression and disease-free survival times

The median time to progression was 11 (range 4–30) months, 7 (range 3–21) months, 4 (range 2–4) months and 4 (range 1–4) months, for stages I, II, III and IV, respectively.

TABLE 2. Patients' demographics

Characteristics of 67 patients	Mean (range) (%)
Age (years)	11.7 (6–16)
Sex ratio (M:F)	37:30
Body weight (kg)	22.6 (3.5–58)
Breeds	
Crossbreed	24 (35.8%)
Labrador retriever	9 (13.4%)
Cocker spaniel	7 (10.4%)
Golden retriever	6 (8.9%)
Beagle	4 (5.9%)
Poodle	4 (5.9%)
Rottweiler	3 (4.4%)
Doberman	2 (2.9%)
Shar-Pei	2 (2.9%)
Dogo	1 (1.4%)
English Mastiff	1 (1.4%)
Chow-Chow	1 (1.4%)
Basset Hound	1 (1.4%)
Pekingese	1 (1.4%)
Dalmatian	1 (1.4%)
Location (Rostral : Caudal)	36 : 31
Stage	
I	11 (16.5%)
II	19 (28.3%)
III	26 (38.8%)
IV	11 (16.4%)
Histologic subtype	
Epithelioid	17 (25.4%)
Mixed	17 (25.4%)
Spindle cell	17 (25.4%)
Anaplastic	9 (13.4%)
Others	7 (10.4%)

The multivariate analysis for the time to progression showed that stages I (p = 0.0005) and II (p = 0.0094), and absence of bone invasion (p = 0.043) were predictive factors for longer times to progression (Table 4).

Considering the patients who achieved a complete response (n = 14), the median disease-free survival time for these patients was 12.5 (range 3–30) months. Among them: 2 (14%) remained alive at the end of the study, 6 (43%) died of unrelated causes. Only one (7%) developed distant metastases during the follow-up, three months after the complete response was obtained. Twelve (86%) were treated with only one session of ECT. Four (29%) had bone involvement.

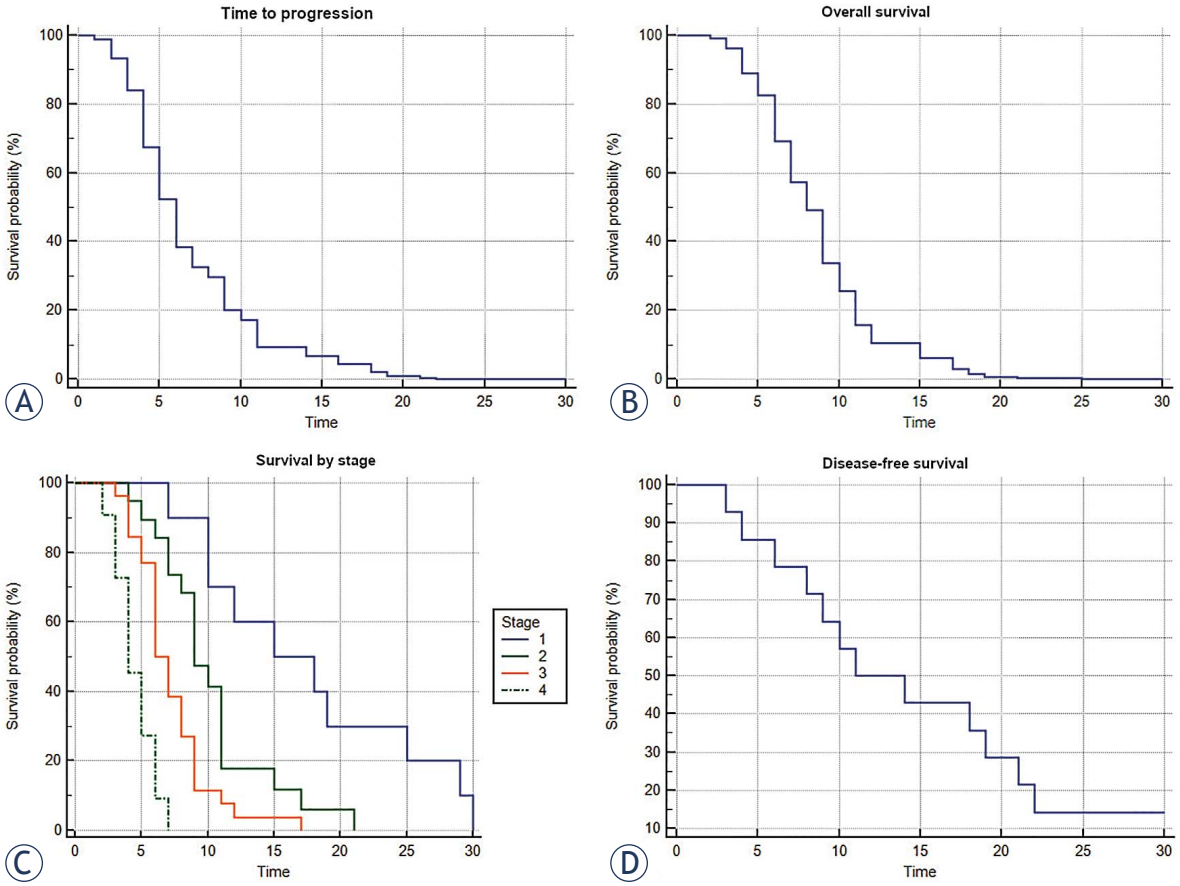

FIGURE 4. (A) Time to progression curve (Cox proportional-hazards regression, n = 67, p < 0.0001). **(B)** Overall survival time (Cox proportional-hazards regression, n = 67, p < 0.0001). **(C)** Survival time by stage (Kaplan-Meier curves of survival, n = 67, p < 0.0001). **(D)** Disease-free survival time (Kaplan-Meier, n = 14).

Overall survival time

Median survival time after the treatment was 16.5 (range 4–30) months, 9 (range 4–21) months, 7.5 (range 3–17) months and 4.5 (range 2–7) months, in stage I, II, III and IV, respectively. Two patients remained alive at the end of the study, therefore, in the statistical analysis were censored (Figure 4).

In multivariate analysis for survival time, stage IV (p = 0.0001) was a negative predictive factor. On the contrary, stage I (p = 0.0083) and the absence of bone involvement (p = 0.0340) were predictive factors for longer survival (Table 4).

Cause of death

Euthanasia was the main cause of death during the course of this study, representing 77.6% (52) of the deceased patients, where 49.3% (33) of those euthanasias were performed due to local progression and 28.4% (19) due to metastatic progression of the disease. Unrelated causes of death reached 17.9% (12) and finally, 3% (2) of the overall enrolled patients remained alive by the end of the follow-up period (24 months).

Metastasis

Among patients with no metastatic lesions at the time of diagnosis, 23.9% (16) developed new metastasis during subsequent follow-up visits. Patients treated in stage I did not develop new metastasis. For stages II, III and IV, the number of patients that developed metastasis after the first visit was also similar, being 31.6%, 36.8% and 31.6%, respectively.

Quality of life

Patients in stage I with the tumor in rostral location had no negative impact on their quality of life before or after the treatment. Patients in stages I (with

TABLE 3. Univariate (Fisher exact test) and multivariate (Logistic regression analysis) test for analyzing the association between OR and different factors

Characteristics	Number of patients with OR	Univariate Fisher exact test	Multivariate OR 95% CI Logistic Regression Analysis	p
Sex (M/F)	37/30	p = 0.602	–	–
Location (Rostral/Caudal)	36/31	p = 0.062	–	–
Bone Involvement (Yes/No)	43/24	**p = 0.026**	automatically excluded	–
Metastasis present, excluding lymph nodes (Yes/No)	10/57	p = 0.150	–	–
Early-stage (stages I+II/stages III+IV)	30/37	**p = 0.00016**	0.08(0.02-0.36)	0.0013

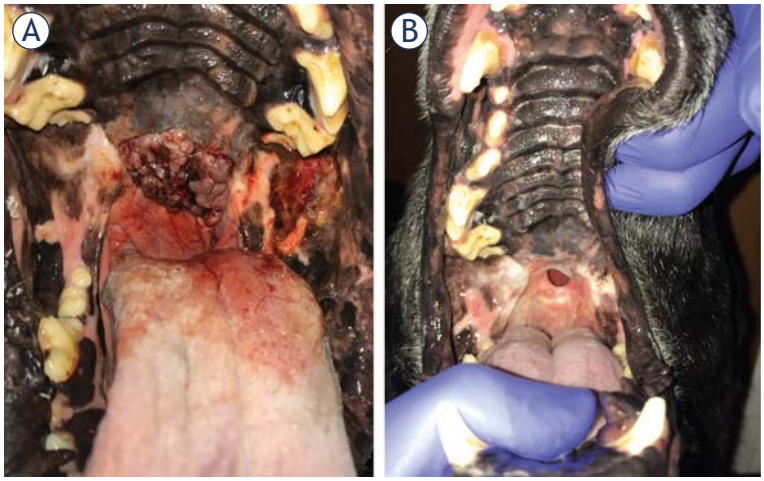

FIGURE 5. (A) Stage II patient with melanoma in the soft palate, before the treatment. **(B)** Final response obtained 30 days after third round of ECT. Completely absent tumoral tissue and a fistula can be seen. Quality of life of the patient improved in spite of the fistula, which was asymptomatic.

the tumor in a caudal location), II and III presented the following signs and symptoms: bleeding, accidental biting of the tumor, pain and impossibility to eat adequately. Among the latter, those who obtained a CR or PR after ECT, had an improvement in their quality of life (Figure 5). On the contrary, patients that obtained a SD or PD, did not. Patients in stage IV did not improve their quality of life regardless of the obtained response.

Discussion

ECT is a very effective local treatment for tumors originating almost any histological type of tissue. In the last few years, the treatment has become readily available in many countries due to its multiple advantages. Among them: high efficacy, low cost and simplicity at time of performing. The aforementioned, combined with the availability of inexpensive devices, made possible its expansion through Latin America, where radiotherapy continuous to be a non-affordable therapeutic alternative. Since often ECT is the only available option, professionals tend to treat indiscriminately, without even taking in consideration if that particular patient is indeed a surgical candidate or not. Therefore, we believe that veterinarians should assess more carefully, in advance, if good results could potentially be achieved with this modality, in order to consider or plan a therapeutic alternative. We think that for an adequate determination who might be considered a good candidate for the treatment, further studies are needed, with the hope that one day ECT could become a first-line therapy for many tumors.

In this study, frequency of breeds, differences in gender predisposition and age at the time of diagnosis were in accordance with different authors.[33,34] Also, melanomas located in the tongue were rare, as reported by others.[35] The largest group of patients enrolled in the study were staged II or III. This is probably related to the fact that owners were not able to recognize the characteristic lesions earlier in the onset of the disease, due to the intra oral location of the tumor. Early signs and symptoms were probably unnoticed by the caretakers, but more prominent symptomatology caused by a more advanced stages of the disease was the red flag that made owner seek for medical advice.[36] We had a similar prevalence of histological subtypes to the one reported by Ramos-Vara *et al*., except for a lower incidence of spindle cell type. Also, we had a higher incidence of amelanotic melanomas.[33]

Best local responses were obtained in patients with tumors smaller than 4 cm in diameter (stages I and II). We came to the conclusion that smaller tumors are easier to treat with ECT than larger ones, not only due to the limitation related to the size of the electrode (lower chances to reach larger tumor extensions), but also because bigger tumors have necrotic areas, and a very inhomogeneous vascularization, affecting the distribution of the drug. We addressed this particular issue in a previous

TABLE 4. Univariable (Kaplan-Meier) and multivariable (Cox proportional-hazards) analysis of patient-related factors for time to progression and overall survival time. Hazard Ratio and 95% Confidence interval is reported

	N. of patients	Overall Survival [Univariable]	P	[Multivariable]	P	TTP [Univariable]	P	[Multivariable]	P
Sex									
Male	35	1.58 (0.95–2.63)	0.0634	–	–	1.67 (0.99–2.75)	**0.0433**	–	–
Female	30			–	–			–	–
Location									
Rostral	34	0.71 (0.43–1.16)	0.1596	–	–	0.77 (0.47–1.26)	0.2921	–	–
Caudal	31			–	–			–	–
Metastasis									
No (or lynph node only)	55	0.29 (0.09–0.88)	**0.0001**	–	–	0.34 (0.12–0.96)	**0.0009**	–	–
Yes	10			–	–			–	–
Bone Involvement									
Yes	42	2.45 (1.50–4.00)	**0.0004**						
No	23			(0.24–0.88)	0.0184	0.42 (0.26–0.68)	**0.0005**	(0.28–0.98)	0.043
Histology									
Epithelioid *vs.* Fusocellular	16 / 16	0.59 (0.31–1.14)	0.1768	–	–	0.60 (0.32–1.14)	0.0534	–	–
Epithelioid *vs.* Mixed	16 / 17	0.54 (0.28–1.05)		–	–	0.49 (0.25–0.96)		–	–
Epithelioid *vs.* Anaplastic	16 / 9	0.46 (0.19–1.11)		–	–	0.35 (0.14–0.93)		–	–
Fusocellular *vs.* Mixed	16 / 17	0.92 (0.44–1.93)		–	–	0.82 (0.39–1.72)		–	–
Fusocellular *vs.* Anaplastic	16 / 9	0.77 (0.30–1.99)		–	–	0.59 (0.22–1.64)		–	–
Mixed *vs.* Anaplastic	17 / 9	0.84 (0.33–2.18)		–	–	0.73 (0.26–2.04)		–	–
Stage									
I *vs.* II	10 / 18	1.86 (1.03–3.34)	<0.0001	Stage I: (0.13–0.74)	0.0083	0.53 (0.30–0.96)	0.0001	Stage I: (0.07–0.47)	0.0005
I *vs.* III	10 / 26	3.28 (1.76–6.09)				0.28 (0.15–0.53)		Stage II: (0.24–0.82)	0.0094
I *vs.* IV	10 / 11	9.05 (2.58–31.75)				0.19 (0.07–0.51)			
II *vs.* III	18 / 26	0.57 (0.30–1.06)				0.52 (0.27–0.99)			
II *vs.* IV	18 / 11	0.21 (0.06–0.72)		Stage IV:(2.04–9.29)	0.0001	0.35 (0.13–0.96)			
III *vs* IV	26 / 11	0.36 (0.10–1.29)				0.67 (0.24–1.90)			

study, by using a combined administration of bleomycin, systemically and locally.[37]

There is still no consensus on when is the best time to perform a second (or subsequent) ECT session. In this work, we considered tumor growth as the main variable to be taken in consideration for that matter. This is based on our 10+ years of experience performing ECT, and by the fact that we were able to identify four different types of outcomes or evolutions after the first session. In the first one, the most common, the tumor gets steadily smaller until reaching a stable size or disappearing completely. In the second type, the so called "two-times response", the lesion reduces its size up to one point where it stops for some time, resuming shrinkage later until reaching a stable size or disappearing. In the third type, the "apparent unresponsive", it never gets smaller, stays apparently unresponsive to the treatment, but it does not grow and can also remain in that steady state for years. And finally, in the fourth type, "the insufficiently treated", after an initial short period of shrinkage, the tumor starts growing back again. It is known that all these types of evolutions are related to the bleomycin's mechanism of action, which consists in cutting the DNA strands and inducing mitotic

cell death. If the tumor cell are treated while dividing, it will die. However, if there is a number of quiescent cells not attempting to enter into cell cycle, they will remain alive. This is the reason why this treatment displays selectivity towards dividing cells.[9] In the light of the above explained, we consider that the only situation that potentially opens the door for a subsequent treatment after the original one is when a regrowth from a previously treated lesion is observed. Otherwise, we can be facing a type two or three evolution.

Absence of bone involvement was associated with longer times to progression and survival. This fact could be attributed partially to how difficult is to insert the electrode inside the bone tissue. Even when the electrode is properly positioned, changes in electric field distribution between bone and tumor tissue can lead to inhomogeneities that will lead to inadequate treatment.[38] This is in accordance with other authors who treated oral malignant melanoma with surgery plus radiotherapy. They had better results treating small tumors without bone involvement, but they also found that rostral location was associated with better responses.[39-41] In this work, location of the tumor did not achieve statistical significance to be associated either with local response, time to progression or survival.

If we compare frequency of use between ECT and other treatment modalities, we find that surgery is the most common one performed. The median survival time for dogs with malignant melanoma treated with surgery alone varies from 5 to 11 months.[42,43] Conservative surgery is only recommended followed by radiotherapy in non-resectable tumors for local control of the disease. Median survival times are similar to the times obtained in this work, and that was expected, since both are local treatments. When surgery is possible and the owner accepts it, we recommend to follow that path, as surgery is a well-established technique in our setting. In these cases, ECT could be used in combination with surgery to extend surgical margins, or even to treat the scar and reduce the recurrence rate (further research is needed to confirm this hypothesis). Recurrence rate after incomplete surgical resection is high (62–65% *vs*. 15–22% recurrence rate with incomplete and complete resection, respectively), and in those cases we choose to perform ECT as a first-line treatment.[44]

Radiation therapy alone is effective to obtain good local control of the disease, especially with patients that are not suitable for complete surgical excision (the addition of chemotherapy provides no additional benefit).[39] The response rates are similar regardless of the protocol that was used. Proux *et al.* achieved 51% CR, 31% PR, 16% SD and 1% PD. If we compare these results with the ones obtained in this work, we find similar OR rates, but with more CR when treating with radiotherapy. However, they report a 51% incidence of new metastasis, while in this work, the incidence of new metastasis was 28.4%. Results of median overall survival time with radiotherapy were 7 months (6–9 months), also similar to the ones obtained in this work, 7.5 months (2–30 months).[39] An adequately designed clinical study should be designed performed to compare both treatment modalities.

It is well known that melanoma is an extremely chemotherapy-resistant tumor, and recent studies have been supporting the idea that chemotherapy brings little to no benefits based on the analysis of results obtained after using the aforementioned therapeutic modality. Boria *et al.*[45] reported a tumor remission of 18% in melanoma patients and a median survival time of 4 months.

In relation to lymph node involvement, fine-needle aspiration (FNA) technique is mandatory for patient staging.[34] Several (FNA) samples obtained from the patients, ended up being positive even after unremarkable lymphadenopathy noticed to palpation at the moment of the exam.

Among the limitations we had to face throughout the making of this study, lack of chances to perform immunohistochemistry in every patient, was probably, one of the most important. Consequently, we had to draw upon the aforementioned technique only when diagnosis of melanoma was not definitive by using simple hematoxylin-eosin stain. Another limitation was imaging. As default, bone involvement was assessed by plain radiographs. CT scan was only performed in selected patients, either due to financial constraints or at times when we felt X-rays were not giving enough information in order to get to a definitive diagnosis.

New immunological approaches are now available in order to improve the results of local therapy. Promising results have been published by Mozillo *et al.* combining ECT with ipilimumab. The ECT session was performed for local control of the disease after treatment with ipilimumab. It seems that ECT triggered a systemic response, which was obtained in 60% of the patients.[46]

In the last decade, DNA-based immunotherapy reached important goals in the treatment of infectious diseases and cancer.[47] In a previous study, we explored the use of ECT in combination with canine IL-2+IL-12 plasmid vector delivered by electroporation, obtaining local control of the disease

in patients with different histological neoplasms. We observed fever, swelling, and lethargy due to the transfection, which had to be treated with corticosteroids. A single systemic response in lung metastasis was obtained[48] but further research is needed to study this combination.

Milevoj *et al*. obtained a 67% CR rate in 9 canine patients with an oral malignant melanoma treated with surgery plus electrochemotherapy plus gene electrotransfer with IL-12. They report a declining in regulatory T cells number that is associated with the treatment.[49] Cemazar *et al*. combined ECT with human IL-12 plasmid vector delivered by electroporation and obtained a 72% CR rate in spontaneous canine mast cell tumors without side effects. They also observed that the therapy prevented local recurrences and distant metastasis.[50]

ECT is a very appealing treatment modality for combination with immunotherapies, especially with those based on gene electrotransfection. This latter technology allows performing the immunotherapy at a fraction of the cost when compared with traditional immunotherapies. In this sense, low income countries may be the most benefited from the combination of ECT and gene electrotransfer.

In conclusion, ECT is an adequate local treatment modality for canine patients with oral malignant melanoma with lesions of up to 4 cm in diameter, i.e. stages I and II, and without bone invasion.

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Author contributions statement

M.N.T. and F.H.M. worked on study design, patient selection and treatment, data analysis and manuscript writing. S.D.M. worked on patient treatment, data analysis and manuscript writing. G.R.M. worked on manuscript writing, group direction and fund administration. E.S. worked on study design, manuscript writing, group coordination and fund administration. All authors reviewed the manuscript.

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research article

Long term response of electrochemotherapy with reduced dose of bleomycin in elderly patients with head and neck non-melanoma skin cancer

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Background. Electrochemotherapy (ECT) is a local cancer treatment based on electroporation where the electric field is used to enhance cell membrane permeability and thereby facilitating the transition of chemotherapeutic agents into the cell. For the treatment of non-melanoma skin cancer, a standard dosage of 15,000 IU/m^2 bleomycin (BLM) is used. The aim of the present study was to evaluate the long-term ECT response in the group of elderly patients with non-melanoma skin cancer treated with a reduced dose of BLM in comparison to the outcome in the patients treated with the standard dose of BLM.

Patients and methods. Twenty-eight patients older than 65 years, with a total of 52 non-melanoma skin lesions were included in the study. Twelve patients (24 lesions) in the experimental group received a reduced dose of BLM (10,000 IU/m^2), 16 patients (28 lesions) were treated with a standard dose of BLM (15,000 IU/m^2).

Results. No statistically significant difference in tumor control was observed between both groups. In the experimental group, tumors recurred in 39.0% of treated lesions in a median follow-up time of 28 months. In the control group, the recurrence rate of treated lesions was 15.4% in a median follow-up time of 40 months.

Conclusions. ECT with a reduced dose of BLM is a feasible treatment option for elderly patients with equal efficacy to standard dose treatment and should be considered as a treatment modality in advanced aged patients with comorbidities, where overall life expectancy is poor.

Key words: electrochemotherapy; bleomycin; non-melanoma skin cancer

Introduction

Electrochemotherapy (ECT) is a local cancer treatment modality with proven antitumor efficacy in various histological types of malignant tumors.[1] During an ECT procedure, electroporation is used to transiently increase cell permeability to a hydrophilic chemotherapeutic agent such as bleomycin (BLM) and cisplatin.[2] Currently, intravenous administration of BLM is the most common way of drug administration utilized in ECT treatment.[3,4]

ECT acts through at least three different mechanisms. The first mechanism is a direct cytotoxic effect driven by the transition of a chemotherapeutic agent into the tumor cells, which is enhanced by electroporation. ECT also has an impact on tumor blood vessels through sympathetic nerve stimulation and subsequent vasoconstriction lasting sev-

eral hours. Consequently, drug washout from the tumor is delayed. Additionally, the cytotoxic effect on endothelial cells of tumor blood vessels results in the late destruction of tumor vasculature by vascular disrupting effect. The third mechanism involves immune response provoked by immunogenic cell death and enhanced tumor antigen expression.[5]

In the ECT treatment of non-melanoma skin cancer (NMSC) compelling results were achieved when using standard dose (15,000 IU/m^2) of intravenously administrated BLM.[6] According to published BLM pharmacokinetics study, an equally good antitumor response might be obtained with a reduced dose of BLM in the elderly population due to the slow elimination rate of BLM.[7] Hence the updated SOP for ECT proposes de-escalation of standard dose due to high age and/or compromised creatinine clearance.[3] Till today only two clinical studies have been focused on ECT effects with a reduced dose of BLM. Both compared treatment response two months after ECT and showed similar efficacy to the standard dose of BLM.[8,9] Even more, in one study authors observed faster healing time and favorable cosmetic outcomes when using the reduced dose.[9]

The aim of the present study was to evaluate the long-term ECT response in the group of elderly patients with NMSC treated with a reduced dose of BLM in comparison to the outcome in the patients treated with the standard dose of BLM.

Patients and methods

Study summary

The study was conducted between June 2014 and June 2019 at the Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana. The study protocol was approved by the Republic of Slovenia National Medical Ethics Committee (182/02/14 and 0120-132/2015-2). Patients were selected according to the inclusion and exclusion criteria listed in Standard Operating Procedures of ECT (SOP) and as previously reported.[3,7,9] All patients had treatment naïve and biopsy-verified primary NMSC in the head and neck region. Each patient was presented to the multidisciplinary head and neck tumor board that confirmed indication. Prior to ECT procedure a written informed consent was obtained from all patients.

The study was conducted as a nonrandomized prospective study. Patients were grouped according to BLM dose they received. The patients who were treated with a standard dose of BLM formed the control group and data from that group was used to determine BLM pharmacokinetics in elderly patients.[7] All subsequently treated patients older than 65 years received the reduced dose and formed the experimental group.

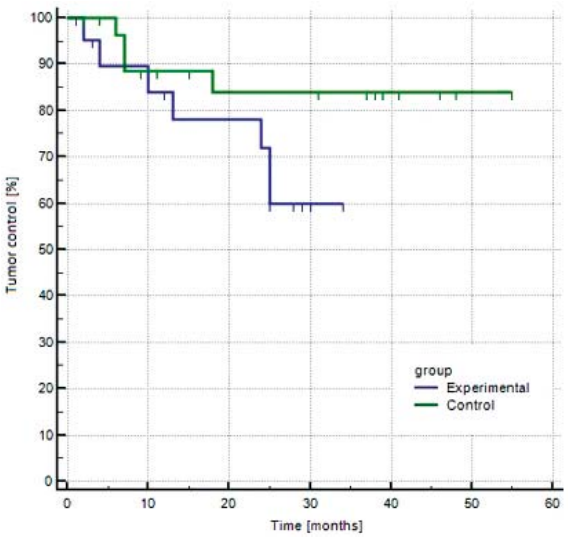

FIGURE 1. Kaplan-Meier curve of tumor control in experimental and control group.

Procedure

ECT was performed under sedation or general or local anesthesia. BLM (Bleomycin medac; Medac, Wedel, Germany) was administered as intravenous bolus injection in 2 minutes at a dose of 15,000 IU/m^2 body surface area in the control group and at a dose of 10,000 IU/m^2 body surface area in the experimental group (1,000 IU is equal to 1 mg of bleomycin activity). In both groups, the electric pulses were applied 8 minutes after the injection of BLM by electrodes with fixed geometry (hexagonal, needle row, or plate electrodes). Electric pulses were generated by Cliniporator Pulse Generator (IGEA, s.r.l., Carpi, Italy), as described previously.[9]

Follow up

Response to the treatment was evaluated according to Response Evaluation Criteria in Solid Tumors criteria, version 1.1.[10] The two-month outcome has already been reported in 2018 in our previous publication.[9] In this study we evaluated treatment outcome at 2, 4, 6, 12, 18, 24 months after ECT, and yearly thereafter. Minimal follow-

TABLE 1. Patients' characteristics, treatment procedure and response to treatment of patients included in experimental group

Patient	Gender	Age (years)	Creatinine (μmol/L)	Total bleomycin dose (IU)	Electrodes used	Last follow up (months after ECT)	Histology	Maximum tumor size (mm)	Recurrence of tumor (months after ECT)
1	M	86	84	20,000	Needle row	30	BCC	20	
							BCC	8	
2	M	67	108	24,000	Needle row	29	BCC	10	
							BCC	8	
3	M	80	106	21,000	Needle row	20	BCC	30	10
4#	M	82	66	20,000	Needle row	2 *	SCC	10	
							SCC	6	
							SCC	10	
5	M	92	88	20,000	Needle row	25	BCC	25	25
							BCC	15	
							BCC	15	25
6	M	79	67	20,000	Plate	34	BCC	80	
							BCC	20	24
							BCC	20	
							BCC	10	
7	F	78	83	20,000	Needle row	30	BCC	15	
8#	M	76	144	21,000	Needle row	6 *	SCC	20	
							SCC	27	
							SCC	35	
9	F	86	74	17,500	Needle row	19 *	BCC	25	
10	M	80	81	21,000	Needle row	28	BCC	10	13
11	M	85	42	20,000	Needle row	24 *	BCC	50	3
12	M	83	94	19,000	Needle row	28	BCC	7	
							SCC	7	4

BCC = basal cell carcinoma; ECT = electrochemotherapy; F = female; M = male; SCC = squamous cell carcinoma; * = patient deceased; # = excluded from analysis

up time of 6 months was required. Based on their presentation at three-year follow-up some patients were deemed disease free and were excluded from further follow-up at our institution. Those patients were referred to dermatologist for future yearly follow-up with instruction to report back in case of suspected recurrence of malignancy in treated area.

Statistical analysis

Statistical comparison between the control and experimental group was performed using the Mann-Whitney test (age of the patients and maximal tumor diameter) and the Chi-square test (sex, histology type, number of recurrent lesions and response to treatment). The results were analyzed using the PC SPSS, release 18.0 (SPSS, Chicago, IL) statistical package. All the tests were 2-sided, and the results were considered significant at a probability level of 5%. Kaplan-Maier plots were drawn, and the log-rang test was performed for the evaluation of long-term tumor control.

Results

Twenty-eight patients, 65 years or older, with histologically proven NMSC in the region of the head and neck, were included in the study. All the patients were treated by ECT, using intravenous bolus injection of BLM in standard (15,000 IU/m^2 body surface area) or reduced (10,000 IU/m^2 body surface area) dose. There were no statistically significant differences between both groups concerning the patients (age and sex) and tumors (diam-

TABLE 2. Patients' characteristics, treatment procedure and response to treatment of patients included in control group

Patient	Gender	Age (years)	Creatinine (μmol/L)	Total bleomycin dose (IU)	Electrodes used	Last follow-up (months after ECT)	Histology	Maximum tumor size (mm)	Recurrence of tumor (months after ECT)
1	M	65	68	30,000	Needle row	39	BCC	22	
2	F	74	86	26,000	Needle row	55	BCC	15	
3	M	83	129	28,000	Plate	19 *	BCC	12	
4	M	81	78	27,000	Needle row	37	BCC	32	7
							BCC	10	7
							BCC	10	
							BCC	5	
							BCC	6	
5	F	82	73	24,000	Plate	41	BCC	4	
							BCC	9	6
							BCC	5	
							BCC	11	
6#	M	88	142	30,000	Plate	2	BCC	39	
7	F	69	84	23,000	Finger	46	BCC	15	
8	F	82	DM	24,000	Hexagonal	41	BCC	50	
9	F	89	69	23,000	Plate	48	BCC	7	
							BCC	20	18
							BCC	6	
							BCC	5	
							BCC	6	
10	M	65	DM	27,000	Plate	31	BCC	24	
11#	F	70	DM	27,000	Plate	4	BCC	7	
12	M	78	DM	23,000	Plate	46	BCC	15	
13	F	74	52	24,000	Needle row	38	BCC	15	
14	F	89	54	25,000	Plate	48	BCC	21	
							SCC	22	
15	M	67	DM	30,000	Plate	11	SCC	25	
16	M	85	100	24,000	Needle row	15 *	SCC	45	

BCC = basal cell carcinoma; ECT = electrochemotherapy; F = female, M = male; SCC = squamous cell carcinoma; * = patient deceased; # = excluded from analysis

eter, histology type, and recurrent lesions) characteristics ($P > 0.05$). Details of patients and tumors treated with reduced and standard BLM doses are presented in Tables 1 and 2.

The experimental group consisted of 12 patients (10 men and 2 women; median age 81 years; range 67-92 years) with 24 lesions (17 BCCs, 7 SCCs), 18 (75%) tumors were treatment naïve. The largest tumor diameter ranged from 6 mm to 80 mm (median 21 mm). In control group 16 patients (8 men and 8 women; median age 78 years; range 65-89 years) had 28 lesions (25 BCCs; 3 SCCs), 24 (86%) tumors were treatment naïve. The largest tumor diameter ranged from 4 mm to 50 mm (median 17 mm).

The complete response rates two months after ECT were observed in control and experimental in 96% and 100%, respectively.[9] All patients were further followed up at regular visits. In the experimental group, two patients died before six months follow up. One died due to the systemic dissemination of previously treated melanoma. The cause of death in another patient was an underlying disease, not related to skin cancer or ECT treatment. These two patients were excluded from the study. Out of

12 patients in the experimental group, 10 (83.0%) were evaluable at 6 months or longer. Median long-term follow up was 28 months in the experimental group (average 24.9 ± 7.4 months). Tumor recurred in 6/10 (60.0%) patients and in 7/18 (39.0%) tumors in median recurrence time 18.5 months (average 26.9 ± 9.0 months).

In the control group, 2/16 patients were excluded from the study. One died 4 months after the treatment due to other causes than cancer. The other patient did not come to a follow-up visit at 6 months and was lost to further follow-up. Fourteen patients (88%) were evaluable at 6 months or longer. Median long-term follow up was 40 months (average 36.0 ± 14.5 months). The recurrence was observed in 3/14 patients (21.4%) and in 4/26 nodules (15.4%) in a median time of 7.0 months (average 9.4 ± 5.6 months).

Overall, 6/28 (21%) patients included in the study died during follow-up due to other comorbidities. In neither of them the cause of death was related to NMSC or ECT treatment. After statistical analysis no significant difference in recurrence rate was observed between groups (Logrank test P=0,104).

No statistically significantly elevated levels of creatinine was detected neither in control (85.0 ± 28.6 μmol/L) nor in experimental group (86.4 ± 25.6 μmol/L, $p > 0.1$).

Discussion

Until now, only a few studies evaluated the long-term efficacy of ECT treatment in NMSC in the head and neck region. Kristiansson *et al.* reported on 71% control rate in a case series of 7 patients with NMSC treated with ECT after a median follow up of 119 months.[11] In the most comprehensive study regarding long–term follow up after ECT treatment of BCCs, 5-year recurrence rate of local and locally advanced BCC was 20% and 38%, respectively.[12] It should be emphasized that ECT in these studies was performed according to the first version of SOP, thus standard dose (15,000 IU/m^2) of intravenous administrated BLM was used.[13] To the best of our knowledge, our clinical study is the only one where long-term effectiveness of ECT in NMSC in the head and neck region after the intravenous administration of a reduced dose of BLM was evaluated and was found to be equal to the effectiveness of ECT with standard dose.

The efficacy of a reduced BLM dose in elderly patients was confirmed in clinical studies after the identification of the main parameters of BLM pharmacokinetics.[7–9] Ageing is related to the impairment of body functions, *e.g.*, impaired renal function, and to a reduction in lean body mass. Both changes lead to reduced clearance of water-soluble drugs (such as BLM) and reduced volume of their distribution. Hence, the plasma concentration of BLM is higher than in younger adults. Thus, the use of the standard dose of BLM in elderly patients could lead to prolonged healing time and a more prominent inflammatory response, as a result of exceeded optimal concentrations of BLM. The rationale for dose de-escalation in elderly patients lays in diminishing possible systemic side effects (*e.g.*, lung fibrosis), improving local healing and keeping total BLM dose as low as possible, especially in circumstances when multiple sessions of ECT are expected.[8,9] According to our previous study complete response rate two months after ECT was almost 100% when using both standard (15,000 IU/m^2) or reduced (10,000 IU/m^2) BLM dose in elderly patients with NMSC.[9] One of the major drawbacks of that study was too short observation time for adequate assessment of clinical response. This is especially important in BCCs because they are slow-growing tumors and it often takes months to years for a tumor to relapse after initial treatment.[14]

After statistical analysis no significant differences were observed between control and experimental groups regarding clinical or tumor parameters. In the control group, 4 of 26 tumors recurred in the median follow up time of 7.0 months. In the experimental group of patients, 7 out of 18 tumors recurred in the median follow up time of 18.5 months. After statistical analysis, no significant differences in long term tumor control between groups were observed (p=0.104). This confirms our hypothesis that long-term tumor control could be achieved with a lowered BLM dose. Our finding is in concordance with results of previous clinical trials on the elderly population, that suggest equal efficacy of reduced dose two months after the treatment even though the concentration of BLM around the tumor immediately after electroporation is lower compared to the standard dose.[8,9] These findings can be explained with the pharmacokinetics of BLM in elderly patients, where higher concentrations are achieved due to reduced BLM clearance by the kidney and by the reduced volume of distribution.[7]

Although differences in the long-term tumor control between both groups were not statistically significant, we observe a trend towards a re-

currence of BCCs in the experimental group. We might speculate that further studies with a larger cohort of patients could show a lower tumor control of BCCs treated with reduced dose, as a result of reduced local inflammatory response. One of the antitumor mechanisms of ECT involves immune response, provoked by immunogenic cell death and enhanced tumor antigen expression.[5] This might be especially important in the treatment of BCCs since these tumors have the greatest mutational burden among all human cancers.[15] Consequently, numerous tumor antigens provoke immune system, which is the most probable reason for the less aggressive nature of BCCs.[15] Taking these facts into consideration, less prominent local immune response after reduced dose ECT might lead to a lower tumor control of BCCs.

The overall long-term tumor control rate at the end of the study was 79%. Deaths were correlated with underlying disease and not with NMSC progression or side effects after ECT. It is important to emphasize that although long term tumor control was not achieved in all patients, reducing possible side effects and consequent quality of life in patients with short life expectancy is of paramount importance. Thus, a reduced BLM dose in ECT should be considered as a treatment modality in elderly patients with comorbidities, where overall life expectancy is poor.

One of the future perspectives of ECT should be an orientation towards individual patients and tumors. For example, some studies have already shown that differences between tumor vascularization have an impact on BLM pharmacokinetics and therefore on antitumor response.[16] The exact dose to achieve maximal antitumor response with minimal side effects should be determined according to tumor histology and patients' overall health. Hence, in Updated Standard Operating Procedures for ECT a compromised creatinine level was proposed as a guide for using a reduced dose of BLM.[3] In our study average creatinine levels during ECT procedure were normal in both groups. It should be noted that the production of creatinine is decreased due to age-related reduction in skeletal muscle mass; thus, plasma creatinine level is an inaccurate indicator of glomerular filtration rate in the elderly population, where the reduction of lean body mass is prominent.[7] We presume that creatinine is not a reliable marker in applying a reduced dose of BLM in the group of elderly patients and it must be interpreted in concordance with other methods such as bioelectrical impedance analysis, which is used for body composition measurements.

We are aware that a low number of patients in our study is one of the drawbacks of the study. Even though this study raises some important clinical questions, which need to be addressed. In the future, randomized studies with a larger cohort of patients, treated with reduced doses of BLM, are needed to evaluate the appropriate indications and clinical significance of de-escalated dose BLM in ECT treatment.

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research article

Evaluation of soluble mesothelin-related peptides and MSLN genetic variability in asbestos-related diseases

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Background. Asbestos exposure is associated with increased risk of several diseases, including malignant mesothelioma (MM). Cell surface glycoprotein mesothelin is overexpressed in MM and serum soluble mesothelin-related peptides (SMRP) were already proposed as a diagnostic or prognostic biomarker in MM. However, interindividual variability in serum SMRP levels limits the clinical usefulness. Our primary objective was to investigate the influence of *MSLN* rs1057147 on serum SMRP levels in asbestos-exposed subjects and patients with asbestos-related diseases as well as on survival in MM.

Subjects and methods. Among 782 asbestos-exposed subjects and patients with asbestos-related diseases, 154 had MM. Serum SMRP levels were determined using sandwich enzyme-linked immunosorbent assay. All subjects were genotyped for *MSLN* rs1057147 polymorphism using competitive allele-specific polymerase chain reaction. Nonparametric tests, logistic and Cox regression were used in statistical analysis to compare different subject groups.

Results. MM patients had significantly higher SMRP levels than all other subjects ($p < 0.001$). Compared to wild-type *MSLN* rs1057147 genotype, both heterozygotes and carriers of two polymorphic alleles had significantly higher SMRP levels among subjects without MM ($p < 0.001$), but not in MM patients ($p = 0.424$). If genotype information was included, specificity of SMRP increased from 88.5% to 92.7% for the optimal cutoff value. Overall survival was significantly shorter in MM patients carrying at least one polymorphic rs1057147 allele (HR = 1.72, 95% CI = 1.15-2.55, $p = 0.008$).

Conclusions. *MSLN* genetic variability affects serum SMRP levels and was associated with shorter survival of MM patients. Combination of genetic and serum factors could therefore serve as a better diagnostic or prognostic biomarker in MM patients.

Key words: malignant mesothelioma; asbestos-related disease; mesothelin, soluble mesothelin-related peptides; polymorphism

Introduction

Occupational and environmental exposure to asbestos is associated with the development of different asbestos-related diseases. Even though several countries have banned the use of asbestos after it was classified as a carcinogen in 1977, it is still being used in some countries and it is also still present in the environment.[1,2] Additionally, there is a long latency period between exposure and development of asbestos-related diseases, which can occur several decades after asbestos exposure even in subjects exposed to relatively low doses.[1] Therefore, the incidence of asbestos-related diseases continues to rise in most countries and they remain one of the major public health issues.[1]

Pleural plaques, diffuse pleural thickening, pleural effusions and asbestosis are classified as benign asbestos-related diseases.[1] Exposure to asbestos also increases the risk of various cancers, including malignant mesothelioma (MM). MM is an extremely aggressive cancer affecting serosal membranes, mostly pleura or peritoneum.[3,4] Due to non-specific symptoms, diagnosis is usually made in the advanced stages of the disease, leading to poor prognosis and short survival of MM patients.[4] Even though the use of chemotherapy increased the survival of MM patients, response rate is still limited.[4-6] Early diagnosis could therefore contribute to a more effective treatment of MM.[7,8] Currently, immunohistochemical analysis investigating a panel of markers on tissue samples is required to confirm the MM diagnosis.[9] New noninvasive biomarkers that would enable earlier diagnosis of MM are thus extensively studied, particularly in pleural MM.

The most frequently investigated biomarker in MM is mesothelin, as many studies have shown it is frequently increased in both tumor tissue and serum of MM patients, especially in epithelioid histological type.[8,10-17] Mesothelin is a cell-surface glycoprotein expressed in mesothelial cells and overexpressed in several cancer types. It is involved in important cellular processes, including cell adhesion, proliferation, invasion, and epithelial-to-mesenchymal transition.[18,19] Mesothelin is a glycophosphatidylinositol–linked membrane protein, but it also has three isoforms that are present in the circulation.[12,15,20] Enzyme-linked immunosorbent assay (ELISA) can detect different isoforms, usually referred to as soluble mesothelin related peptides (SMRP).[12,20]

Meta-analyses focusing on SMRP as a diagnostic marker of MM showed that high SMRP has high specificity, but limited sensitivity, suggesting that positive results should lead to further diagnostic steps, but negative results do not exclude MM and therefore additional biomarkers are needed.[12,14]

Several studies also investigated SMRP as a prognostic biomarker in MM.[18,21-23] SMRP levels were markedly increased at disease progression.[21] In a meta-analysis, increased SMRP was associated with shorter overall survival and worse prognosis.[18] Numerous studies also noted that serial longitudinal SMRP measurements may be more informative than SMRP levels at diagnosis.[22,23] Mesomark™ ELISA kit was already approved by the FDA for the measurement of SMRP and monitoring of MM.[20,24]

A few previous studies have shown that genetic variability also influences SMRP levels. Single nucleotide polymorphisms (SNPs) in the 5′ and 3′ untranslated region (UTR) of the mesothelin gene (*MSLN*) can contribute to the observed SMRP variability. Polymorphic alleles of rs3764247 (c.-894A>C), rs3764246 (c.-790A>G), and rs2235503 (c.-340C>A) in the 5′ UTR were associated with increased SMRP levels in healthy asbestos-exposed subjects or subjects with benign asbestos-related diseases, but not in MM patients.[25,26] Similarly, rs1057147 (c.*69G>A) in the 3′ UTR that affects miR-611 binding was also associated with increased SMRP levels in healthy asbestos-exposed subjects or subjects with benign asbestos-related diseases.[27,28] *MSLN* genetic variability was thus shown to improve specificity of SMRP as a diagnostic marker and could help redefine and improve the predictive ability of currently used cutoff values.[25,26,28] However, no data is available regarding the association of *MSLN* genetic variability with the prognosis of MM.

The aim of our study was to determine serum SMRP levels in patients with asbestos-related diseases and in asbestos-exposed subjects without asbestos-related disease and to assess the association of *MSLN* rs1057147 with serum SMRP levels. We also investigated the association of serum SMRP levels and *MSLN* rs1057147 with the survival of MM patients.

Subjects and methods

Subjects

The study included 782 subjects with different asbestos-related diseases (pleural plaques, asbestosis and MM) and asbestos-exposed subjects with no asbestos-related disease.

Patients with MM were treated at the Institute of Oncology Ljubljana in the period between 1 January 2004 and 31 December 2012. The diagnosis of pleural MM was performed by thoracoscopy and the diagnosis of peritoneal MM by laparoscopy. In both cases, the diagnosis of MM was confirmed histologically by an experienced pathologist. The MM stage was determined according to the TNM staging system for pleural MM, while performance status was evaluated according to Eastern Cooperative Oncology Group (ECOG) scores.

All patients with pleural plaques, asbestosis and subjects with no asbestos-related disease were occupationally exposed to asbestos and presented at the State Board for the Recognition of Occupational Asbestos Diseases in the period from 1 January 1998 to 31 December 2007. The diagnosis of pleural

plaques, asbestosis or "no asbestos-related disease" was based on the Helsinki Criteria for Diagnosis and Attribution of Asbestos Diseases[29] and on the American Thoracic Society recommendations[30] and was confirmed by two groups of experts each consisting of a skilled occupational physician, a radiologist, and a pulmonologist. Follow-up was performed in all patients in 2018 to confirm they did not develop any other asbestos-related disease.

Demographic and clinical data were obtained from the medical records. Data on smoking were obtained using a standardized questionnaire[31,32] and during the interview. Patients were classified as ever/never smokers. All subjects provided written informed consent. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia and was carried out according to the Helsinki Declaration.

Serum SMRP measurement

Serum samples were collected at diagnosis for patients with MM and at inclusion in the study for all other subjects. Serum samples were prepared within 6 hours after blood sampling, aliquoted and stored at -20°C. For determining serum SMRP levels, sandwich ELISA assay (Mesomark™) using two monoclonal antibodies (4H3 and OV569) was used according to the manufacturer's protocol (Fujirebio Europe BV, Breda, The Netherlands).[24]

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using Qiagen FlexiGene Kit (Qiagen, Hilden, Germany). *MSLN* rs1057147 (c.*69G>A) genotype was determined for all subjects using a fluorescent-based competitive allele-specific polymerase chain reaction (KASPar) assay (LGC Genomics, UK) or real-time PCR-based Taqman assay (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. In 15% of the subjects, samples were genotyped in duplicates and the duplicate call rate was 100%.

Statistical analysis

Continuous variables were described using median with interquartile range (25%–75%) or 95% confidence intervals (CIs), while categorical variables were described using frequencies. Fisher's exact test was used to compare categorical variables among different groups. Nonparametric Mann-Whitney or Kruskal-Wallis tests were used to compare distribution of continuous variables. Pairwise comparisons with *post hoc* Bonferroni corrections were used with Kruskal-Wallis test to obtain adjusted p values (p_{adj}). Deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated using the standard chi-square test. Both dominant and additive genetic models were used in the analysis. Logistic regression models were used to calculate non-adjusted and adjusted odds ratios (ORs) and 95% CIs for comparison of genotype frequencies between groups. Clinical characteristics, significant in univariable analysis, were used for further adjustment. A receiver operating characteristic (ROC) curve was used to analyze the predictive value and the area under the curve (AUC).

In the survival analysis, progression-free survival (PFS) and overall survival (OS) were assessed. PFS was defined as the time to the day of documented disease progression, or death, whichever occured first and OS was defined as the time to death from any cause. Patients without progression or death at the time of the analysis were censored at the date of the last follow-up. The data on vital status were obtained from medical records or from the Slovenian Cancer Registry. Kaplan-Meier analysis was used to calculate median survival or follow-up time, while Cox regression was used to calculate the hazard ratios (HR) with the 95% CIs.

The statistical analyses were carried out by using IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY, USA). All statistical tests were two-sided and the level of significance was set at 0.05.

Results

Among 782 subjects included in our study, 154 (19.7%) patients had MM. Among 628 non-MM subjects that were occupationally exposed to asbestos, 69 did not develop any asbestos-related disease, 410 subjects had pleural plaques, and 149 patients had asbestosis. Characteristics of each subject group are presented in Table 1. Gender and smoking distributions were similar across all groups (p = 0.479 and 0.740, respectively). On the other hand, age at diagnosis or inclusion in the study differed significantly between subject groups (Kruskal-Wallis test statistic = 88.602; $p < 0.001$). MM patients were significantly older compared to all other subjects (Mann-Whitney U = 69560.5; $p < 0.001$).

Among 138 patients with pleural MM, 8 (5.8%) had stage 1, 41 (29.7%) stage 2, 43 (31.2%) stage 3, and 41 (29.7%) stage 4 disease, while the stage could not be determined in 5 (3.6%) patients.

TABLE 1. Clinical characteristics of study groups

Characteristic		All subjects (N =782)	No disease (N = 69)	Pleural plaques (N = 410)	Asbestosis (N = 149)	MM (N = 154)	MM with SMRP at diagnosis (N = 86)
Gender	Male, N (%)	581 (74.3)	52 (75.4)	295 (72.0)	115 (77.2)	119 (77.3)	69 (80.2)
	Female, N (%)	201 (25.7)	17 (24.6)	115 (28.0)	34 (22.8)	35 (22.7)	17 (19.8)
Age	Years, median (25%–75%)	56.9 (50.2–64.9)	52.9 (48.2–59.2)	54.6 (48.8–62.2)	59.1 (51.2–65.2)	65 (57–70)	66 (59–72)
Smoking	No, N (%)	375 (49.0) [17]	31 (45.6) [1]	203 (50.9) [11]	71 (48.0) [1]	70 (46.7) [4]	43 (51.2) [2]
	Yes, N (%)	390 (51.0)	37 (54.4)	196 (49.1)	77 (52.0)	80 (53.3)	41 (48.8)
SMRP	nmol/l, median (25%-75%)		0.30 (0.00–0.85) [1]	0.14 (0.00–0.58) [6]	0.03 (0.00–0.39) [5]		2.43 (0.44–8.62)

Number of missing data is presented in [] brackets.
MM = malignant mesothelioma; SMRP = soluble mesothelin-related peptides

Additionally, 16 (10.4%) patients had peritoneal MM. Most patients had epithelioid MM (114, 74.0%), 15 (9.7%) patients had biphasic and 14 (9.1%) patients had sarcomatoid MM. In the remaining 11 (7.1%) patients histological type could not be determined. ECOG performance status at diagnosis was 0 in 4 (2.6%) patients, 1 in 82 (53.2%), 2 in 60 (39.2%) and 3 in 3 (1.9%) MM patients, while no data on performance status were available for 5 (3.2%) patients.

Serum samples of 86 (55.8%) MM patients were available at diagnosis, therefore the SMRP level was measured only in these patients. MM patients with available data on serum SMRP at diagnosis did not differ significantly from the rest of MM patients regarding gender, smoking, stage, location (pleura or peritoneum) and histological type (all $p > 0.05$). However, these patients were older ($p = 0.005$) and had worse ECOG performance status ($p = 0.019$).

Serum SMRP levels

SMRP serum levels differed significantly between subject groups presented in Table 1 (Kruskal-Wallis test statistic = 96.470; $p < 0.001$; Figure 1A). MM patients had significantly higher SMRP levels at diagnosis after Bonferroni correction for multiple comparisons than subjects with no disease (Kruskal-Wallis test statistic = – 158.068; $p_{adj} < 0.001$), subjects with pleural plaques (Kruskal-Wallis test statistic = – 209.046; $p_{adj} < 0.001$) and patients with asbestosis (Kruskal-Wallis test statistic = – 246.125; $p_{adj} < 0.001$). Additionally, the difference between patients with asbestosis and subjects with no disease remained significant after Bonferroni correction (Kruskal-Wallis test statistic 88.058; $p_{adj} = 0.015$), while no significant differences after adjustment were observed between subjects with pleural plaques and subjects with no disease (Kruskal-Wallis test statistic = 50.979; $p_{adj} = 0.294$) or patients with asbestosis (Kruskal-Wallis test statistic = 37.079; $p_{adj} = 0.318$).

When comparing MM patients with all other subjects, MM patients had significantly higher SMRP levels (2.43 (0.44–8.62) nmol/l compared to 0.13 (0.00–0.55) nmol/l; Mann-Whitney U = 42493; $p < 0.001$). In the ROC curve analysis, the AUC for serum SMRP predicting MM was 0.802 (95%CI = 0.740–0.864; $p < 0.001$, Figure 1B). At the cutoff

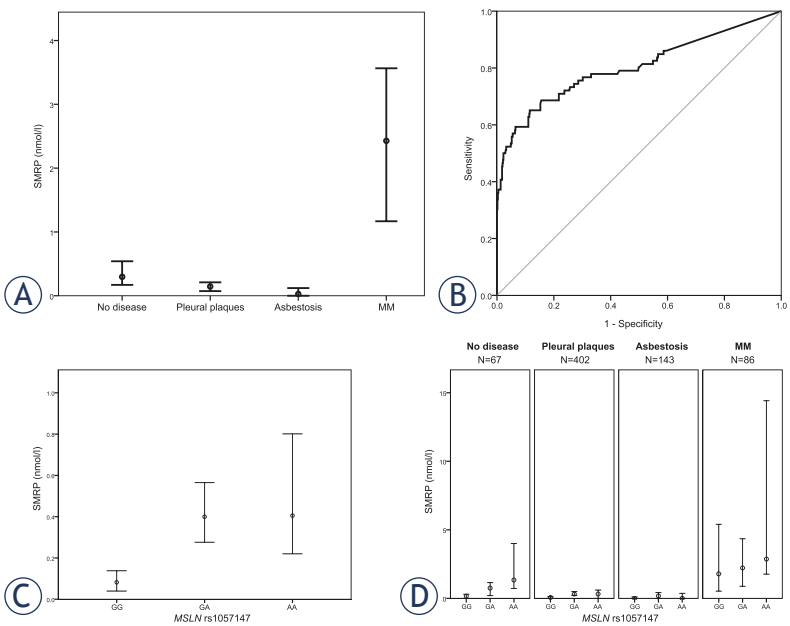

FIGURE 1. Median soluble mesothelin-related peptides (SMRP) levels in different groups **(A)**, receiver operating characteristic (ROC) curve for SMRP predicting malignant mesothelioma (MM) **(B)** and association of *MSLN* rs1057147 genotype with serum SMRP levels at inclusion in the study in the whole cohort of 698 subjects **(C)** and for each subject group **(D)**. Data are presented as median with 95% confidence intervals.

TABLE 2. ROC curve analysis according to *MSLN* rs1057147 genotype

Comparison	AUC (95% CI)	p	SMRP cutoff (nmol/l)	Sensitivity	Specificity
MM vs. all other subjects	0.802 (0.740–0.864)	< 0.001	1.5	0.593	0.930
			1	0.651	0.864
			1.11[¤]	0.651	0.885
MM vs. all other subjects with *MSLN* rs1057147 GG genotype	0.827 (0.767–0.888)	< 0.001	1.5	0.593	0.967
			1	0.651	0.925
			0.87[¤]	0.686	0.911
MM vs. all other subjects with *MSLN* rs1057147 GA genotype	0.765 (0.697–0.834)	< 0.001	1.5	0.593	0.882
			1	0.651	0.783
			1.57[¤]	0.593	0.896
MM vs. all other subjects with *MSLN* rs1057147 AA genotype	0.780 (0.702–0.858)	< 0.001	1.5	0.593	0.878
			1	0.651	0.780
			1.68[¤]	0.570	0.927

[¤] Cutoff with the highest sum of sensitivity and specificity.
AUC = area under the curve; MM = malignant mesothelioma; ROC = receiver operating characteristic; SMRP = soluble mesothelin-related peptides

TABLE 3. Odds for MM in subjects with different *MSLN* rs1057147 genotypes

Genotype	No disease (N = 69) N (%)	Pleural plaques (N = 410) N (%)	Asbestosis (N = 149) N (%)	MM (N = 154) N (%)	MM vs all other subjects			
					OR (95% CI)	p	OR_{adj} (95% CI)	p_{adj}
GG	41 (60.3) [1]	241 (59.1) [2]	84 (56.8) [1]	81 (52.6)	reference		reference	
GA	21 (30.9)	147 (36.0)	49 (33.1)	60 (39.0)	1.25 (0.86–1.82)	0.243	1.28 (0.87–1.90)	0.215
AA	6 (8.8)	20 (4.9)	15 (10.1)	13 (8.4)	1.43 (0.73–2.80)	0.292	1.30 (0.64–2.64)	0.472
GA+AA	27 (39.7)	167 (40.9)	64 (43.2)	73 (47.4)	1.28 (0.90–1.82)	0.174	1.29 (0.89–1.87)	0.187

Number of missing data is presented in [] brackets.
adj = adjusted for age; CI = confidence interval; MM = malignant mesothelioma; OR = odds ratio

value of 1.5 nmol/l suggested by the ELISA assay manufacturer, specificity for predicting MM was 0.930, while sensitivity was 0.593. However, we observed the highest sum of specificity and sensitivity at the cutoff value of 1.11 nmol/l, resulting in a specificity of 0.885 and sensitivity of 0.651 (Table 2).

Serum SMRP was not associated with gender (Mann-Whitney U = 48033.5; $p = 0.756$) or smoking (Mann-Whitney U = 61928.5; $p = 0.276$). In the whole study group, higher age was weakly correlated with slightly higher SMRP (Spearman's rho = 0.105; $p = 0.005$), but within each group, there was no association between age and SMRP (no disease: $p = 0.127$; pleural plaques: $p = 0.856$; asbestosis: $p = 0.846$; MM: $p = 0.866$).

In MM patients, 10 patients with peritoneal MM had significantly higher SMRP levels than patients with pleural MM (8.72 (3.65–12.25) nmol/l *vs*. 1.78 (0.267.74) nmol/l, Mann-Whitney U = 569; $p = 0.011$). Additionally, patients with epithelioid histological type had significantly higher SMRP levels than patients with other histological types (2.87 (0.51–10.47) nmol/l *vs*. 1.14 (0.10–3.60) nmol/l; Mann-Whitney U = 540.5; $p = 0.035$).

MSLN rs1057147 genotype frequencies

Genotype frequencies of *MSLN* rs1057147 are presented in Table 3. Minor allele frequency was 24.6% in the whole cohort. Genotype frequencies were in agreement with HWE in subjects without any disease as well as in the whole cohort (all $p > 0.05$). *MSLN* rs1057147 was not associated with the odds for developing MM and there were no differences between non-adjusted and age-adjusted ORs (Table 3). Similarly, *MSLN* rs1057147 was not associated with the odds for developing any asbestos-related disease, individually or combined, compared to subjects with no disease (all $p > 0.05$, unadjusted ORs in Supplementary Table S1).

MSLN rs1057147 and serum SMRP levels

SMRP levels differed significantly between *MSLN* rs1057147 genotype groups in all 698 subjects with

TABLE 4. Serum SMRP levels according to *MSLN* rs1057147 genotype

	MSLN rs1057147					*MSLN* rs1057147		
Group	**GG genotype**	**GA genotype**	**AA genotype**	**Kruskal-Wallis test statistic**	**p^a**	**GA+AA genotype**	**Mann-Whitney U**	**p^b**
	SMRP (nmol/l, median (25%–75%)	**SMRP (nmol/l, median (25%–75%)**	**SMRP (nmol/l, median (25%–75%)**			**SMRP (nmol/l, median (25%–75%)**		
All subjects N = 698	0.08 (0.00–0.45)	0.40 (0.00–1.11)	0.41 (0.00–1.61)	31.617	< 0.001	0.40 (0.00–1.15)	73900	< 0.001
No disease N = 67	0.16 (0.00–0.44)	0.75 (0.19–1.40)	1.34 (0.98–2.41)	18.657	< 0.001	0.84 (0.25–2.55)	836.5	< 0.001
Pleural plaques N = 402	0.07 (0.00–0.33)	0.32 (0.00–0.88)	0.32 (0.00–0.75)	18.839	< 0.001	0.32 (0.00–0.88)	24272.5	< 0.001
Asbestosis N = 143	0.00 (0.00–0.21)	0.18 (0.00–0.64)	0.00 (0.00–0.37)	5.817	0.055	0.14 (0.00–0.55)	2951	0.067
No disease or pleural plaques or asbestosis N = 612	0.07 (0.00–0.33)	0.33 (0.00–0.88)	0.33 (0.00–0.76)	31.125	< 0.001	0.33 (0.00–0.87)	56942.5	< 0.001
MM N = 86	1.79 (0.34–8.47)	2.22 (0.52–8.61)	2.87 (1.80–12.14)	1.716	0.424	2.62 (0.88–9.07)	986	0.595

[a] additive model; [b] dominant model
MM = malignant mesothelioma; SMRP = soluble mesothelin-related peptides

available SMRP levels and *MSLN* genotype (p < 0.001; Table 4; Figure 1C). Compared to carriers of two wild-type alleles, both heterozygotes and carriers of two polymorphic alleles had significantly higher SMRP levels after Bonferroni correction (Kruskal-Wallis test statistic = – 83.315, p_{adj} < 0.001 and Kruskal-Wallis test statistic = – 90.291; p_{adj}= 0.007, respectively). The difference was also significant in the dominant model (p < 0.001). When we analyzed the role of *MSLN* rs1057147 in each group separately (Table 4, Figure 1D), SMRP levels differed significantly between genotype groups in subjects without any disease, all subjects without MM or subjects with pleural plaques in both additive and dominant model (all p < 0.001). However, in patients with asbestosis or MM, *MSLN* rs1057147 was not significantly associated with SMRP levels at diagnosis in additive (p = 0.055 and p = 0.424, respectively) or dominant model (p = 0.067 and p = 0.595, respectively), even though carriers of polymorphic allele had higher SMRP levels.

As genetic variability only influenced the SMRP levels in non-MM subjects, we performed separate ROC curve analyses stratified by genotype, comparing all MM patients to non-MM subjects with individual genotypes. The predictive capacity of SMRP was genotype-dependent. The AUC was the highest when comparing the MM patients to all other subjects with *MSLN* rs1057147 GG genotype and SMRP had the highest specificity (Table 2). Similarly, the cutoff value with the highest sum of specificity and sensitivity differed according to genotype. The cutoff was 0.87 nmol/l in subjects with GG genotype, but 1.68 nmol/l in subjects with AA genotype (Table 2).

MSLN rs1057147 and survival of MM patients

Median PFS of MM patients was 7.8 (5.5–13.6) months, while median OS was 18.0 (9.8–29.6) months. The follow-up time of censored patients was 43.5 (21.8–94.3) months.

Compared to *MSLN* rs1057147 GG genotype, patients with GA and AA genotypes had non-significantly shorter PFS, which is supported by the slightly elevated HRs (HR = 1.39, 95% CI = 0.96–2.01; p = 0.084 and HR = 1.40, 95% CI = 0.74–2.66;

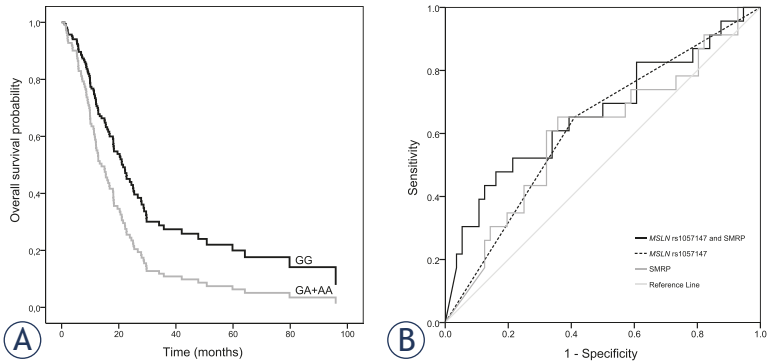

FIGURE 2. The influence of *MSLN* rs1057147 genotype on overall survival **(A)** and receiver operating characteristic (ROC) curve for *MSLN* rs1057147 and soluble mesothelin-related peptides (SMRP) predicting overall survival above median **(B)**.

p = 0.306 for GA and AA genotypes, respectively). Similar results were observed in the dominant model (HR = 1.39, 95% CI = 0.98–1.98; p = 0.068) (Supplementary Table S2).

Compared to *MSLN* rs1057147 GG genotype, patients with GA and AA genotypes had shorter OS (Supplementary Table S2). The difference was statistically significant for GA genotype (HR = 1.68, 95% CI = 1.11–2.54; p = 0.015), while only a trend was seen in AA genotype (HR = 1.91, 95% CI = 0.96–3.81; p = 0.066). In the dominant model, median OS was significantly shorter in carriers of at least one polymorphic allele compared to carriers of two wild-type alleles (15.1 months *vs*. 22.2 months, HR = 1.72, 95% CI = 1.15–2.55; p = 0.008; Figure 2A).

In MM patients whose SMRP levels were available at diagnosis, the serum SMRP level was not significantly associated with PFS or OS in our study group (HR = 0.99, 95% CI = 0.96–1.02; p = 0.574 and HR = 1.03, 95% CI = 0.99–1.06; p = 0.110, respectively). Still, patients with SMRP above 1.5 nmol/l had slightly though not significantly shorter OS (12.1 months *vs*. 20.6 months, HR = 1.45, 95% CI = 0.80–2.61; p = 0.220). In a model combining the serum SMRP level at diagnosis and *MSLN* rs1057147, *MSLN* rs1057147 was the most important predictor of OS (HR = 2.65, 95% CI = 1.41–4.99; p = 0.002).

Using the ROC curve analysis, we assessed how well *MSLN* rs1057147 can predict OS above the median of 18 months in MM patients whose SMRP levels were available at diagnosis (Figure 2B). For *MSLN* rs1057147 in the dominant model, the AUC was 0.621 (0.485–0.757) and did not reach statistical significance (p = 0.093). For the SMRP level at diagnosis, the AUC was 0.602 (0.460–0.743) and did not reach statistical significance (p = 0.157). On the other hand, additive combination of both *MSLN* rs1057147 and the SMRP level at diagnosis based on the Cox regression model that included both variables increased the AUC to 0.662 (0.521–0.803) and was a significant predictor of OS above 18 months (p= 0.024).

Discussion

In the present study, we evaluated serum SMRP as a biomarker in the context of *MSLN* genetic variability in a large group of subjects with asbestos-related diseases. We also evaluated the association of SMRP and *MSLN* genetic variability with the survival of MM patients. We showed that serum SMRP is increased in MM patients and that *MSLN* rs1057147 affects serum SMRP levels in subjects without MM, which could influence the selection of optimal cutoff values for MM diagnosis. Additionally, *MSLN* rs1057147 was an independent predictor of OS in MM patients.

Our study showed that serum SMRP is significantly increased in MM patients compared to patients with other asbestos-related diseases or asbestos-exposed subjects without any disease. All available meta-analyses confirmed that SMRP is elevated in the serum of MM patients as compared to healthy individuals, patients with asbestos-related diseases, benign respiratory diseases or lung cancer.[12,14,16] Previous studies suggest that serum SMRP levels are the highest in epithelioid MM, which is consistent with our results.[12,14] Our present study also included patients with peritoneal MM who were rarely represented in other studies or meta-analyses that focused on pleural MM. Interestingly, we observed that patients with peritoneal MM had significantly higher SMRP than patients with pleural MM. Our results are in agreement with a recent study that also reported elevated serum SMRP in patients with peritoneal MM compared to controls.[33] This suggests that studies on a larger numbers of patients with peritoneal MM are needed to elucidate if SMRP could also serve as a diagnostic biomarker in these patients.

Studies therefore suggest that SMRP measurement could be a potential screening tool for the detection of MM, but clinical use is still limited due to the probability of false positive and especially false negative results.[12,14,16] Some issues related to sensitivity may be associated with a difficult selection of an appropriate cutoff value and different studies found different optimal thresholds.[12,14,16] In our study, the standard cutoff of 1.5 nmol/l had 93% specificity and 59.3% sensitivity for distinguishing MM patients from other subjects. The cutoff with the best sum of specificity and sensitivity was 1.11 nmol/l, with 88.5% specificity and 65.1% sensitivity.

Genetic factors that affect gene expression may account for some of the observed interindividual variability in serum SMRP levels and affect the usefulness of SMRP as a biomarker. In the present study, polymorphic *MSLN* rs1057147 allele was associated with significantly higher serum SMRP levels in both additive and dominant models in the entire cohort. When analyzing each subject group separately, a significant association between *MSLN* rs1057147 and serum SMRP was seen in subjects without a disease and with pleural plaques, while only a trend was observed in asbestosis patients.

Among MM patients, carriers of polymorphic allele had slightly higher serum SMRP, however, there was a large variability in serum SMRP levels and the differences among genotypes were not significant. Both previously published studies focusing on *MSLN* rs1057147 have shown that this polymorphism is associated with increased serum SMRP levels among subjects without MM.[27,28] Consistent with our results, this SNP was not associated with SMRP levels in MM patients.[28] *MSLN* rs1057147 is located in the 3′ UTR region within a binding site for miR-611.[28] Polymorphic allele could reduce the strength of binding to miRNA, limiting the inhibition of gene translation that could lead to the observed increased SMRP levels.[27]

However, other mechanisms might be more important for the increased SMRP expression seen in MM.[28] *MSLN* rs1057147 as a standalone genetic marker was also not associated with MM risk in our study. We observed similar genotype distribution in all subject groups, which is consistent with previously published results.[28]

An important finding is that when rs1057147 genotype was taken into account when determining the serum SMRP cutoff value, the diagnostic predictive value was improved both in our present and in the previously published study.[28] Cutoff values with the highest sum of specificity and sensitivity differed in respective genotype groups. Similar results were reported for SNPs in the 5′ UTR of the *MSLN* gene that may affect binding of transcription factors: polymorphic alleles were associated with increased SMRP levels only in subjects without MM and accounting for the genotype improved the diagnostic performance of SMRP.[25,26] These results suggest that cutoff values should be redefined taking into account the *MSLN* genetic variability to facilitate the interpretation of SMRP measurements.[26] For example, a lower cutoff used in subjects with the wild-type genotype would decrease false negative results.

We also investigated serum SMRP levels and *MSLN* rs1057147 as a prognostic biomarker in MM. In our study, serum SMRP alone was not significantly associated with the survival of MM patients, even though patients with SMRP above 1.5 nmol/l had somewhat shorter OS. Discrepant results were observed in previous studies where very different cutoff values were used. Still, in a meta-analysis, increased SMRP was associated with shorter overall survival, but more comprehensive studies are still needed.[18] However, several studies suggested that a better predictor than baseline serum SMRP would be the change in serum SMRP determined by longitudinal follow-up of MM patients.[22,23,34,35] Serum SMRP could thus be used for monitoring disease progression allowing for early reintroduction of chemotherapy.[36] In Slovenian MM patients, SMRP levels were also increased at disease progression.[21]

On the other hand, *MSLN* rs1057147 was an independent predictor of OS in MM patients in our study. Carriers of at least one polymorphic allele had significantly shorter OS and tended to have shorter PFS. To the best of our knowledge, this is the first report showing that *MSLN* genetic variability can influence the survival of MM patients and further studies are needed to explain the influence of this SNP on survival. As the polymorphic *MSLN* rs1057147 allele was associated with higher serum SMRP levels, this is in agreement with the suggested prognostic role of serum SMRP. The combination of both serum SMRP levels and *MSLN* genetic variability was the best predictor of OS in our study and could therefore serve as a better composite prognostic biomarker in MM.

One of the shortcomings of the present study was that we did not include SNPs in *MSLN* 5′ UTR that could further contribute to the variability in SMRP levels. Apart from that, serum samples were not available for all MM patients at diagnosis, although we do not believe that any systematic bias was involved with this reduction. Additionally, some studies have discussed that other confounding factors, such as age, body mass index and glomerular filtration rate, may affect SMRP levels.[22,37,38] In our study, age, gender and smoking were not associated with serum SMRP levels, but data on body mass index and kidney function were not available. However, not all studies confirmed that these factors influence SMRP levels.[22,37,38] On the other hand, we included a large number of subjects, evaluated different asbestos-related diseases separately and evaluated both serum SMRP levels and *MSLN* rs1057147 as potential diagnostic and prognostic biomarkers.

Mesothelin is by far the most studied biomarker in MM; however, other diagnostic and prognostic biomarkers were also reported. Among serum biomarkers, especially fibulin-3 and osteopontin are promising candidates, but novel biomarkers, such as plasma biomarkers, HMGB1, mRNA and microRNA expression, are also emerging.[16,39-45] Additionally, recent studies suggest that a combination of different biomarkers could improve the predictive capacity of SMRP.[41,42,44,46,47] Our present results show that genetic variability affecting the expression of these biomarkers should also be taken into account.

In conclusion, our results confirm that *MSLN* genetic variability affects serum SMRP levels and could contribute to a better definition of cutoff values for MM diagnosis and screening. We were also the first to show that *MSLN* genetic variability may influence the survival of MM patients. The combination of genetic data and serum biomarkers could therefore serve as a better diagnostic and prognostic biomarker in asbestos-exposed subjects and MM patients.

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research article

The kinetics of γ-H2AX during radiotherapy of head and neck cancer potentially allow for prediction of severe mucositis

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Background. The aim of the study was to evaluate the changes in γ-H2AX expression in peripheral blood lymphocytes (PBL) according to severity of radiation-induced mucositis.

Patients and method. Fifty patients with head and neck cancer treated with radiotherapy (RT) or chemoradiation were included in the study. Blood samples were collected before treatment to measure baseline γ-H2AX levels. Second sample was taken 45 minutes after the first RT fraction and then once a week, 45 min after irradiation. In patients treated with chemoradiation the blood sample was taken the day after chemotherapy. Mucositis was evaluated once a week and reported according to CTCAE v4 and RTOG/EORTC scales. PBL were analyzed with flow cytometry and level of H2AX phosphorylation at every time point was evaluated.

Results. In 35 patients mild to moderate (grade 1–2) mucositis was observed and 15 patients developed severe (grade 3) mucositis. No cases of grade 4 mucositis were observed. The difference in baseline levels of γ-H2AX between groups with mild and severe mucositis was statistically insignificant ($p = 0.25$). The statistically significant difference in γ-H2AX level was observed in week 7 of treatment ($p = 0.01$). No significant differences in γ-H2AX level were found neither between group treated with concomitant chemoradiation or RT alone neither between groups with and without common comorbidities. In the analysis of the kinetics of γ-H2AX during treatment, a statistically significant difference ($p = 0.0088$) between groups with mild and severe mucositis was observed. After fourth week of treatment levels of γ-H2AX decreased significantly in the group with severe mucositis and increased in patients with mild side effects. The observed difference was not caused by the decrease in peripheral lymphocyte count, which was similar in both groups.

Conclusions. Presented results indicate that severity of radiation-induced mucositis does not correlate directly with γ-H2AX levels measured *in vivo* in PBL. Prediction of mucositis grade based on γ-H2AX level is not yet possible, either before treatment or early during treatment, but preliminary results, indicating significant differences in γ-H2AX kinetics between groups, encourage further studies.

Keywords: γ-H2AX; peripheral blood lymphocytes; mucositis

Introduction

Radiotherapy and chemoradiation remain the major treatment modalities of head and neck cancer either as a primary treatment or in postoperative adjuvant setting.

Despite the clear therapeutic benefits, acute and late side effects of radiation and chemora-

diation still limit the quality of life of patients. Furthermore, there is significant heterogeneity in response to radiation, both in tumours and healthy tissues. It would therefore be of great value for the clinicians to predict the response not only in the primary tumour volume, but also in the surrounding normal tissues.

Radiation damage in any tissue occurs primarily through single or double DNA strand break (DSB), which lead to cell death due to chromosomal aberrations and apoptosis if not repaired. Phosphorylated histone H2AX (called γ-H2AX) is an indirect protein marker of DSB. It has been shown that DSB in cell nucleus triggers H2AX phosphorylation in position Ser139 (*i.e.* γ-H2AX). Phosphorylated H2AX is a trigger for DNA repair signaling pathways (*e.g.* through ATM, MRE11, DNA-PK, Rad50, Nbs1). The maximum level of unrepaired damage is observed approximately 30 min after radiation.[1,2]

In this paper, we suggest that assessment of γ-H2AX levels after irradiation could serve as a marker of sensitivity to radiation damage in normal tissue, enabling the estimation of the individual damage repair potential. Peripheral blood lymphocytes (PBL) could serve as effective and easily accessible surrogates of normal tissue. Previous studies have focused on lymphocytes irradiated *in vitro*, which fails to account for the tissue microenvironment and comorbidities. *In vivo* analysis using samples of fresh blood taken from patients immediately after irradiation offers a more reliable way to assess H2AX phosphorylation.[3,4] In the future, predictive factors showing high individual regenerative potential could allow for patient selection for escalated radiotherapy. Additionally, prediction of higher risk for acute and late effects would enable the clinician to intensify supportive care, *e.g.* nutrition and hydration, to maintain as good quality of life (QoL) and survival as possible.

The main objective of the study was to examine the clinical usefulness of assessing radiation effects based on the γ-H2AX evaluation in the PBL. The secondary goal was to assess the changes of γ-H2AX expression in PBL according to the severity of radiation-induced mucositis (M) during radiotherapy or chemoradiation. Mucositis is evaluated in clinic using many scales, most widely used are Common Terminology Criteria for Adverse Events v.4 (CTCAE) and European Organisation for Research and Treatment of Cancer/Radiation Therapy Oncology Group (EORTC/RTOG) scales.[6,7]

Table 1 presents grading of toxicity, using oral mucositis as an example, with two most commonly used scales.

Patients and method

In this prospective study, we included patients with head and neck cancers treated with postoperative or definitive radiotherapy or chemoradiation. Patients treated with palliative intent were excluded from the study. The study was approved by the Ethics Committee of University of Medical Sciences in Poznan (No723/14) and written informed consent was provided by all the participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Treatment

Patients were treated with adjuvant radiotherapy or chemoradiation to 60-66 Gy, if the known features of risk of relapse were present in pathology report.

Patients with inoperable tumors were treated mostly with concomitant chemoradiation to dose 70 Gy (fractions of 2 Gy once daily, 5 times a week) concurrently with cisplatin 40 mg/m^2 once a week

TABLE 1 Grading of radiation-induced oral mucositis according to CTCAE v4 and EORTC/RTOG scales.[6,7]

Mucositis (oral)	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
CTCAE v4	Asymptomatic/mild symptoms, intervention not indicated	Moderate pain not interfering with oral intake, modified diet indicated	Severe pain interfering with oral intake	Life-threating consequences, urgent intervention indicated	Death
EORTC/ RTOG	Irritation, may experience slight pain, not requiring analgesic	Patchy mucositis that may produce inflammatory serosanquinitis discharge, may experience moderate pain requiring analgesia	Confluent, fibrinous mucositis, may include severe pain requiring narcotic	Ulceration, haemorrhage or necrosis	N/A

TABLE 2. Demographics, staging and treatment information of studied patients

Characteristics		Total N = 50 (%)	M 0-2 N = 35 (%)	M 3 N = 15 (%)	*p*-value
Sex	Male	44 (88)	30 (85.7)	14 (93.3)	0.44
	Female	6 (12)	5 (14.2)	1 (6.6)	
Primary site	Parotid	5 (10)	5 (14.3)	0 (0)	0.27
	Oral cavity	11 (22)	7 (20)	4 (26.7)	
	Larynx	14 (28)	10 (28.6)	4 (26.7)	
	Hypopharynx	4 (8)	4 (11.4)	0 (0)	
	Oropharynx	12 (24)	6 (17.1)	6 (40)	
	CUP	2 (4)	1 (2.8)	1 (6.7)	
	Nasalcavity/paranasal sinuses	2 (4)	2 (5.7)	0 (0)	
T classification	T1	4 (8)	2 (5.7)	2 (13.3)	0.49
	T2	17 (34)	11 (31.4)	6 (40)	
	T3	8 (16)	7 (20)	1 (6.7)	
	T4	19 (38)	14 (40)	5 (33.3)	
	Tx	2 (4)	1 (2.8)	1 (6.7)	
N classification	N0	26 (52)	19 (54.3)	7 (46.7)	0.25
	N1	4 (8)	4 (11.4)	0 (0)	
	N2	20 (40)	12 (34.3)	8 (53.3)	
Surgery	Yes	28 (56)	22 (62.85)	6 (40)	0.13
	No	22 (44)	13 (37.1)	9 (60)	
Concomitant chemotherapy	Yes	33 (66)	26 (74.3)	7 (46.7)	0.06
	No	17 (34)	9 (25.7)	8 (53.3)	
Comorbidities	Yes	34 (68)	21 (60)	13 (86.7)	0.06
	No	16 (32)	14 (40)	2 (13.3)	
Selected comorbidities	Diabetes type II	6 (12)	5 (14.3)	1 (6.7)	0.09
	Hypertension	17 (34)	12 (34.3)	5 (33.3)	
	Ischemic heart disease	11 (22)	4 (11.1)	7 (46.7)	

CUP = cancer unknown primary; M = mucositis; grade 0-2 or grade 3 (of mucositis).
No grade 4 mucositis was observed. The statistical differences between groups were assessed with the use of Chi-square test.

or 100 mg/m^2 every three weeks of treatment, on days 1, 21, and 42 of radiotherapy (fraction 1, 16, 31). In every patient mucositis was evaluated once a week, independently, by radiation oncologist and head and neck surgeon, using the Common Terminology Criteria for Adverse Events version 4 (CTCAE v.4) and the Radiation Therapy Oncology Group (RTOG) scales.[6,7] For the purpose of this study, grading results based on the CTCAE scale were employed because it is more commonly used in clinic. The maximum score observed during treatment was used for correlation with γ-H2AX level.

Blood samples and flow cytometry analysis

The level of γ-H2AX was analyzed in PBL. The first blood sample was collected before treatment, to measure basic level of γ-H2AX in every patient. The second sample was taken 45 minutes after the first fraction and then once a week, 45 minutes after radiotherapy fraction. The sampling time was selected according to observation from a pilot study, showing that the level of γ-H2AX in PBL was highest 45 min after irradiation. In patients treated with concurrent chemotherapy, the blood sample was taken the day after chemotherapy. The collected blood samples were stabilized by Transfix (Cytomark, UK) administration according to manufacturer protocol. H2AX phosphorylation levels in the leucocytes were examined by flow cytometry according to manufacturer protocol (Apoptosis, DNA Damage, and Cell Proliferation Kit, BD, USA). Briefly, isolated leucocytes (centrifuging 1200g at 20°C for 20 min) were fixed with BD Cytofix/Cytoperm Fixation/Permeabilization Solution for 30 min at room temperature. Subsequently, cells were incubated with BD Cytofix/Cytoperm Plus Permeabilization Buffer for 10 min at 4ºC, and re-fixed, with BD Cytofix/Cytoperm Fixation/Permeabilization Solution (5 min, at room temperature). Next, the cells were incubated with Alexa Fluor 647 Mouse Anti-H2AX antibody (pSer139). Staining with isotype control (APC Mouse IgG2b κ Isotype Control; Becton Dickinson, USA) was per-

formed to assess the threshold of positive staining. Data acquisition was performed using a BD Accuri C6 Plus flow cytometer (BD Biosciences, USA), and analyzed using FlowJo software (FlowJo, LLC, USA).

Statistical analysis

Statistical analysis was performed using STATISTICA (StatSoft, Inc. USA version 12, 2014). The differences between groups were assessed with the use of Chi-square test (Table 2). Two-sided Welch's t-test, and two-way ANOVA were applied to assess the statistical significance of differences between γ-H2AX levels. The result were evaluated at $\alpha = 0.05$ significance level.

Results

Fifty patients with head and neck cancers treated with adjuvant or primary radiotherapy or chemo-radiation were included in the study. Mild (grade 1) to moderate (grade 2) mucositis was observed in 35 patients and severe (grade 3) in 15. No cases grade 4 mucositis were observed. No patient was lost from follow up. Median follow-up was 51 months (range, 2–60 months), median overall survival (OS) for whole group reached 51 months. For patients with mild and severe mucositis median OS was 50.5 and 55 months respectively. We did not observe any unexpectedly severe acute reaction *i.e.*, necrosis in the study. Mean radiotherapy dose for neck lymph nodes was 52 Gy (range 50–60 Gy) and mean total volume of irradiated tissue (Planning Target Volume, PTV) was 750.8 cm^3 (range 121.0–1022.0). Table 2 shows demographics, staging and treatment details of the patients.

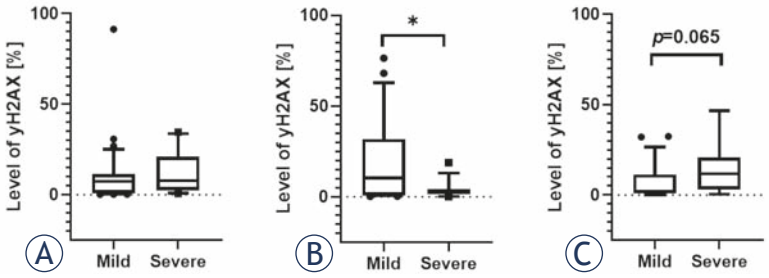

FIGURE 1. Level of γ-H2AX in peripheral blood lymphocytes before radiotherapy **(A)**; in week 7 **(B)**; week 2 **(C)** of treatment. Mild = grade 1 or 2 mucositis; Severe = grade 3. No grade 4 mucositis was observed.

The difference in basic level of γ-H2AX between the groups with mild and severe mucositis was statistically insignificant (p = 0.25) (Figure 1A). However, we observed a significantly lower level of H2AX phosphorylation in lymphocytes in week 7 (p = 0.011) in the group with severe mucositis in comparison to the mild one (Figure 1B).

Interestingly, we noticed an opposite statistical trend in week 2 (p = 0.065) (Figure 1C). Analyzing the kinetics of γ-H2AX during treatment, we observed a statistically significant difference (p = 0.0088) between groups with mild and severe mucositis (Figure 2A, Table 3, Table S1 in Supplementary material). Between treatment weeks 4 and 7, levels of γ-H2AX decreased significantly in the group with severe mucositis and increased in patients with mild side effects. The observed difference is not caused by the expected decreasing of peripheral lymphocytes level during radiotherapy which was similar in both groups (Figure 2B).

TABLE 3. Level of γ-H2AX in peripheral blood lymphocytes before and during treatment

Week of radiotherapy	Mild mucositis		Severe mucositis		No. of samples	*p*-value
	Mean	SD	Mean	SD		
Before	10.64	16.38	11.40	11.71	48	0.868
Week 1	8.48	8.99	11.24	10.78	48	0.347
Week 2	7.22	9.86	13.27	11.94	48	0.065
Week 3	9.45	11.46	12.44	12.16	48	0.403
Week 4	10.81	14.10	11.87	15.46	48	0.812
Week 5	14.95	19.88	11.97	19.60	47	0.624
Week 6	18.25	20.96	8.90	15.62	47	0.121
Week 7	20.50	23.13	3.81	4.78	39	0.011*
Week 8	13.23	10.82	5.46	8.83	8	0.268

SD = standard deviation

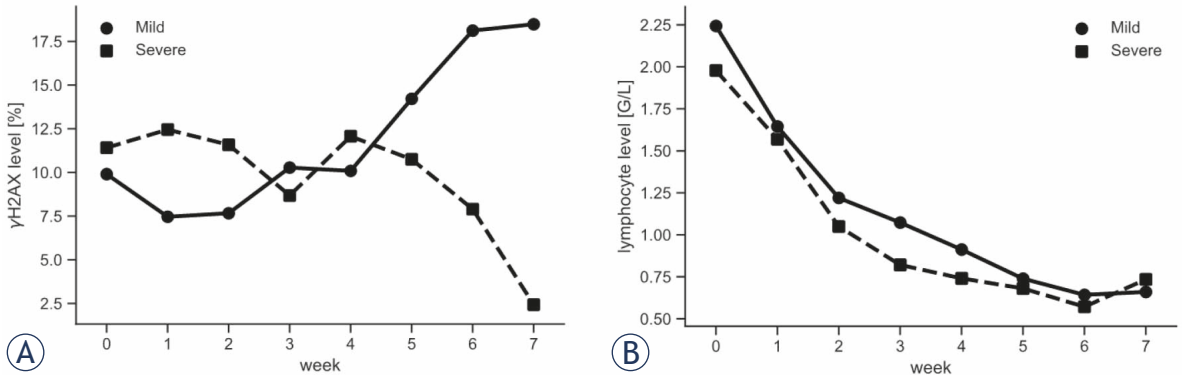

FIGURE 2. Kinetic of γ-H2AX level in peripheral blood lymphocytes **(A)**. Level of Peripheral Blood Lymphocytes during treatment **(B)**. Mild = grade 1 or 2 mucositis; severe = grade 3. No grade 4 mucositis was observed.

Further analysis showed no statistical differences in γ-H2AX levels neither between the groups treated with concomitant treatment and radiotherapy alone nor between groups with and without common comorbidities like hypertension or diabetes, and habits like smoking. Furthermore, we did not notice correlation between γ-H2AX levels and survival, staging or tumor primary site.

Discussion

The aim of this study was to test the usefulness of γ-H2AX as a clinically feasible predictor of severity of acute side effects caused by radiotherapy *i.e.*, mucositis. Such predictor would be of great value during the planning of treatment and supportive care and in selecting potential over-reactors.

γ-H2AX is a marker of DNA damage and kinetics of repair. Thus, residual γ-H2AX can be valuable for evaluation of radiosensitivity and accumulation of unrepaired DNA damage. Due to difficulties in obtaining mucosa or skin cells during radiotherapy for *in vivo* studies, especially in the presence of acute mucositis, we used peripheral blood lymphocytes as a surrogate of normal cells, including skin and mucosa.[8]

To assess γ-H2AX levels, we employed flow cytometry. Although sensitivity of flow cytometry has been questioned by Beaton *et al.* in study attempting to identify radiation-sensitive patients with prostate cancer, the method is fast, accurate and easy to apply in daily clinical setting.[9]

The only significant difference in γ-H2AX levels between patients with mild and severe mucositis was found in the seventh week of radiotherapy. Our results are similar to findings of Vasireddy *et al.* and Li *et al.*, who suggested that the formation of γ-H2AX foci measured *in vivo* was not significantly different between patients with severe and mild mucositis.[4,10] Li analyzed the formation of γ-H2AX, both *in vitro* and *in vivo*, and although the correlation was seen in *in vitro* settings, there was no similar correlation in *in vivo* study.

Other published reports analysing H2AX phosphorylation during acute phase of radiotherapy side effects are conflicting. Fleckenstein *et al.* did not find a direct correlation between mucositis and H2AX phosphorylation, However, according to Burton *et al.*, the residual γ-HAX levels are higher in over reactors' PBLs *in vitro* at 24 hours after fraction or *in vivo*, after 3–6 hours.[3,11,12] Goutham *et al.* observed increasing residual DSB with increasing severity of the reaction but they did not find a clear correlation with grade of mucositis.

Many publications assess γ-H2AX formation and its 24-hours kinetics after one dose of irradiation, *in vivo* or *in vitro*. For example, Li *et al.* suggested that only a single fraction of irradiation as high as 8 Gy *in vitro* induces different levels of γ-HAX between patients with severe and mild mucositis.[4] Patients included in our study were irradiated with 2 Gy fraction according to the standard protocol for head and neck radical treatment, to dose 66-70 Gy (33-35 fractions). Thus, conclusions from previous experiments with one fraction may not hold, especially at the end of fractionated treatment. Formation of γ-H2AX after multiple therapeutic fractions separated by more than 12 hours is still a subject of debate.[14,15]

We also did not find significant differences in baseline γ-H2AX levels between the individual patients before start of radiotherapy or chemoradiation. The mean baseline level of γ-H2AX was higher for patients with severe mucositis but observed difference was not statistically significant.

An interesting finding from our study is a rapid decrease in γ-H2AX levels after fourth week of therapy in group with severe mucositis in contrast to mild mucositis group, where an increase was observed. This phenomenon can be explained by ineffective DNA damage repair system (DDR) responsible for recognition and repair of DSB in most sensitive patients and changes in the conformation of chromatin after irradiation. Such changes can affect DSB signaling after the next fraction, resulting in lower phosphorylation of H2AX or lower detection of phosphorylated histones.[15] However, this phenomenon has been observed when the next fraction was applied 5–6 hours following the first. So far little is known about repair processes and γ-H2AX formation when the time between fractions is longer than 12 hours. Bouquet *et al.* showed that γ-H2AX levels reflect DSB repair activity only at doses of cytotoxic agent or irradiation producing less than 100-150 DSBs breaks per genome.[16]

Finally, we did not find γ-H2AX levels to be predictive for severe risk of mucositis before or early during treatment. Kinetics of γ-H2AX during treatment could potentially allow for such discrimination by evaluation of changes in subsequent γ-H2AX levels after 4 weeks of therapy. To our knowledge, this is the only study evaluating the kinetics of γ-H2AX during 7-week treatment.

In our study, we also considered selected, patient-related factors with potential impact on severity of mucositis like comorbidities, age and staging. Smoking was not included in the analysis as it was very challenging to obtain reliable information about smoking during treatment. We did not find any correlation between γ-H2AX levels and studied factors, as well as cisplatin use or radiotherapy setting. Similarly to the Werbrouck *et al.*, we did not observe any relationship between γ-H2AX levels during treatment and grade of late toxicity like skin fibrosis.[17]

The limitations of current *in vivo* studies are small sample sizes and heterogeneity of evaluated reactions, from acute mucositis to chronic reactions, like ulceration and fatigue. Moreover, different scales of evaluation of side effects are commonly used, making comparison between studies very difficult.[3] Standardization of evaluation and reporting of acute and late effects would be of a value not only for patients' benefit but also for the future studies and audits to prevent potential overreaction due to errors in irradiation.[13]

In this study, we tested the PBL of relatively large group of patients during radiotherapy and chemoradiation. In line with other studies, we were not able to prove that assessment γ-H2AX with flow cytometry is a method ready to be used clinically for identification of patients with high risk for mucositis before or early during treatment.[9,11] The probable reason for this is the multifactorial clinical and radiobiological background of acute mucositis, which depends among others on site of disease, total dose, dose per fraction, irradiated volume, comorbidities, concomitant chemoradiation, hydration, nutrition, and repair potential of normal tissue.[5] Furthermore, circulating PBLs receive variable doses of radiation and highly damaged cells might be eliminated from circulation, potentially biasing the results.

Conclusions

Based on the results presented, it is not yet possible to predict the severity of mucositis induced by radiation using γ-H2AX. However, preliminary results indicating significant differences in kinetics of γ-H2AX levels between groups after fourth week of treatment encourage further studies.

Acknowledgements

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research article

Molecular heterogeneity in breast carcinoma cells with increased invasive capacities

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Giulia Negro and Bertram Aschenbrenner contributed equally to this work.

Background. Metastatic progression of breast cancer is still a challenge in clinical oncology. Therefore, an elucidation how carcinoma cells belonging to different breast cancer subtypes realize their metastatic capacities is needed. The aim of this study was to elucidate a similarity of activated molecular pathways underlying an enhancement of invasiveness of carcinoma cells belonging to different breast carcinoma subtypes.

Materials and methods. In order to reach this aim, parental and invasive (INV) MDA-MB-231 (triple-negative), T47D (hormone receptor-positive), and Au565 (Her2-positive) breast carcinoma cells were used and their molecular phenotypes were compared using a proteomic approach.

Results. Independently from breast cancer subtypes, INV cells have demonstrated fibroblast-like morphology accompanied by enhancement of invasive and migratory capacities, increased expression of cancer stem cell markers, and delayed tumor growth in *in vivo* animal models. However, the global proteomic analysis has highlighted that INV cells were different in protein expressions from the parental cells, and Her2-positive Au565-INV cells showed the most pronounced molecular differences compared to the triple-negative MDA-MB-231-INV and hormone receptor-positive T47D-INV cells. Although Au565-INV breast carcinoma cells possessed the highest number of deregulated proteins, they had the lowest overlapping in proteins commonly expressed in MDA-MB-231-INV and T47D-INV cells.

Conclusions. We can conclude that hormone receptor-positive cells with increased invasiveness acquire the molecular characteristics of triple-negative breast cancer cells, whereas Her2-positive INV cells specifically changed their own molecular phenotype with very limited partaking in the involved pathways found in the MDA-MB-231-INV and T47D-INV cells. Since hormone receptor-positive invasive cells share their molecular properties with triple-negative breast cancer cells, we assume that these types of metastatic disease can be treated rather equally with an option to add anti-hormonal agents. In contrast, Her2-positive metastasis should be carefully evaluated for more effective therapeutic approaches which are distinct from the triple-negative and hormone-positive metastatic breast cancers.

Key words: breast cancer cells; cancer stem cells; invasiveness; migration; metastasis; molecular profiling

Introduction

According to the World Health Organization (WHO) reports, 2.1 million women worldwide are diagnosed with breast cancer every year. In 2018, it was estimated that 627000 deaths were counted for breast cancer and it was approximately 15% of all cancer-related deaths among women (www.who.int). It is believed that metastatic progression is responsible for most breast cancer deaths.[1] Therefore, elucidation of molecular properties of carcinoma cells responsible for metastatic spread can help in the development of biomarkers or therapeutic targets to predict or improve clinical outcome in breast cancer patients.

Metastasis cascade contains a number of steps such as cancer cell invasion in surrounding tissues followed by cell intravasation into the blood and/or lymphatic vessels, extravasation into the tissue of distant organs, and, finally, colonization and growth in the targeted organs.[2] Since metastatic spread begins from cancer cell invasion through the biological membranes and extracellular matrix (ECM), it is logical to suggest that breast carcinoma cells with enhanced metastatic capacities should possess an augmented invasiveness.

It is currently known that different breast cancer phenotypes reveal different propensities for cancer cell dissemination. Thus, hormone-dependent breast carcinomas demonstrate lower metastatic potential in comparison with Her2-positive and triple-negative breast cancers.[3] However, it is not fully understood whether carcinoma cells belonging to different breast cancer subtypes have an activation of similar or different pathways involved in metastatic progression. There is a limited number of reports on the molecular diversities of primary tumors and metastatic lesions. Therefore, it is difficult to study the molecular perturbations that occur in breast cancer cells during metastatic progression.

Since triple-negative breast cancer possesses the most aggressive metastatic potential, other types of breast carcinomas (hormone receptor-positive and Her2-positive) are considered as metastatically active if they demonstrate an overexpression of molecules upregulated in triple-negative tumors (EGFR, cytokeratines 5/6, 14, 17, etc.).[4] Therefore, it is assumed that different subtypes of metastatic breast cancer cells can have the same therapeutic targets.[5] This hypothesis leads to the current therapeutic strategy which does not really stratify the patients depending on the molecular patterns of the secondary tumors. Nowadays, a chemotherapeutic approach contains an arsenal of cytotoxic compounds nonspecifically killing the rapidly dividing carcinoma cells.[6] There are only few additional options to use anti-hormonal and anti-Her2 agents in the treatment of patients with appropriate tumor subtypes.[7] This strategy could be logical and acceptable, if metastatic breast cancer cells of different histopathological subtypes reveal the unified molecular properties and common molecular pathways are involved in metastatic cascade.

This study aimed to elucidate a similarity of activated molecular pathways underlying an enhancement of invasiveness in carcinoma cells belonging to different breast carcinoma subtypes.

Materials and methods

Cells and cell cultures

MDA-MB-231 (triple-negative type (TNBC): estrogen, progesterone and HER2/*neu* receptor negative (ER-, PR-, HER2/*neu*-), T47D (luminal A type: ER+, PR+, HER2/*neu*-), and Au565 (Her2/*neu*-positive cells: ER-, PR-, HER2/*neu*+) cells were purchased from the American Type Culture Collection. All cell lines were grown in RPMI1640 medium supplemented with 2 mM L-glutamine, 50 U/mL penicillin, 50 μg/mL streptomycin, and 10% fetal calf serum (FCS) (Thermo Fisher Scientific, Vienna, Austria). Cell cultures were maintained in a 5% CO_2 humidified atmosphere.

Breast carcinoma cells with increased invasive abilities (INV cells) were obtained from parental breast carcinoma cells after repetitive cell migration through the uncoated 8 μm-pore membrane in the Boyden chamber (Corning Life Sciences, Berlin, Germany) toward the cell culture medium containing an attractant (10% FCS). Cell migration was repeated ten times every 2 weeks. In order to support and control the invasive abilities of the INV cells, cell migration through the membrane with 8 μm pores was repeated and evaluated every month.

3D tomographic microscopy

Parental and invasive MDA-MB-231, T47D, and Au565 cells were seeded into the glass-bottom dishes with diameter of 35 mm (Ibidi, Switzerland). Seeded cells were incubated for 24 hours at 37°C and 5% CO_2 humidified atmosphere. Next, cells were analysed for their morphology using 3D tomographic microscope (3D Cell Exlplorer, Nanolive SA, Switzerland), and 3D tomographic images (z-stacks) were collected for further compu-

tational analysis using STEVE software (Nanolive SA, Switzerland).

Migration assay

Migratory capacities of the investigated breast cancer cells were assessed using the Boyden chambers and automated live cell imager Lionheart FX (BioTek, Vermont, USA). Parental and INV breast carcinoma cells were serum-starved for 24 hours, next harvested and seeded ($5x10^5$ cells in 2.5 mL RPMI 1640 serum-free medium) into the upper insert with an 8-μm pore size polycarbonate membrane. The lower chamber contained the same volume of cell culture medium with chemoattractant (10% FCS). After 24 hours of cell incubation at 37°C in a 5% CO_2 humidified atmosphere, the inserts were removed and the migrated cells were stained with the Hoechst 33342 (1,5 μg). Using the Gen 5 3.0 software, the images of the stained cells were captured and a number of migrated cells were counted. All cell migration assays were performed in triplicates, and repeated at least three times.

Invasive abilities of breast carcinoma cells

Cell Biolabs CytoSelect TM Laminin and Collagen I Cell Invasion Assay Kits (Cell Biolabs, Inc., San Diego, USA) were used to study the invasive abilities of the investigated parental and newly obtained INV breast carcinoma cells. Briefly, breast carcinoma cells harvested in the RPMI1640 serum-free quenching medium were placed in the upper insert with the laminin- or collagen I-coated membranes. The lower chamber contained RPMI1640 medium with chemoattractant (10% FCS). Prepared plates were incubated for 24 hours at 37°C in a 5% CO_2 humidified atmosphere. Cells migrated through the laminin- or collagen I-coated membranes were detached using the cell detachment solution, and then lysed in the lysis buffer containing CyQuant® GR dye solution. Fluorescence of the investigated samples was measured with a fluorescence microplate reader Synergy H1M (BioTek, Vermont, USA) at 480nm/520nm. Background readings from buffers were subtracted from the readings of each sample. Invasion assay was repeated at least three times for each investigated cell line.

Cell growth

Cell proliferation rate was determined as previously described.[8] Parental MDA-MB-231, T47D and Au565 and their invasive pairs MDA-MB-231-INV, T47D-INV and Au565-INV breast cancer cells were seeded (1×10^4) in triplicates in 4.0-mL in 6-well plates. Cells were incubated for 24 hours at 37°C and then trypsinized and counted using Beckman Coulter Vi-CELL AS cell viability analyzer (Beckman Coulter, Fullerton, CA, USA). Cell population doubling time (DT) in hours was determined using the computerized tool (http://www.doubling-time.com/compute.php) and following equation:

$DT\ (hours) = duration\ (24\ hours) * log(2) / log\ (N_1) - log\ (N_0)$, where N_1 – final cell number; N_0 – initial cell number. Each experiment was repeated at least three times to ensure the reproducibility of the results.

Flow cytometry

For analysis of CD44 and CD24 expression in parental and invasive MDA-MB-231, T47D, and Au565 cells by flow cytometry (BD FACS Canto II, Becton Dickinson, San Jose, USA) $1x10^6$ cells per tube were pelleted at 200xg, resuspended and stained with 4 μg/mL human monoclonal anti-CD44-PE and anti-CD24-FITC antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany). An equivalent amount of PE- and FITC-conjugated IgG1k (Miltenyi Biotec, Bergisch Gladbach, Germany) were used as isotype controls. The labeled cells were washed in the FACS buffer and then analyzed by flow cytometry, and results were evaluated by the subsequent FACS DIVA Software 7.0. All measurements were repeated at least three times.

ALDEFLUOR Assay for detection of the ALDH1 activity

To detect ALDH1 enzymatic activities in the investigated parental and INV breast carcinoma cells, ALDEFLUOR assay kit was used according to the manufacturer instructions (STEMCELL Technologies, Koeln, Germany). Briefly, 10^6 cells/mL were harvested from cell cultures, centrifuged at 200xg, and resuspended in 1 mL of ALDEFLUOR Buffer. For ALDEFLUOR staining, 5 μL/mL of the activated ALDEFLUOR™ Reagent was added into each investigated sample. Each experimental sample was accompanied by the negative control after adding 5 μL of diethylaminobenzaldehyde (DEAB), the ALDH1 inhibitor. All prepared test and negative control samples were incubated at 37°C for 30 minutes. Following incubation, the samples were centrifuged for 5 minutes at 250 xg.

Supernatants were discarded, and cells were resuspended in 0.5 mL of ALDEFLUOR™ Assay Buffer and stored on for 30 minutes. Next fluorescence intensity of the stained cells was analyzed by the flow cytometry, and a percentage of breast carcinoma cells with ALDH1 activity was estimated by the subsequent FACS DIVA Software 7.0. All experiments were repeated three times.

Western blot analysis

Western blot was performed as published previously[9] using ALDH1A3 rabbit polyclonal antibody (ab129815, Abcam, Cambridge, UK) or GAPDH rabbit monoclonal antibody (Cell Signaling Technologies, Frankfurt am Mein, Germany) as a loading control. For evaluation of protein expression, X-ray films (GE Healthcare, Chicago, IL, USA) were scanned and analyzed by the Image StudioTM Lite 5.0 (LI-COR Biotechnology, Lincoln, NB, USA). The Integrated Density Value (IDV) was obtained as a ratio of normalized protein band densities in parental and INV cells after background correction.

Proteomic analysis

Materials used for the analysis of protein profiling

TMT 6 plex™ Isobaric Label Reagent Set (6-plex TMT) was obtained from Life Technologies (Grand Island, NY, USA). Trypsin (Sequencing grade modified porcine) was obtained from Promega. Acetonitrile was purchased from JT Baker, and formic acid was obtained from EMD Millipore (Billerica, MA, USA). MyProt-Buffer 1, MyProt-Buffer 2, MyProt-Buffer 3, MyProt-PhosphoWASH Buffer, and MyProt-PhosphoELU Buffer were utilized by MyOmicsDx, Inc (Towson, MD, USA).

Protein preparation

Protein samples were processed by MyOmicsDx, Inc (Towson, MD, USA) using "Filter Assisted Sample Preparation" (FASP) method. Briefly, protein samples in 9M UREA were reduced with 5 mM TCEP at 37°C for 45 min and reduced cysteines were blocked using 50 mM iodoacetamide at 25°C for 15 min. Protein samples (100 µg each) were then cleaned using 10 kDa Amicon Filter (UFC501096, Millipore, USA) three times using 9M urea and two times using MyProt-Buffer 1 (MyOmicsDx, Inc). Samples were then proteolyzed with trypsin (V5111, Promega, USA) for 12 hrs at 37°C. The peptide solution then was acidified by adding 1% trifluoroacetic acid (TFA) and was incubated at room temperature for 15 min. A Sep-Pak light C18 cartridge (Waters Corporation, USA) was activated by loading 5 mL 100% (vol/vol) acetonitrile and was washed by 3.5 mL 0.1% TFA solution two times. Acidified digested peptide solution was centrifuged at 1,800 × g for 5 min, and the supernatant was loaded into the cartridge. To desalt the peptides bound to the cartridge, 1 mL, 3 mL, and 4 mL of 0.1% TFA were used sequentially. To elute the peptides from the cartridge, 2 mL of 40% (vol/vol) acetonitrile with 0.1% TFA was used, and this elution was repeated two more times (for a total of 6 mL of eluate). It was important to ensure that the cartridge had stopped dripping before each sequential wash and elution solution was applied. The eluted peptides were lyophilized overnight and reconstituted in 100 µL of MyProt-Buffer 2 (MyOmicsDx, Inc, MD, USA).

Multiplexed TMT labeling

Digested peptides from samples in a volume of 100 µl MyProt-Buffer 2 were labeled using 6-plex TMT reagents. After 2 hours, 8 µL of 5% hydroxylamine were added to the sample and incubated for 15 minutes to quench the reaction. Labeled peptides were dried to remove organic solvents and reconstituted in 500ul MyProt-Buffer 3 (MyOmicsDx, Inc), combined and fractionated on a bRPLC (basic Reverse Phase Liquid Chromatography) column (XBridge BEH C18 Column, 5 µm, 2.1 x 100 mm) via XBridge BEH C18 Guard Column (Waters Corporation, USA) using an Agilent 1260 HPLC system. Peptides in each fraction were dried.

Strong cation exchange chromatography and TiO2-based phosphopeptide enrichment

Peptides were fractionated by strong cation exchange (SCX) chromatography. Briefly, 10 mg of lyophilized peptides mixture was resuspended in 1 ml of SCX solvent A (5 mM KH2PO4 pH 2.7, 30% ACN) and fractionated by SCX chromatography on a PolySULPHOETHYL A (5 µm, 200 Å) column (200 × 9.4 mm; PolyLC Inc., Columbia, MD) by employing an increasing gradient of SCX solvent B (5 mM KH2PO4 pH 2.7, 30% ACN, 350 mM KCl) on an Agilent 1100 LC system. For each experiment, a total of 90 fractions were initially collected, which were pooled into fractions of various sizes (13, 16, or 22 fractions for each respective experiment), and dried using speedvac (Eppendorf, USA). Each fraction was subjected to TiO2-based phosphopeptide enrichment. Briefly, TiO2 beads were incubated with DHB solution (80% ACN, 1%

TFA, 3% 2,5-dihydroxybenzoic acid (DHB)) for 2–4 hours at room temperature. Each fraction was re-suspended in DHB and incubated with pretreated TiO2 beads (5 mg) overnight at room temperature. Phosphopeptide-bound TiO2 beads were washed three times with DHB solution and twice with MyProt-PhosphoWASH buffer. Peptides were eluted three times with 40 µl of MyProt-PhosphoELU buffer.

Nanoflow electrospray ionization tandem mass spectrometry analysis

Data dependent MS/MS analyses of the TMT labeled peptides were carried out on a Thermo Scientific™ EASY-nLC 1000™ HPLC system and Thermo Scientific EASYSpray™ source with analytical nanoflow column system includes 2 cm trap column and 75 µm x 20 cm analytical column both packed with Magic C18 AQ, 5 µm, 100 Å (Michrom Bioresources, USA). Samples were analyzed on a Thermo Scientific™ Q Exactive Mass Spectrometer using FT HCD MS2 fragmentation mode. Peptides were electrosprayed through a 15 µm emitter (PF3360-75-15-N-5, New Objective) at a voltage of 2.0 kV spray voltage. Reversed phase solvent gradient consisted of 0.1% formic acid with increasing levels of 90% acetonitrile in 0.1% formic acid over a period of 90 minutes. The Q Exactive instrument was operated to automatically switch between full scan MS and MS/MS acquisition. Survey full scan MS spectra (m/z 350–1800) was acquired in the Orbitrap with 35,000 resolution after accumulation of ions to a 3×10^6 target value based on predictive AGC from the previous full scan. Dynamic exclusion was set to 15 s. The 10 most intense multiply-charged ions ($z \geq 2$) were sequentially isolated and fragmented in the Axial Higher energy Collision-induced Dissociation (HCD) cell using normalized HCD collision energy at 30% with an AGC target 1e5 and a maxima injection time of 400 ms at 35,000 resolution.

MS data analysis

Mass spectrometry raw files were automatically processed through Proteome Discoverer 2.1 software using Xtract in addition to default spectrum selector node. The searches were performed using Mascot search engine together with Sequest HT interfaced with different processing nodes of Proteome Discoverer 2.1 SP1. Search parameter included oxidation on methionine, deamidation on residues N and Q as different variable modifications and TMT 6-plex on N-terminus and lysine residue, carbamidomethyl on cysteine residue as different fixed modifications. Mass tolerances on precursor and fragment masses were set to 15 ppm and 0.03 Da, respectively. Peptide validator node was used for peptide validation with a stringent cutoff of 0.01 and relaxed cutoff of 0.05 (false discover rate, FDR) and 1% FDR cutoff was used to filter the data. High confidence (0.1% FDR) and top-ranked peptide were considered with protein grouping option. Protein ratios were normalized through *MyProt-QuantiR (MyOmicDx, Inc*) software package and peptides with >30% isolation interference were excluded from the protein quantification to avoid potential interference of reporter ions from contaminant peaks.

Bioinformatic analysis of proteomic data

Alterations in activation of intracellular molecular pathways relatively to normal samples were quantitatively assessed using the bioinformatical platform Oncobox.[10] The structures of 3121 molecular pathways were extracted from the following public databases: Reactome,[11] NCI Pathway Interaction Database,[12] Kyoto Encyclopedia of Genes and Genomes,[13] HumanCyc,[14] Biocarta [www.biocarta.com] and Qiagen (www.qiagen.com/ us/shop/genes-and-pathways/pathway-central/). Molecular pathways were visualized using Oncobox software.[15]

Pathway activation level (PAL) for each molecular pathway was calculated according to the formula:

$$PAL_p = \sum_n ARR_{np} \times \ln(CNR_n) \div \sum_n |ARR_n|,$$

where PAL_p - molecular pathway p activation level; CNR_n (case-to-normal ratio) - ratio of protein n product concentrations in the test sample and in the norm (average value in the control group); ln – natural logarithm; discrete value ARR_{np} (activator/repressor role) for protein n in pathway p is defined as follows:

$$ARR_{np} = \begin{cases} -1; \text{ protein product } n \text{ is repressor of pathway } p \\ -0.5; \text{ protein product n is rather repressor of pathway } p \\ 0; \text{ activator/repressor role of protein product } n \text{ in pathway } p \text{ is unclear or unknown} \\ 0.5; \text{ protein product } n \textit{ is} \text{ rather activator of pathway } p \\ 1; \text{ protein product } n \text{ is activator of pathway } p \end{cases}$$

Visualization of the molecular pathways was performed using R "ggplot2" and "VennDiagram" packages. 1000 random permutations were made in order to test significance of overlaps for top deregulated proteins or molecular pathways.

Protein set enrichment analysis was performed as previously described.[16] Top 10% of up- or

down-regulated proteins were analyzed using "GSEAPreranked" module of the software using the following gene sets databases: c2.cp.kegg.v6.2.symbols.gmt, c2.cp.reactome.v6.2.symbols.gmt, c5.all.v6.2.symbols.gmt.

Animal experiments

Animal experiments were approved by the Ministry of Agriculture, Forestry and Food of the Republic of Slovenia No. 34401-15/2017/8 based on the approval of the National Ethics Committee for Experiments on Laboratory Animals, and were in compliance with the standards required by the EU Directive 2010/63/EU for animal experiments. Female NUDE (HSD: Athymic Nude-Foxn1^{NU}, Envigo RMS Srl, San Pietro al Natisone, Italy) mice were maintained on a 12 h light–dark schedule under specific pathogen-free conditions at constant room temperature and humidity. Food and water were provided *ad libitum*. In order to estimate the tumorigenic capacities of the investigated cells, parental and INV breast carcinoma cells were injected at a concentration of 1×10^6 in 0.1 ml NaCl subcutaneously for the induction of subcutaneous tumors in 6 weeks old NUDE mice (6 animals per group). Tumor formation was monitored every day until tumors became palpable. Afterward tumors were measured every second day using Vernier Caliper in three perpendicular diameters (a, b, c) and tumor volumes were calculated according to formula $V = (\pi \times a \times b \times c)/6$. The tumor doubling times were calculated as the time in which tumor reaches its double volume; *i.e.* from 40 mm^3 to 80 mm^3. When tumors reached 250 mm^3 or significant palpable axillary or inguinal lymph nodes were detected, animals were sacrificed and autopsied. Lungs, liver, kidney, intestine, colon, ovarium, spleen, lymph nodes were visually inspected for macrometastases. Tumors and axillary and inguinal lymph nodes were excised for histological analysis. The tumors and lymph nodes were fixed in IHC zinc fixative (BD Biosciences, San Diego, CA, USA), embedded to paraffin blocks and cut into three consecutive 2-μm-thick sections. The first section of tumor and lymph nodes was H&E stained to evaluate the morphology. The second section of tumor was stained with Masson`s trichrome in order to determine collagen. To determine cell proliferation, we incubated the third tumor section with a monoclonal mouse anti-human antibody against Ki-67 (1:200; clone MIB-1 M7240; DAKO Agilent technologies inc., Denmark). The sections were stained on an automated slide stainer with an indirect, biotin-free system (Optiview DAB IHC Detection kits, 760-700; Ventana Medical Systems, Roche, USA). The other two sections of lymph nodes were stained with a monoclonal rabbit anti-human E-cadherin antibody (1:2500, ab40772, Abcam) and a polyclonal rabbit anti-human pan-cytokeratin antibody (1:3000, ab217916, Abcam). These primary antibodies were then detected with a peroxidase-conjugated streptavidin–biotin secondary antibody (Rabbit specific HRP/DAB detection IHC kit, ab64261, Abcam) and counterstained with hematoxylin. Ten images of each immunohistochemically stained sections were captured with a DP72 CCD camera (Olympus; Hamburg, Germany) connected to a BX-51 microscope (Olympus, Hamburg, Germany) under the 200x or 600x magnification. The number of Ki67-positive cells and the area of collagen were determined on the acquired images using CellSens Dimensions software (Olympus, Hamburg, Germany).

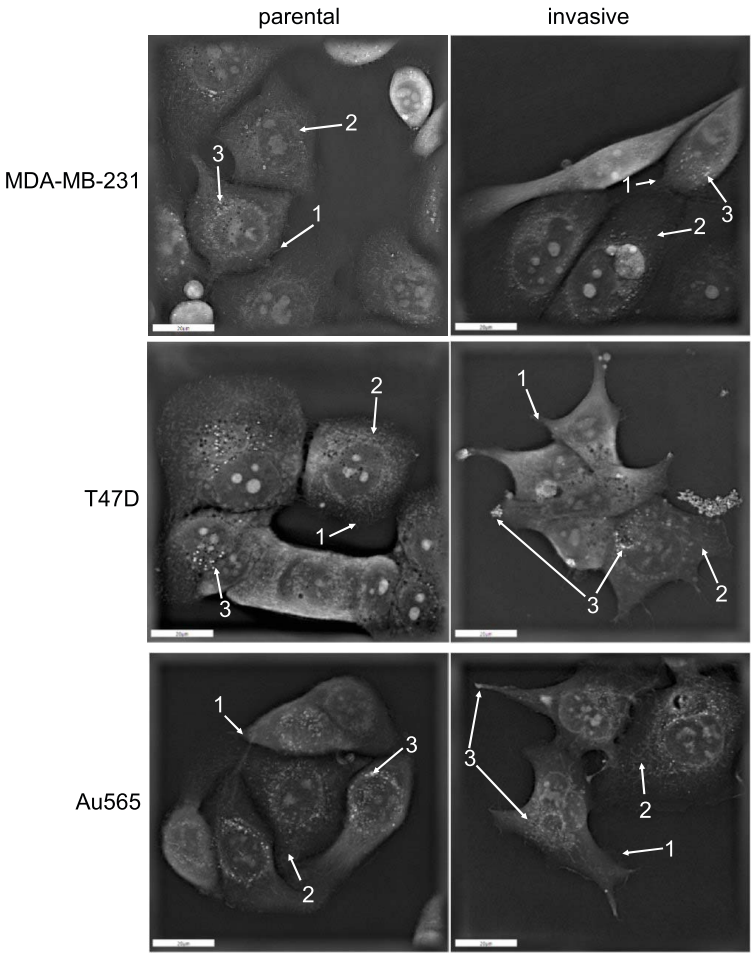

FIGURE 1. Holographic microscopy of breast cancer cells. Parental and invasive MDA-MB-231, T47D, and Au565 cells were analysed for their morphology using 3D tomographic microscope as described in Materials and Methods. Arrow 1: filopodia; Arrow 2: mitochondria; Arrow 3: lipid droplets.

Results

Cellular morphology

As can be seen in Figure 1, INV breast carcinoma cells with enhanced invasive abilities have markedly changed their morphological phenotype toward the formation of fibroblast-like and spindle-shaped cells. INV carcinoma cells increased their cellular surfaces due to membrane ruffling accompanied by lamellipodia and filopodia formation. Independently from the breast cancer subtype, all INV cells were characterized by more pronounced mitochondria fission compared to parental breast carcinoma cells. Interesting to note that all INV cells were characterized by the lower number of lipid droplets in comparison with their parental counterparts. Additionally, T47D-INV and Au565-INV cells were characterized by the lipid droplets translocation to the filopodia tips, whereas a perinuclear lipid droplet localization was detected in parental cells only.

Migratory and invasive abilities of the investigated breast carcinoma cells

To determine whether newly obtained breast carcinoma INV cells possess affected invasive and migratory abilities, appropriate assays were performed.

The investigated cells were evaluated for their migratory abilities through the uncoated 8-μm pore size polycarbonate membrane (Figure 2A). Among the parental MDA-MB-231, T47D, and Au565 breast carcinoma cells, triple-negative MDA-MB-231 and Her2-positive Au565 cells showed the more pronounced cell migration than hormone-dependent T47D cells. Thus, 901.7 + 109.4 MDA-MB-231 cells and 1089.7 + 360.8 Au565 cells migrated toward the RPMI 1640 medium containing attractant (10% FCS), whereas parental T47D cells demonstrated 176,3 + 54.4 migrated cells. All newly obtained MDA-MB-231-INV, T47D-INV Au565-INV invasive breast carcinoma cells were more migratory than their parental counterparts (5308.3 + 1513.5, 1261.7 + 776.6 and 19864.0 + 7830.6 migrated MDA-MB-231-INV, T47D-INV and Au565-INV cells, respectively).

As it was detected, all INV breast carcinoma cells demonstrated an enhancement of their capacities to invade through the collagen 1- and laminin-coated membranes (Figure 2B). Triple-negative MDA-MB-231-INV and hormone-dependent T47D-INV cells showed a ~ 2.38- and ~ 1.72-fold and ~ 1.8- and ~ 2.67-fold enhancement of invasiveness over that in parental cells through the collagen 1- and laminin-coated membranes, respectively. Invasive capacities of Her2-positive Au565-INV breast carcinoma cells through the collagen 1- and laminin-based matrix was estimated as a ~4.29- and ~13.85-fold higher than in their parental counterparts Au565 cells, respectively. Among all investigated INV

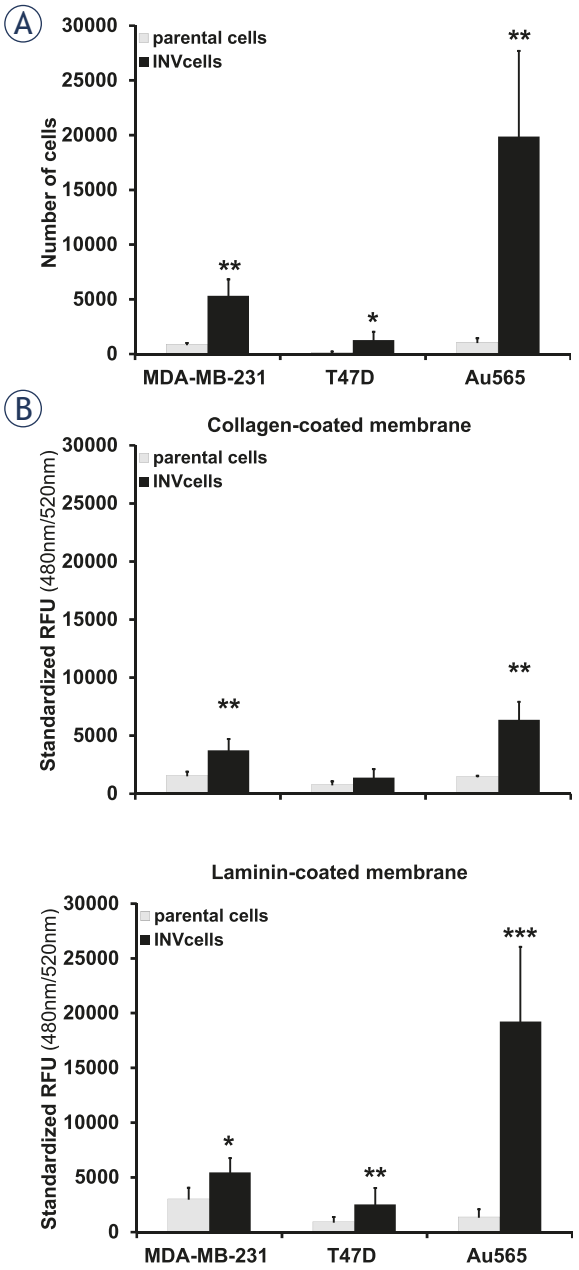

FIGURE 2. Breast cancer cell migration and invasion. **(A)** Differences in migration of parental and INV breast carcinoma cells. **(B)** Invasion of parental and invasive breast carcinoma cells through the collagen- and laminin coated membranes. Columns represent the mean value including standard deviation obtained from three independent experiments (*p < 0.05; **p < 0.01; ***p < 0.001).

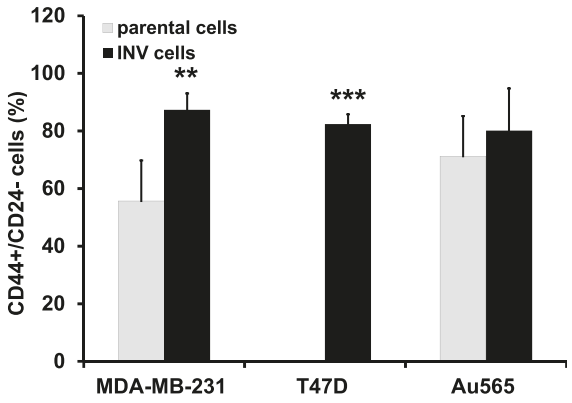

FIGURE 3. Expression of CD44+/CD24- cancer stem cell markers in breast carcinoma cells. Columns represent the mean value including standard deviation obtained from three independent experiments (**p < 0.01; ***p < 0.001).

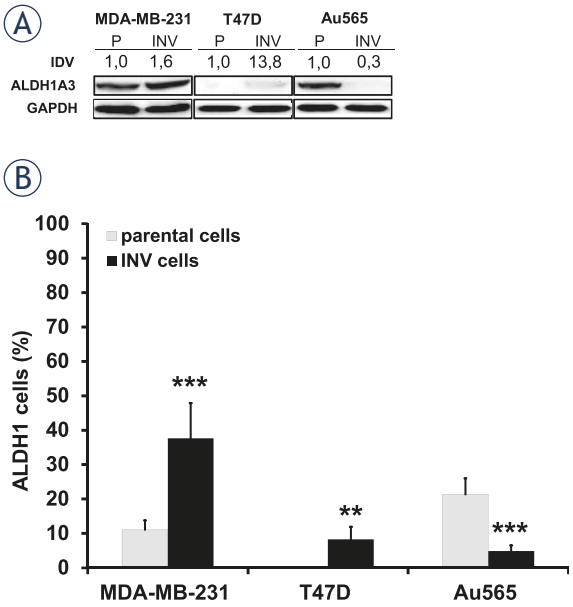

FIGURE 4. ALDH1 expression and activity in breast cancer cells. **(A)** Exponentially growing parental and INV breast carcinoma cells were collected for Western blot analysis. Total protein extracts were prepared from the cells and then processed for immunoblotting using antibodies to detect ALDH1A3. GAPDH was used as a loading control. IDV was determined as described in the section Materials and Methods. **(B)** ALDH1 activities were determined in the investigated parental and INV cells. Columns represent the mean value including standard deviation obtained from three independent experiments (**p < 0.01; ***p < 0.001).

cells, hormone-positive T47D-INV cells revealed the lowest invasive potential compared to triple-negative MDA-MB-231-INV and Her2-positive Au565-INV breast carcinoma cells. Thus, the invasiveness of T47D-INV cells through the collagen 1-coated membranes was ~2.7- and ~4.6-fold lower than of MDA-MB-231-INV and Au565-INV, respectively. Similarly, T47D-INV cells were ~2.2-fold less invasive through the laminin-coated membrane than MDA-MB-231-INV cells. In contrast, T47D-INV cells demonstrated ~7.6-fold lower invasiveness through the laminin-coated membrane than Au565-INV breast carcinoma cells.

Breast cancer stem cell (CSC) markers CD24, CD44 and ALDH1

Since an enhancement of the invasive abilities of carcinoma cells could be associated with altered expression of CD24 and CD44 markers[17,18], we have evaluated their expressions in the INV breast cancer cells in comparison with parental cells (Figure 3). It was found that only MDA-MB-231-INV and T47D-INV cells changed their phenotype toward the loss CD44-/CD24+ subpopulation and increased number of $CD44^{+}/CD24^{-/low}$ cells. Thus, ~ 90% and ~ 80% of MDA-MB-231-INV and T47D-INV cells possessed CD44+/CD24- phenotype, whereas parental MDA-MB-231 showed CD44+/CD24 expression in ~ 50% cells and parental T47D cell line did not contain CD44+/CD24- cells. Interesting to note that Her2-positive Au565-INV cells did not markedly differ in CD44+/CD24- expression from their parental counterparts (~70% in parental Au565 versus ~ 80% in Au565-INV cells).

It is known that enhanced ALDH1 expression and activity can be associated with breast carcinoma cells aggressiveness, invasiveness and metastasis.[19] Therefore, we next determined the ALDH1A1 and ALDH1A3 expressions and ALDH1 activity in all investigated breast carcinoma cells (Figure 4). Although ALDH1A1 was expressed neither in parental nor in INV breast carcinoma cells (data not shown), ALDH1A3 was differently presented in parental and INV cells (Figure 4A).

Parental MDA-MB-231 and Au565 cells demonstrated equal ALDH1A3 expression, whereas parental T47D possessed very weak ALDH1A3 expression. Both invasive MDA-MB-231-INV and T47D-INV cells revealed an upregulation of ALDH1A3. In contrast, Au565-INV cells showed significant downregulation of this protein. ALDH1 activity was detected in parental MDA-MB-231 and Au565 cells only (11.13 + 2.65 % and 21.42 + 4.59 %, respectively) (Figure 4B), whereas parental T47D cell line did not contain cells with ALDH1 activity. Similarly to ALDH1A3 expression, ALDH1 activity was increased in MDA-MB-231-INV and T47D-INV cells (37.70 + 10.2 % and 8.31 + 3.62 %,

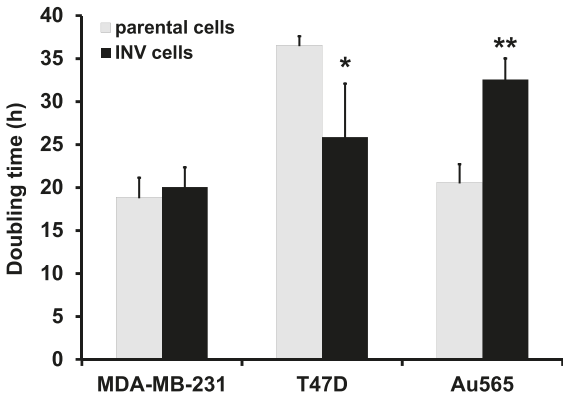

FIGURE 5. Cell proliferation rate in breast carcinoma cells. Parental and INV cells were evaluated for their doubling time as described in the Materials and Methods. For statistical evaluation, mean values and SD were calculated using at least three independent experiments; significance was determined by paired Student's *t*-test (*p < 0.05; **p < 0.01).

respectively). Again, Au565-INV cells showed a repression of the ALDH1 activity (4.92 + 1.61 %) compared to parental Au565 cells.

Cell proliferation

To estimate the proliferation rates of the parental (MDA-MB-231, T47D, Au565) and INV cells (MDA-MB-231-INV, T47D-INV, Au565-INV), cell growth was evaluated and the doubling time of each investigated cell line was determined (Figure 5). Hormone-dependent T47D cells had the slowest proliferation capacities with a doubling time of 36.6 + 1.8 hours. MDA-MB-231 and Au565 breast carcinoma cells revealed shorter doubling times of 18.9 + 3.9 hours and 20.6 + 3.7 hours, respectively. Although invasive triple-negative MDA-MB-231-INV cells did not demonstrate the significant differences with their parental counterparts, hormone receptor-positive T47D-INV and Her2-positive Au565-INV cells showed an affected doubling time (~ 1.5-fold shorter time for T47D-INV and ~1.6-fold longer doubling time for Au565-INV compared to their parental counterparts).

Protein expression analysis and molecular pathway activation

To investigate molecular features linked to breast cancer cells invasiveness, we intersected top 10% differentially regulated proteins in all three cell lines, up- and downregulated proteins were intersected separately. Only six proteins were common for top upregulated ones in all the cell lines

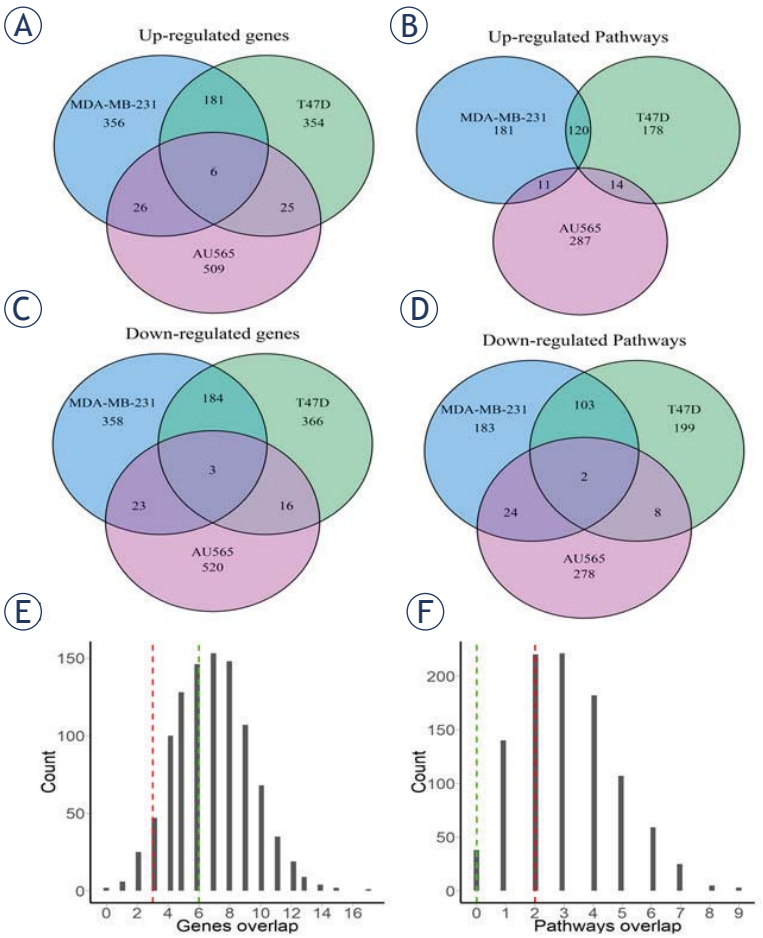

FIGURE 6. Bioinformatic analysis of proteins and pathways dysregulated in the INV compared to parental breast carcinoma cells. Overlap of up-regulated **(A, B)** or down-regulated **(C, D)** genes **(A, C)** and molecular pathways **(B, D)** between the investigated INV breast carcinoma cells. Top 10% features (proteins or pathways) were selected for the analysis. **(E, F)** Distribution of overlapped proteins for randomly selected groups (1000 random permutations).

(Figure 6A), and three – for downregulated proteins (Figure 6C).

We then tested whether this intersection is statistically significant by intersecting three random sets (576 out of 5755 proteins) 1000 times, the resulting histogram is shown in Figure 6E. The observed real intersection of top differential proteins didn't exceed values obtained during random permutations, thus suggesting that the intersections observed may be random. This suggested also that the differential protein profiles were strongly different between the cell lines investigated.

Similarly, we calculated activation levels of 3118 molecular pathways using Oncobox algorithm that quantitatively estimates activation of molecular pathways.[10,20] Pathway activation level (PAL) values were calculated for three cell lines tested in

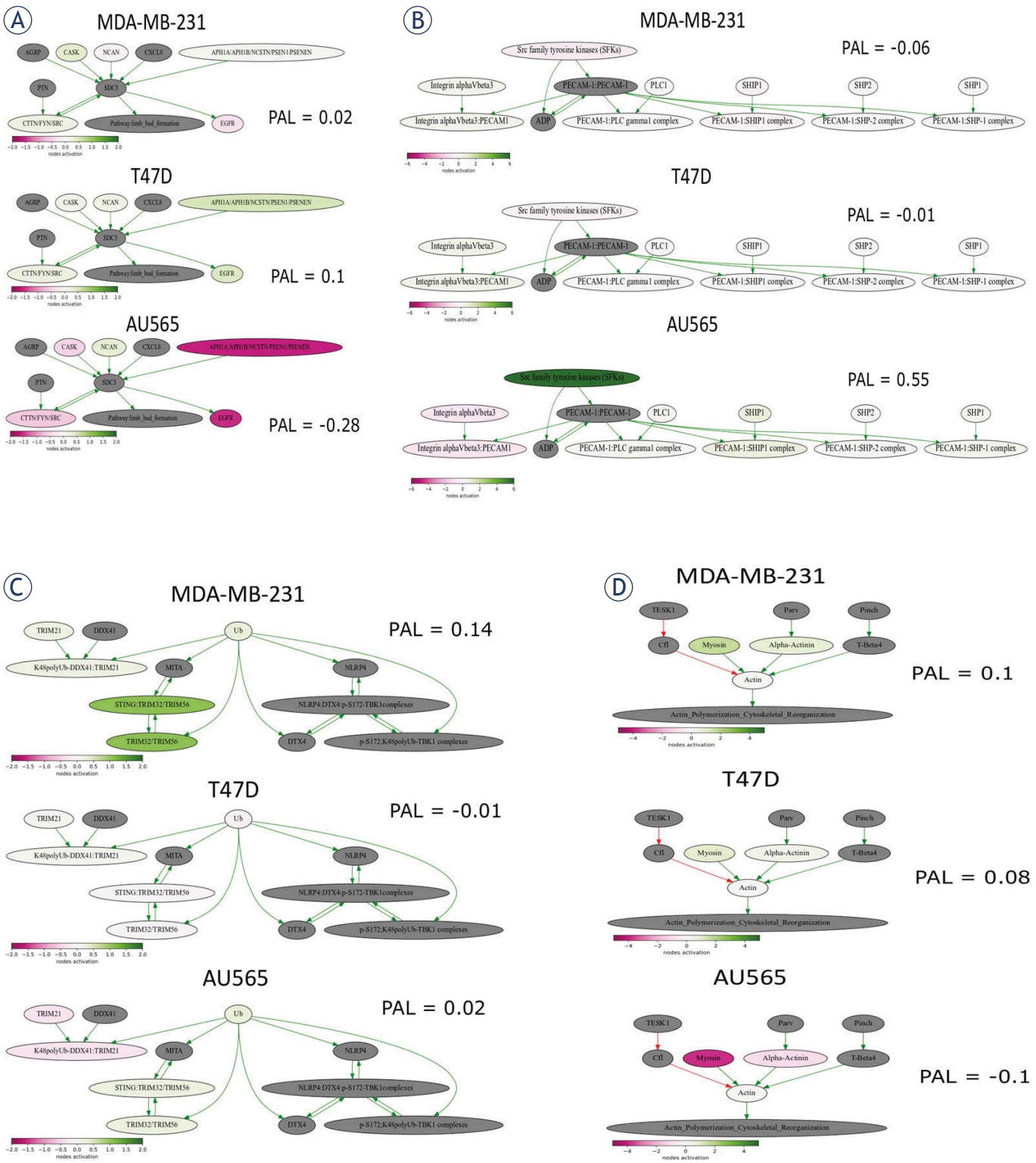

FIGURE 7. Visualization of pathways involved in breast cancer cell invasiveness. **(A)** "NCI Syndecan 3 mediated signaling events Main Pathway" **(B)** "PECAM1_interactions_Main_Pathway"; **(C)** "reactome Regulation of innate immune responses to cytosolic DNA Main Pathway"; **(D)** "ILK Signaling Pathway Actin Polymerization Cytoskeletal Reorganization". The pathway is shown as an interacting network, where green arrows indicate activation, red arrows indicate inhibition. Color depth of each node of the network corresponds to the logarithms of the case-to-normal (CNR) expression rate for each node, where "normal" is expression level in the control group, the scale represents extent of up/downregulation.

TABLE 1. Proliferation abilities and collagen content in breast tumor xenografts

Tumor cell line	% of proliferating cells (mean ±se)	% of collagen (mean ±se)
MDA-MB-231	46.1 ± 3.1	18.7 ± 2.0
MDA-MB-231-INV	69.8 ± 4.7*	22.9 ± 3.5
T47D	43.3 ± 2.3	3.3 ± 0.7
T47D-INV	22.9 ± 2.4***	21.6 ±1 .9***
Au565	24.3 ± 1.7	32.3 ± 1.8
Au565-INV	57.7 ± 3.5***	6.1 ± 1.4***

* p < 0.5; *** P < 0.001

comparison with the control samples using quantitative proteomics expression data (supplementary Table 1). Positive values of PAL score indicate down-regulation of a pathway, and vice versa, negative values mean downregulation of a pathway.[21] We then separately intersected top 10% of up- and downregulated pathways and found no common upregulated pathways in the three cell lines tested (Figure 6B), whereas two pathways were found to be strongly downregulated in all three cell lines (Figure 6D). As in the above protein intersection significance test, we intersected three random sets of pathways (312 out of 3118) for 1000 times, obtained histogram is shown on Figure 6F. We found that the real intersection of top deregulated pathways between the cell lines tested didn't exceed values for these random permutations. Again, this suggested that the intersections observed may be random and that the differential pathway profiles shared little identity for the cell lines investigated.

In order to study the mechanisms of different cell line invasiveness, we focused on top differentially regulated pathways specific for each cell line. For this analysis we selected only pathways with >9 genes and used the following criteria: the pathway should be up-regulated in one cell line but down-regulated or at least 5-fold less activated in other lines. The most activated and satisfying this requirement pathway in T47D-INV cell line was "NCI Syndecan 3 mediated signaling events Main Pathway" (Figure 7A).

It was not up-regulated in MDA-MB-231-INV but instead was down-regulated in AU565-INV cells. Syndecans are membrane proteins controlling cell proliferation, differentiation, adhesion and migration [22] and were recently shown to promote breast cancer metastasis.[23] Thus, invasiveness of T47D-INV cells may potentially be linked with activation of this pathway.

The top up-regulated pathway in AU565-INV cells was "PECAM1_interactions_Main_Pathway" (Figure 7B), but it was not upregulated in MDA-MB-231-INV and T47D-INV cells. This pathway appeared to be activated mostly due to over-expression of Src family tyrosine kinases (SFKs). These kinases are known to be deregulated in many cancer types and their inhibitors were tested in clinical trials.[24] Indeed, inhibiting Src was previously associated with inhibited breast cancer cell migration and invasion and therefore may be linked with Au565-INV cell invasiveness in our case.[25]

The most up-regulated pathway in MDA-MB-231-INV cell line was "reactome Regulation of innate immune responses to cytosolic DNA Main Pathway" (Figure 7C), mostly due to over-expression of TRIM32. TRIM32 expression was previously found to be up-regulated in breast cancer tissues and cell lines, promoting cell proliferation and colony formation.[26] High TRIM32 expression was also positively associated with lymph node metastasis.[26]

As MDA-MB-231-INV and T47D-INV cells had much more common up-regulated pathways, when compared to AU565-INV, we also studied which pathway is most up-regulated in both cell lines. It appeared that top pathway was "ILK Signaling Pathway Actin Polymerization Cytoskeletal Reorganization" (Myosin and actin were over-expressed, Figure 7D), which indicates that cytoskeletal re-arrangements may be common in these cell lines.

In addition we performed gene set enrichment analysis (GSEA) for top 10% of up- or down-regulated proteins. It appeared that, there no common gene sets at nominal p-value cut-off of 0.05 for up-regulated genes (Figure S1A), and 1 common gene set for down-regulated genes (Figure S1B), namely „GO_Neurological_system_process". Detailed results of GSEA can be found supplementary Table S2.

In vivo

Next, we have evaluated the tumorigenic capacities of INV cells compared to their parental counterparts. It was observed that tumors originated from the INV cells demonstrated significantly delayed growth rates. Thus, parental and invasive Her2-positive Au565 had the slowest growth with tumor doubling times of 11.4 ± 1.6 days and 19.7 ± 0.9 days, respectively. The tumor doubling times of MDA-MD-231 and MDA-MB-231-INV tumors were 2.9 ± 0.2 and 10.7 ± 1.0 days, respectively.

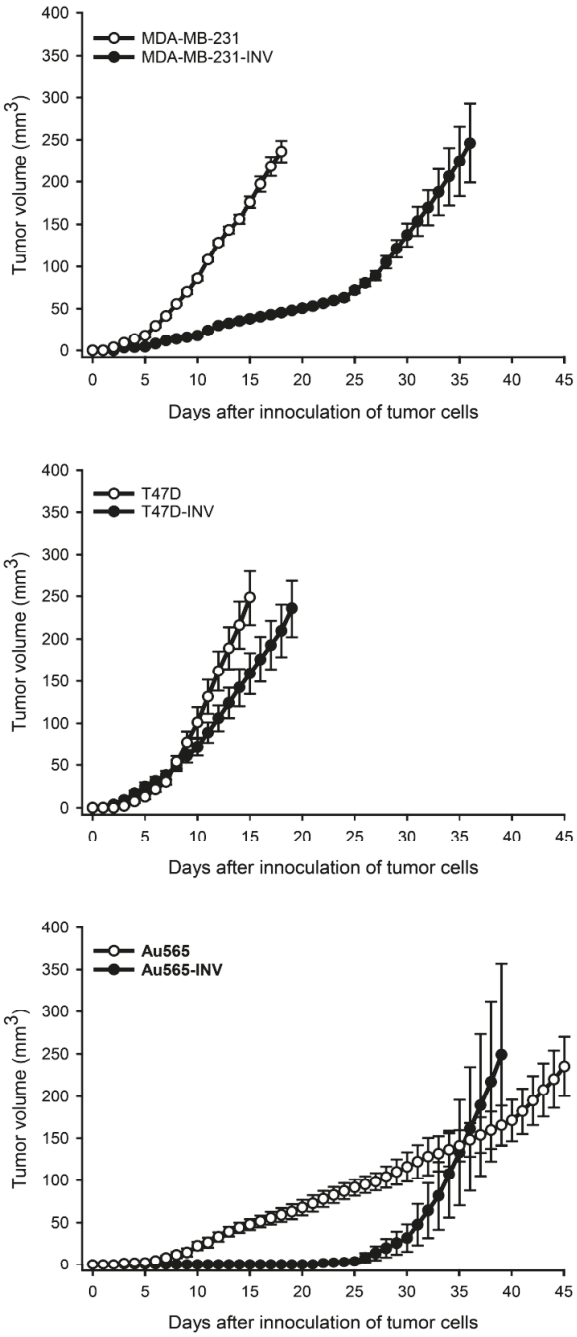

FIGURE 8. Tumorigenic abilities of parental and INV breast cancer cells. Tumor growth curves of breast cancer xenografts, parental (MDA-MB-231, T47D, Au565,) and INV counterparts (MDA-MB-231-INV, T47D-INV, Au565-INV) are received as descibed in the section Materials and Methods.

Surprisingly, hormone receptor-positive T47D and T47D-INV tumors revealed the fastest growth rates compared to other tumor xenografts, with a tumor doubling times of 1.5 ± 0.3 and 3.1 ± 0.4 days, respectively (Figure 8).

Histological analysis of the investigated xenografts has shown that both parental and invasive T47D tumors were more differentiated (presence of nests) compared to parental and invasive Au565 and MDA-MB-565 tumors (Figure 9). Although MDA-MB-231-INV and Au565-INV xenografts were markedly delayed in their initial growth, they have demonstrated an exponential increase of tumor volume after day 25, and their proliferative potential was up to 70% (Figure 9, 10A, Table 1).

In contrast, in T47D-INV tumors demonstrated a lower number of proliferating cells (~ 20%) and increased desmoplasia compared to parental T47D tumors (Figure 9, 10B; Table 1). There was an opposite observation in parental and invasive Au565 xenografts: ~24% versus ~57% of proliferating parental Au565 and invasive Au565-INV cells, respectively; ~32% versus ~6% of stromal compartment with collagen in parental and invasive Au565 tumor, respectively (Figure 9,10; Table 1).

In addition, the lymph nodes corresponding to the invasive MDA-MB-231, T47D, and Au565 xenografts were markedly but not significantly increased in sizes and weight compared to their parental counterparts (Figure 11). Histological analysis has shown that all investigated lymph nodes from both parental and invasive xenografts revealed medullar sinus histiocytosis and plasmacytosis (Figure 12). However, they were more pronounced in the lymph nodes obtained from animals bearing the tumors originated from INV breast carcinoma cells. Among the parental breast carcinoma xenografts, only Au565 tumors have given a growth of microscopic metastasis in the lymph node parenchyma in 8% of all investigated lymph nodes, whereas parental MDA-MB-231 and T47D tumors were not characterized with any suspicious lymph node metastasis. In contrast, growth of all tumors originated from the MDA-MB-231-INV, T47D-INV, and Au565-INV was accompanied by appearance of carcinoma cells in lymph nodes. Thus, few individual MDA-MB-231-INV, T47D-INV, and Au565-INV tumor cells were found in the subcapsular sinus in 17%, 8%, and 8% of all analyzed lymph nodes, preferentially inguinal, respectively.

Discussion

Breast cancer metastases are still a challenge in oncology and one of the main causes of cancer-related deaths in women. Carcinoma cells entering the metastatic cascade should reveal an increased

invasive potential to pass a number of biological barriers to reach the target organ. It seems that invasive abilities of carcinoma cells are required during the whole process of metastatic spread of carcinoma cells. Although there is a number of reports discussing an involvement of a variety of the molecules and pathways in cell invasiveness resulting in metastasis development, only limited data on the global molecular patterns in metastatic breast cancer cells are available.[4,27] Furthermore, the majority of publications indicate that initiation of metastasis is uniformly regulated independently from the molecular subtypes of breast cancer. However, these reports demonstrated an up-regulation of the known metastasis-related pathways (EMT markers, TGF, VEGF, IL6, etc.) which were detected in the metastatic lesions.[1,4,23,28] In our opinion, these pathways are the final "downstream" events in the chain of molecular perturbations resulting in metastasis development. Unfortunately, it is still not fully elucidated which molecules and pathways can be considered as initiators of metastatic cascade in breast cancer. Furthermore, it is still unclear, whether pathways-initiators are common for all breast cancer subtypes.

Hence, the main aim of our study was to determine how an enhancement of invasive and metastatic patterns in breast carcinoma cells is regulated in different breast cancer subtypes. It was assumed that breast cancer cell invasiveness should be accompanied by the acquiring of similar cellular morphology and molecular phenotype. Indeed, the newly obtained cells have demonstrated equal morphology which is specific for carcinoma cells possessing the augmented invasive and migratory capacities. Spindle-like form of the INV cells with more pronounced filopodia formation, diminished number of lipid droplets and their translocation to the filopodia tips result in cell abilities to pass the biological barriers and use the energy from the lipid droplets.[29,30] As it was expected, all three INV cell lines belonging to different subtypes of breast cancer showed the enhanced migratory and invasive abilities. Although the parental breast carcinoma cells had equal invasive and migratory properties, and the procedure to obtain new cell lines with increased invasiveness was common for all investigated cells, Her2-positive Au565-INV cells revealed the most pronounced augmentation of the capacities to invade and migrate through the collagen- and laminin-coated membranes compared to other INV cells. Our experimental findings fully correlate with the clinical data describing the higher frequencies of positive lymph nodes in patients

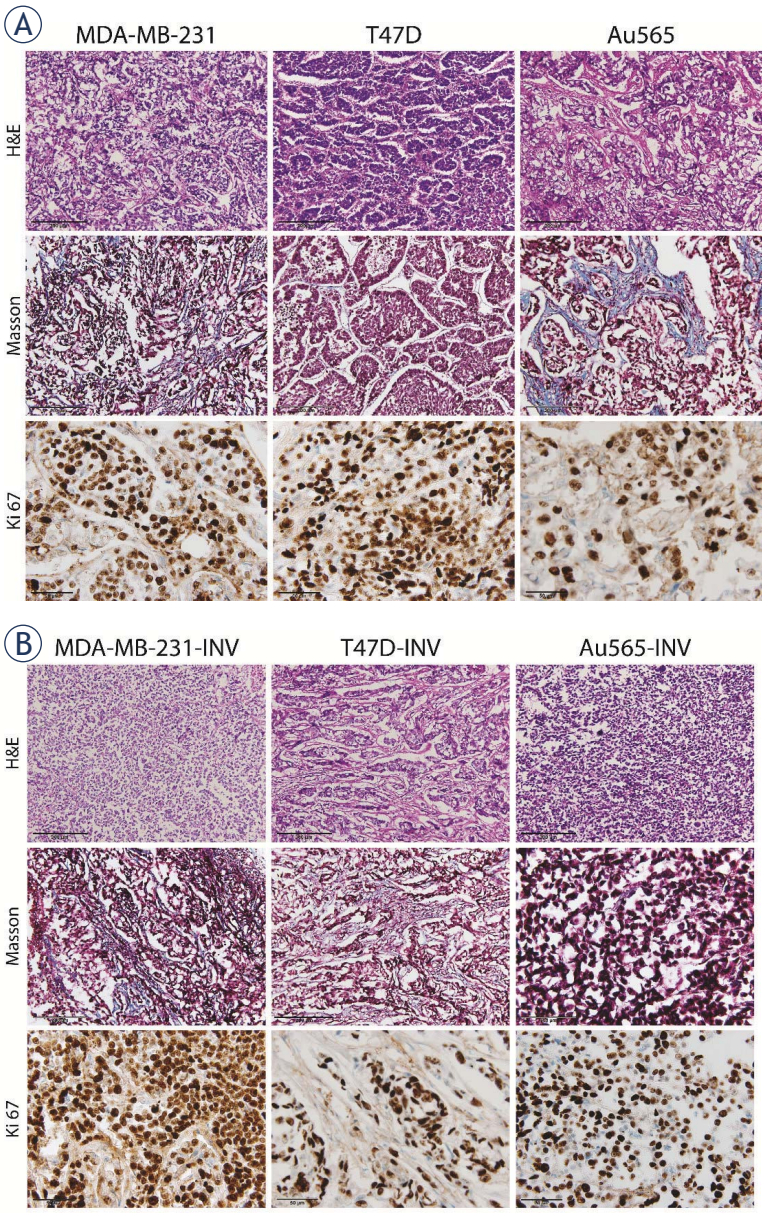

FIGURE 9. Representative histological images of breast cancer xenografts. Tumors originating from parental **(A)** and invasive **(B)** MDA-MB-231, T47D and Au565 breast carcinoma cells were stained with H&E for evaluation of tumor morphology (200× magnification). Collagen (blue) was stained using Masson`s staining (200× magnification). Ki-67 proliferative tumor cells are stained brown (600× magnification).

with Her2-positive tumors compared to triple-negative and hormone receptor-positive ones.[31] Furthermore, Her2-positive breast cancers have a strong correlation with the margin positivity after operation.[32] We suppose that Her2-positive cells with increased invasiveness can be found in the surgical margins due to enhanced capabilities to invade the surrounding tissues and migrate markedly more actively compared to the cells of other morphological subtypes of breast cancer. These

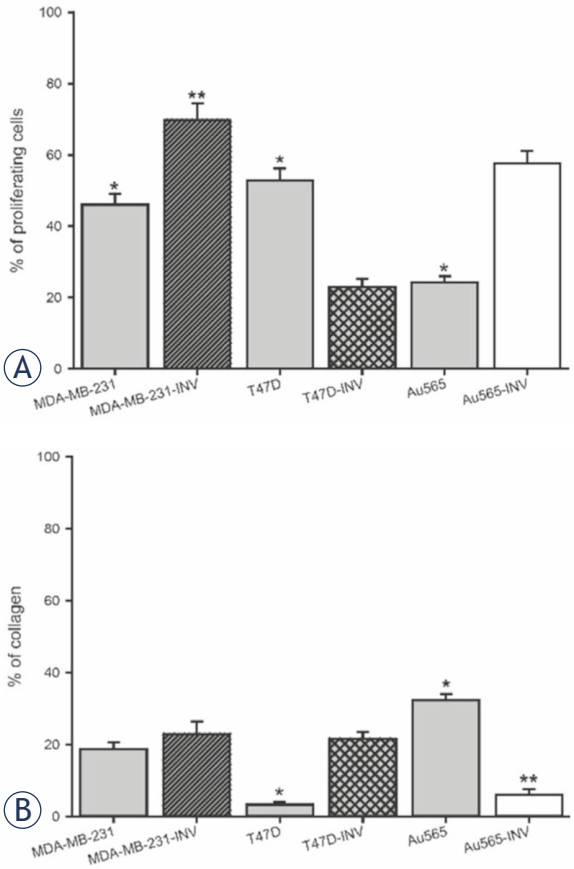

FIGURE 10. Histological analysis of parental and INV breast xenografts. Tumors originated from the parental (MDA-MB-231, T47D, Au565) and INV breast carcinoma cells (MDA-MB-231-INV, T47D-INV, Au565-INV) were evalueated for desmoplasia (intratumoral collagen content) and percentage of proliferative cells (Ki67 expression) (*p < 0.05; **p < 0.01; ***p < 0.001).

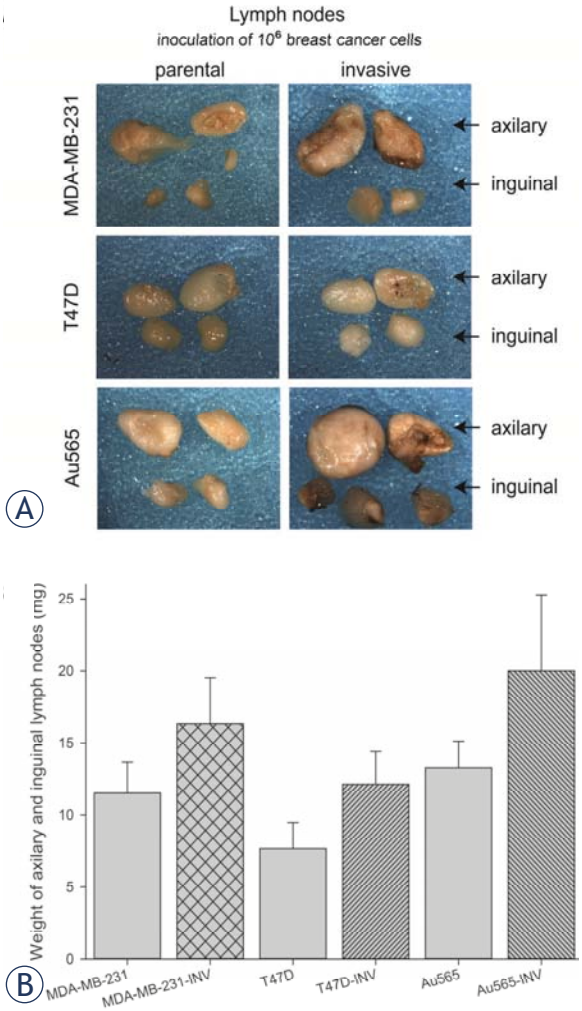

FIGURE 11. Lymph nodes of mice bearing breast cancer xenografts originated from parental (MDA-MB-231, T47D, Au565) and INV counterparts (Au565-INV, MDA-MB-231-INV, T47D-INV). **(A)** Representative images of macromorphology of the lymph nodes; **(B)** Cumulative weight of lymph nodes (AM+SE of 6 mice per group).

differences in invasion and migration allowed us to assume that other molecular properties of invasive carcinoma cells belonging to different subtypes of breast cancer are also distinct.

It is assumed that tumors containing carcinoma cells with stemness features and mesenchymal phenotype demonstrate a higher probability for metastatic progression.[17-19,28,33] Again, all investigated INV breast carcinoma cells have shown different enrichment for cells carrying stemness markers CD44+/CD24- and ALDH1. Despite an increased content of carcinoma cells with stemness capabilities, the tumorigenic capacities of all INV cells were comparable. Therefore, it is possible to conclude that neither CD44+/CD2- nor ALDH1 are the determinants for tumorigenic capacities of the investigated cells. However, the delayed tumor growth of the INV xenografts could be explained by a higher number of the injected CD44+/CD24-/ALDH1+ cells that are affected in their proliferation and can be dormant compared to the parental xenografts.[17,34-37] We would like to emphasize that the balance between CD44, CD24, and ALDH1 expression was differently affected in INV cells compared to the parental cells belonging to different subtypes of breast cancer. In accordance with the report by Sulaiman *et al.*[38], MDA-MB-231-INV and T47D-INV cells acquired a hybrid stemness phenotype, and Au565-INV cells switched their phenotype from hybrid to mesenchymal one. Differences in the acquired molecular phenotypes of INV cells were not limited to the observed distinctions in the invasiveness, migratory and stemness abilities, it was also found that INV cells not equally mobilized the molecular pathways potentially in-

volved in the metastasis development. Taking into account the bioinformatic analysis of the proteomic data of the investigated INV cells, we can conclude that hormone receptor-positive T47D-INVcells with increased invasiveness acquire the molecular characteristics of triple-negative breast cancer cells, whereas Her2-positive Au565-INV cells specifically changed their own molecular phenotype with very limited partaking in the involved pathways found in the MDA-MB-231-INV and T47D-INV cells. These data demonstrate that triple-negative, hormone receptor-positive and Her2-positive breast carcinoma cells realize their invasive potential through activation or repression of different molecular pathways. To our knowledge, there are no reports on the differences in molecular patterns underlying an enhancement of breast cancer cell invasiveness depending on the type of breast cancer. Undoubtedly, further detailed investigation of the molecular pathways involved in breast cancer cell invasiveness is required and clinically important.

Taking together, we conclude that breast carcinoma cells having the same cellular morphology accompanied by the increased invasiveness and migratory capacities, reveal an activation of distinct pathways associated with increased metastatic properties. These pathways should be further elucidated to develop the biomarkers and potential therapeutic targets to predict, prevent or combat the metastatic spread of carcinoma cells belonging to different subtypes of breast cancer. Since hormone receptor-positive invasive cells share their molecular properties with triple-negative breast cancer cells, we assume that these types of metastatic disease can be treated rather equally with an option to add anti-hormonal agents. In contrast, Her2-positive metastasis should be carefully evaluated for more effective therapeutic approaches which are distinct from the triple-negative and hormone-positive metastatic cancers.

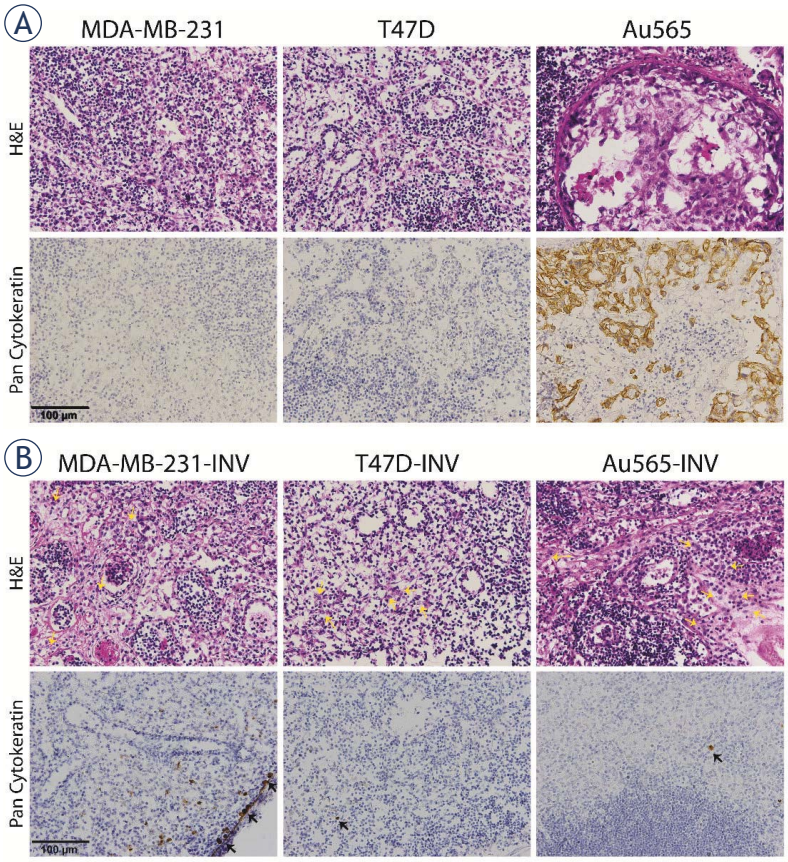

FIGURE 12. Histology of lymph nodes of mice bearing breast cancer xenografts. **(A)** HE staining in lymph nodes corresponding to the parental MDA-MB-231, T47D, Au565 and **(B)** invasive MDA-MB-231-INV, T47D-INV, Au565-INV xenografts. Representative images of lymph nodes taken under 400 × magnification. Brown staining represent pan-cytokeratin positive tumor cells in lymph node.

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research article

Retrospective analysis of treatment-naive Slovenian patients with metastatic melanoma treated with pembrolizumab – real-world experience

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Background. Based on recent data from clinical trials, the immune checkpoint inhibitor pembrolizumab prolongs survival and has a good toxicity profile in patients with advanced or metastatic melanoma. However, the question remains whether these results are transmitted into daily clinical practice. The aim of this study was to assess the efficacy and toxicity of pembrolizumab in treatment-naive patients with metastatic melanoma in everyday clinical practice in Slovenia and compare it to the results from clinical trials.

Patients and methods. This observational retrospective cohort study included 138 consecutive metastatic treatment-naive melanoma patients treated with pembrolizumab at the Institute of Oncology Ljubljana in Slovenia, from January 2016 to December 2018. Patient and treatment characteristics were retrospectively collected from hospital data base. Statistical data was obtained using the SPSS software version 22. Survival rate was calculated with the Kaplan-Meier method. Observation period took place between January 2016 and the end of June 2019.

Results. The estimated median overall survival (OS) was 25.1 months (95% CI, 14.6–35.6) and the median progression-free survival (PFS) was 10.7 months (95% CI, 5.9–15.4). Among all patients, 29 (21.0%) achieved complete response, 31 (22.5%) partial response and 23 (16.7%) reached stable disease. The number of organs with metastatic involvement and the level of baseline lactate dehydrogenase (LDH) concentration had significant influence on survival rates. Immune-related adverse events (irAE) were reported in 88 (63%) patients, while grade 3–4 irAE occurred in 12 (8.7%). Due to toxicity, 16 (11.6%) patients discontinued the treatment.

Conclusions. Our real-world data from single centre retrospective analysis of treatment-naive metastatic melanoma patients treated with pembrolizumab showed inferior median OS and similar median PFS, compared to the results from clinical trials. However, patients with normal serum levels of LDH and a small number of organs with metastatic involvement had comparable survival outcomes. Toxicity rates of pembrolizumab were quite similar. These results further support the use of pembrolizumab for metastatic treatment-naive melanoma patients.

Key words: immunotherapy; pembrolizumab; metastatic melanoma; treatment-naive

Introduction

The annual incidence of malignant melanoma is still rising steadily; in Europe it varies between 3 to 5 people per 100.000 in Mediterranean countries and 12 to 35 people per 100.000 in Nordic countries.[1] As for Slovenia, the average annual melanoma incidence rate is estimated to increase to 34 men and 26 women per 100.000 (95% prediction interval) for the year 2019. That makes Slovenia one

of the European countries with the highest annual incidence of malignant melanoma. Approximately 78% of Slovenian patients with melanoma initially present with localized disease, 19% with regional disease and 3% with distant metastatic disease.[2] All Slovenian melanoma patients in stage III and IV are treated with systemic treatment at the Institute of Oncology Ljubljana.

Historically, patients with advanced melanoma had a median overall survival of around 8 months, with a 5 year overall survival of less than 10%.[3] New treatment options, such as immunotherapy and targeted therapy are changing the landscape for these patients. Programmed cell death 1 (PD-1) blockade is now a standard of care for all advanced and metastatic melanoma patients in the first-line setting.[1] A recent publication about the 5-year outcomes from a randomised, phase 3 trial Keynote-006 of pembrolizumab for ipilimumab-naive advanced or metastatic melanoma patients, showed a median overall survival (OS) of 38.7 months (95% CI, 27.3–50.7 months), median progression-free survival (PFS) of 11.6 months (95% CI, 8.2–16.4), 5 year OS rate of 43.2% and 46% (95% CI, 41.0–51.4) objective response rate in an analysis of a subgroup of patients who received first-line treatment. They also showed a good toxicity profile, with grade 3–4 immune-related adverse events (irAE) reported by 17% of patients treated with pembrolizumab mono-immunotherapy.[4]

In melanoma patients treated with immunotherapy or targeted treatment, it was shown that serum lactate dehydrogenase (LDH) and the number of organs with metastatic involvement have the strongest predictive value for clinical outcome and for durable benefit.[5,6] These factors were not presented in recently published papers on patients treated with pembrolizumab.[4,7,8]

However, it is still unclear whether these remarkable results are also obtained in daily clinical practice. In this paper, we aim to assess the efficacy and the toxicity of pembrolizumab in treatment-naive patients with metastatic melanoma in daily clinical practice and compare these parameters to those reported in clinical studies.

Patients and methods

We conducted an observational retrospective cohort study analyzing 138 consecutive treatment-naive patients with metastatic melanoma, who received pembrolizumab at the Institute of Oncology Ljubljana between January 2016 and December 2018. Patients received pembrolizumab in two different dosages: either 2 mg per kilogram of body weight every 3 weeks or a flat dose of 200 mg every 3 weeks (flat dose since May 2018). Patients with prior systemic therapy and patients treated with a combination of PD-1/ cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors were excluded from the analysis. The period of data collection took place from January 2016 to July 2019.

All relevant data was collected from medical files and entered into a data base. Baseline data was analysed with regard to age, anatomic site of primary melanoma, actionable mutation, baseline serum LDH, number of organs with metastatic involvement and metastatic stage (M1a(0/1)-d(0/1), determined by using 8th version of the American Joint Committee on Cancer (AJCC) tumour, node, metastases (TNM) classification system).[9,10] Efficacy was evaluated according to the response evaluation criteria in solid tumours (RECIST, version 1.1) by using computed tomography (CT) scan, positron emission tomography using 18F-fluorodeoxyglucose (FDG-PET), magnetic resonance scans (MRI), clinical examination and laboratory tests.[11] Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE), version 5.0.[12]

Statistical data was obtained using the SPSS software version 22 and survival rate was calculated by Kaplan-Meier method and compared using log-rank tests.

The study was approved by the Institutional Review Board Committee (Approval number: ERIDEK-0084/2019) and was conducted in accordance with the ethical standards defined by the Declaration of Helsinki. The study was conducted with acknowledgement and consent of the subjects. Prior to treatment, patients have signed an informed consent for treatment and a consent allowing the usage of their data for scientific purposes.

Results

Patients and treatment

Demographic and disease characteristics are detailed in Table 1. All patients were Caucasians. The median age was 65.4 years (range 25–87), the majority of patients (60.9%) were males and in ECOG performance status 1 (51.4%). Among all patients, 116 (84.1%) had cutaneous subtype of melanoma. Twenty-five (18.1%) patients had BRAF V600 mutation, 21 (84%) of which had normal baseline lactate

TABLE 1. Demographic and disease characteristics of the patients

Median age (range) – years	65.4 (25-87)
Older than 70 years – n (%)	47 (48.9%)
Male gender – n (%)	84 (60.9)
Average body weight (range) – kilograms	79.5 (46 – 138)
ECOG performance status – n (%)	
0	53 (38.4)
1	71 (51.4)
2	12 (8.7)
3	2 (1.4)
Anatomic site of primary	
Cutaneous	116 (84.1)
Ocular	8 (5.8)
Mucosal	7 (5.1)
Unknown primary	7 (5.1)
Actionable mutation – n (%)	
Wild type	94 (68.1)
BRAF V600E	22 (15.9)
BRAF V600K/M	3 (2.2)
NRAS	3 (2.2)
Not provided	16 (11.6)
Elevated baseline LDH level (> 4.31 microkat/L) – n (%)	36 (26.1)
Elevated baseline S100 level (> 0.105 microg/L) – n (%)	72 (52.2)
Metastatic stage – n (%)*	
M1a (0)	28 (20.3)
M1a (1)	6 (4.3)
M1b (0)	29 (21.0)
M1b (1)	2 (1.4)
M1c (0)	32 (23.2)
M1c (1)	22 (15.9)
M1d (0)	13 (9.4)
M1d (1)	6 (4.3)
Organs with metastatic involvement – n (%)	
1	47 (34.1)
2	52 (37.7)
3	19 (13.8)
>3	20 (14.5)
Further lines of systemic therapy – n (%)	41 (29.7)
Radiotherapy during immunotherapy – n (%)	38 (27.5)

LDH = lactate dehydrogenase

*Following the 8th edition of the American Joint Committee on Cancer (AJCC) tumour, node, metastases (TNM) classification, to cases with normal level of the LDH are given the suffix (0) and to cases with elevated LDH level suffix (1).

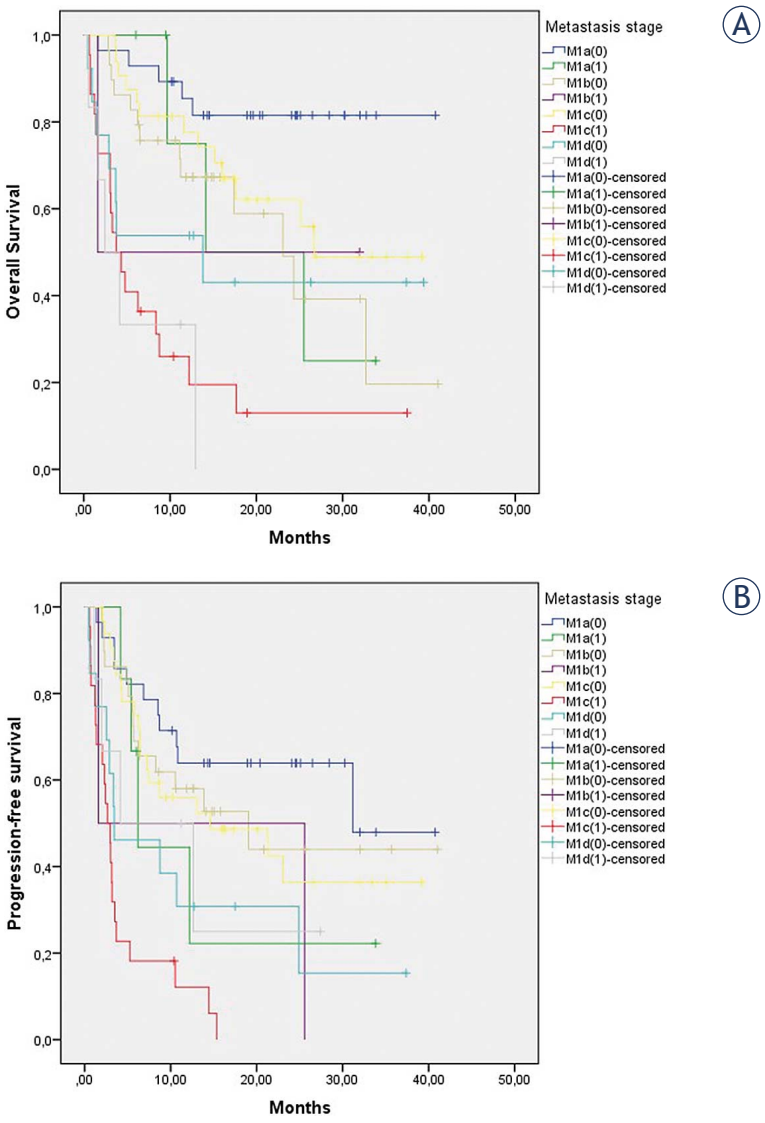

FIGURE 1. Kaplan-Meier estimates for overall survival **(A)** and progression free survival **(B)** according to different metastatic stages ($p < 0.001$).

dehydrogenase (LDH). Serum LDH was elevated in 26.1% of all melanoma patients. The majority – 52 (37.7%) patients – had two organs with metastatic involvement.

Median duration of exposure to pembrolizumab was 6.7 months. (range: 1 day – 36 months). At the time of data cut-off, 38 (27.5%) patients were still receiving pembrolizumab, others discontinued due to progressive disease (PD; n = 78; 56.5%), immune-related adverse events (n = 17; 12.3%) or physician decision (n = 5, 3.6%). Only two (1.4%) patients were retreated with pembrolizumab.

There were five (3.6%) patients with underlying autoimmune or inflammatory disease (AID), two of which had psoriasis, one had psoriatic arthritis,

one suffered from sarcoidosis and one from chronic inflammatory bowel disease. Only the patient with psoriatic arthritis had a flare of his AID.

Most of the patients (n=118, 85.5%) received pembrolizumab per kilogram of body weight and only a minority (n=20, 14.5%) received a flat dose of 200 mg.

Efficacy

At data cut-off, 65 (47.1%) patients died. Estimated median OS was 25.1 months (95% CI, 14.7–35.6) and median PFS was 10.7 months (95% CI, 5.9–15.5) for all patients. In Figure 1, median OS and PFS according to different metastatic stages are shown.

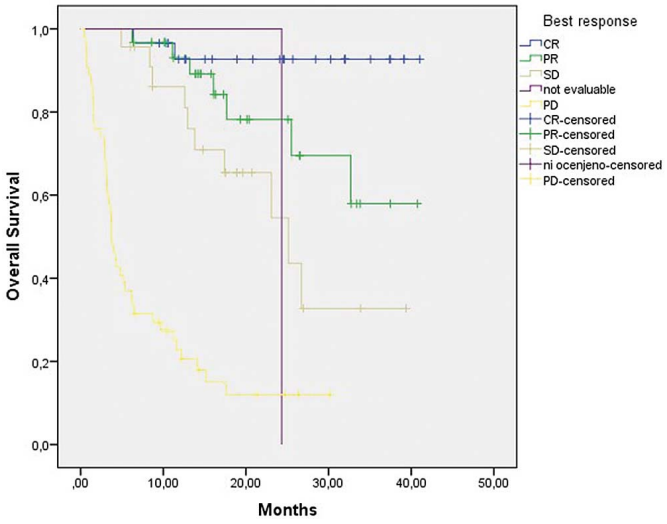

FIGURE 2. Kaplan-Meier estimates of overall survival according to best overall response ($p < 0.001$).

TABLE 2. Best overall responses and median overall survival for each group

Response	n (%)
ORR	60 (43.5)
DCR	83 (60.2)
Best response	
CR	29 (21.0)
PR	31 (22.5)
SD	23 (16.7)
PD	54 (39.1)
No assessment	1 (0.7)

CR = complete response; DCR = disease control rate; ORR = overall response rate; PD = progressive disease; PR = partial response; SD = stable disease

Among all of the 138 treated patients, 29 (21.0%) achieved complete response (CR), 31 (22.5%) achieved partial response (PR) and 23 (16.7%) achieved stable disease (SD). In total, 54 (39.1%) patients experienced a progression disease (PD). In survival rate analysis, median OS in patients with CR in PR was not yet reached, while median OS in patients with SD was 25.1 months (95% CI, 15.1–35.2) and in patients with PD was 3.8 months (95% CI, 3.1–4.4) (Table 2, Figure 2).

A survival rate analysis according to age (older or younger than 70 years), anatomic site of primary melanoma, actionable mutations, baseline LDH level and the number of organs with metastatic involvement was carried out. Older age, different anatomic sites of the primary melanoma and BRAF mutation were not associated with lower survival rates.

The differences in survival rates according to anatomical site were not statistically significant (p=0.071). Namely, patients with cutaneous melanoma had an estimated medium OS of 32.7 months (95% CI non-estimable). Patients with ocular melanoma had medium OS of 11.6 months (95% CI, 2.8–21.4) and patients with mucosal melanoma 4.4 months (95% CI, 2.8–5.9).

However, the survival rate differences were statistically significant (p=0.04) according to the number of organs with metastatic involvement. The median OS for patients with one organ site containing metastases was not reached, while patients with two organs involved had the median OS of 23.1 months (95% CI, 14.4–31.6), patients with three organs involved had 17.7 months (95% CI non-estimable), and patients with more than 3 organs with metastatic involvement had 8.8 months (95% CI, 1.3–16.2) (Figure 3). Similarly, the differences in survival rate according to the baseline levels of LDH were statistically significant (Figure 4, $p < 0.001$). The estimated median OS for patients with normal baseline LDH level was 32.7 months (95% CI non-estimable), whereas for patients with elevated baseline LDH the median OS was 4.8 (95% CI, 0.0. –11.2).

Responses for patients with BRAF mutations were analysed in more details, out of all 25 (18.1%) patients, 7 (28%) achieved CR, 4 (16%) achieved PR and 5 (20%) patients SD, while 9 (36%) patients progressed.

As for the subsequent lines of therapy, 41 (29.7%) patients received it. Most of them, namely 35 (85.4%) patients received chemotherapy, 5 (12.2%) received targeted therapy with BRAF and MEK inhibitors and only 1 (2.4%) patient received CTLA-4 inhibitor ipilimumab monotherapy.

Toxicity

Table 3 presents the reported immune-related adverse events (irAE). They occurred in 88 (63%) patients, 12 (8.7) patients experienced grade 3 to 4 irAE and 16 (11.6%) patients permanently discontinued treatment due to irAE. There were no treatment-related deaths known from the data base.

The most common treatment-related adverse events of any grade were elevation in liver transaminase levels (25.4%), hypothyroidism (23.9%) and pruritus (20.3%). Grade 3 to 4 events that were reported in more than 1% of the patients were elevation in liver transaminase levels (2.2%), arthralgia (1.4%) and pneumonitis (1.4%). There were a few cases of rare irAE. One patient developed limbic encephalitis, another adrenal insufficiency and two had documented nephritis.

TABLE 3. Immune related adverse events

Adverse event*	Any grade – no. (%)	Grade 3-4 – no. (%)
Any	88 (63.8)	12 (8.7)
High AST, ALT	35 (25.4)	3 (2.2)
Hypothyroidism	33 (23.9)	0
Pruritus	28 (20.3)	0
Rash	25 (18.1)	1 (0.7)
Arthralgia	14 (10)	2 (1.4)
Diarrhoea	13 (9.4)	1 (0.7)
Fatigue	8 (5.8)	0
Pneumonitis	7 (5.1)	2 (1.4)
Vitiligo	7 (5.1)	0
Other	12 (8.7)	4 (2.9)

AST = aspartate transaminase; ALT = alanine aminotransferase

*Events are listed in order of descending frequency.

Discussion

Results from this one-country, single centre retrospective analysis showed inferior median OS, similar median PFS and comparable ORR for the whole group of melanoma patients receiving pembrolizumab in first line setting, compared to reported data from clinical studies.

There are more possible reasons for these results. Firstly, medium follow-up in our retrospective analysis was shorter in comparison to published clinical trials. Secondly, the characteristics of our patients differ from those in the clinical trials. Only patients with metastatic disease were included in our analysis. No patient in our research had an advanced or non-metastatic operable melanoma, unlike the Keynote-006 trial, where 3.2% of patients were without distant metastasis (M0). With regard to ECOG performance status, our patients were mainly in ECOG performance status 1, but some of them were also in performance status 2 or 3, probably due to comorbidities or higher tumour burden. There were 19 (13.8%) patients with brain metastases, some of them even had symptomatic brain metastases, which was an exclusion criteria in the Keynote-006 study. We know that patients with active brain metastases not only have a detrimental survival due to their disease, but also require systemic glucocorticoids.[13] That condition was shown to be associated with inferior outcomes for treatment with programmed cell death ligand 1 (PD-L1) blockade as corticosteroids play an important role in feedback inhibition of inflammatory response and immune system homeostasis.[14]

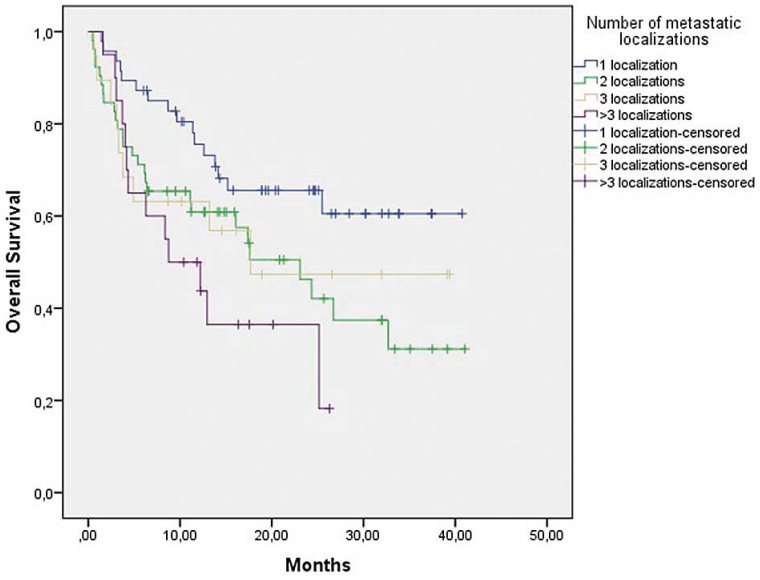

FIGURE 3. Kaplan-Meier estimates of overall survival according to number of organs with metastatic involvement (p = 0.04).

A few patients with ocular and mucosal subtype of melanoma were included in our analysis, which also differs from the Keynote-006 trial, where ocular melanoma patients were excluded.[15] However, these patients were included in the Keynote-001 trial.[7] These two subgroups of melanoma patients usually have worse results and they rarely confer durable remissions with immunotherapy. In patients with metastatic ocular melanoma treated

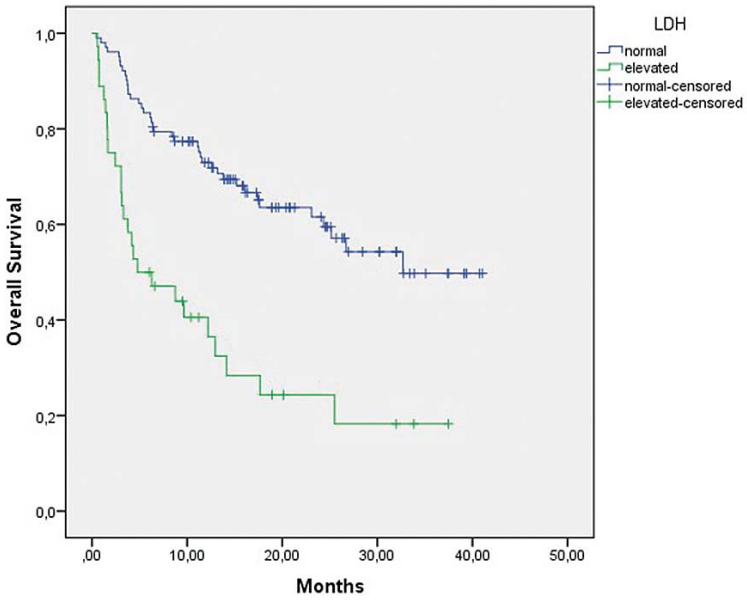

FIGURE 4. Kaplan-Meier estimates of overall survival according to serum lactate dehydrogenase (LDH) ($p < 0.001$).

with PD-(L)1 antibodies, including pembrolizumab, a disease control rate (DCR) of 12.5% and OS of 7.6 months was reported in a retrospective series.[16] As for the mucosal melanoma ipilimumab-naive patients, a post-hoc analysis of Keynote-001, 002 and 006 showed an ORR of 22% and a median OS of 14.0 months.[17] Furthermore, in our analysis these patients performed worse compared to cutaneous melanoma. Ocular melanoma patients had a medium OS of 11.6 (95% CI, 2.8–21.4) and mucosal melanoma patients had a medium OS of 4.4 months (95% CI, 2.8–5.9). The difference was not statistically significant, probably due to a low number of patients with ocular or mucosal primary melanoma.

However, for certain groups of patients, the results are outstanding. Patients with one organ with metastatic involvement and patients with stage M1a(0) had the best survival rate, with median overall survival not reached at the data cut-off time. Patients with normal serum levels of LDH also had a significantly better survival rate than those with elevated levels. These results confirm that the number of organs with metastatic involvement and the level of serum LDH are important prognostic factors.[5,6]

Furthermore, ORR of 43.5% was comparable to ORR in Keynote-006 and Keynote-001, where it amounted to 46% (95% CI, 41.0–51.4) and 52% (95% CI, 43–60) respectively in the first line treatment. In these studies, some unique patterns of response were reported. One of them is pseudoprogression, which is described as a radiological progression that is followed by stabilization or response on the next imaging.[18] In Keynote-001 trial, the incidence of tumour pseudoprogression was 7.3%.[19] In our analysis, we did not collect data on this atypical response, therefore its influence on the response rate cannot be established.

Actionable mutations BRAF and NRAS were present in only 28 (20.3%) patients. Additionally, quite a significant number of patients (n=16, 11.6%) did not receive molecular testing for actionable mutations. The percentage is higher than reported in the clinical trials. For example, in the Keynote-006 trial only 1% of patients had undetermined BRAF status. In our analysis, these were patients with contraindications for the targeted therapy, with ocular primary melanoma, where a BRAF mutation is very rare, and patients where testing was not possible due to lack of tumour tissue. At present, liquid biopsy is not performed at our institute.

A comparison between patients with BRAF mutated melanoma and wild type melanoma showed no statistical differences in median OS, PFS and ORR. Due to a low number of patients with BRAF mutation (n=25, 18.1%), the conclusions are highly questionable. However, looking at the patients' characteristics, most of them (84%) had normal LDH concentration, probably reflecting less aggressive disease and/or metastatic burden and making them the more suitable for immunotherapy. The type of the first line therapy in our BRAF mutated patients depended entirely on the physician's decision. Currently, there are several prospective trials evaluating the best first line approach for the patients with BRAF mutated tumours: immunotherapy, targeted therapy or switching from the latter to the former after certain time. For the time being, only exploratory analysis have shown that immunotherapy might result in a better survival rate after one year of treatment.[20] The patients with tumours threatening important organs or functions and those with high tumour burden and rapid progression are advised to start with targeted therapy, which provides faster responses.

As for older patients, the survival rate was not significantly different from that of younger ones. That gives us another confirmation that we can safely treat older patients with mono-immunotherapy.[20]

Subsequent lines of systemic treatment of metastatic melanoma patients are not evidence-based at this time.[1] A combination of BRAF and MEK in-

hibitors is a good therapeutic option in the second line of systemic treatment only for patients with actionable mutation BRAF that have been treated with immunotherapy in the first line. In our group of patients, 5 (12.2%) of the patients who received second line therapy also received targeted therapy afterwards. The majority of other patients were treated with chemotherapy.

The toxicity profile according to our retrospective analysis is, as reported in clinical trials, very good. Only 8.7% of patients experienced grade 3 to 4 irAE and, more importantly, there were no treatment-related deaths. The incidence of irAE could be underestimated due to retrospective design and unfamiliarity of clinicians with irAE at the beginning of using PD-1 treatment. We had limited experience with CTLA4 antibody ipilimumab usage. Available literature was of great help, as first position papers were published online very soon.[22] With more literature becoming available and with our increasing clinical experience, we learned to recognize irAE and to treat them more effectively.[23-25] This also stands for patients with underlying AID, which we now know is not a contraindication for treatment with immunotherapy.[26-28] Five (3.6%) patients from our group had AID and only one had a transient exacerbation of his autoimmune condition.

Another important question is the financial toxicity. The average body weight of our patients was 80 kg, meaning the average dose per kilogram was 160 mg. With a flat dose regimen we actually spent more money on the treatment than we did with a dose per kilogram. The financial difference between these two regimens is substantial, especially for a country with limited resources, such as Slovenia. Due to a low number of patients that were treated with a flat dose in our study, the comparison regarding the efficacy was not possible. We need prospective data to validate different doses of treatment, which could potentially lead to much wider access to these drugs.[29] This highly effective treatment should stay affordable for countries such as ours, so we should continue searching for more optimal treatments with this medicine. The time spent on a treatment is also an important factor. An optimal duration of treatment has not been established yet, but data shows that patients in complete remission after being treated for more than 6 months have a low risk of relapse after discontinuation. This is not true for patients in partial response or those with stable disease, where the risk is higher. The optimal duration of treatment needs further prospective studies.[30-32]

This study contains some limitations. Firstly, the retrospective design of the study results in the lack of some important or interesting data. For example, the testing on PD-L1 expression was not performed, as it is not part of standard practice. Its clinical use in melanoma patients is limited at the time being, because the treatment with checkpoint inhibitors is effective regardless of the state of PD-L1.[33] Second important limitation of our analysis is a short follow-up time compared to recent publications, which reported 5-year outcomes. Our future perspective is to update the data, especially regarding the survival rate and the responses to treatment. We hope to see the same ongoing antitumor activity of pembrolizumab as it was seen in all randomized clinical trials with this drug.[4,7] Another important limitation that could have impacted our results is the radiological evaluation using RECIST criteria, instead of immune RECIST (iRECIST).[34]

Nivolumab is another PD-1 inhibitor that is indicated for treatment of advanced or metastatic treatment-naive melanoma patients.[35] In January 2016, when PD-1 inhibitor pembrolizumab started to be used for melanoma patients in Slovenia, this was the only PD-1 inhibitor that was reimbursed by medical insurance. Even when nivolumab was first reimbursed in June 2018, pembrolizumab continued to be used in this setting, due to less frequent applications of pembrolizumab at that time (every three weeks for pembrolizumab vs. every two weeks for nivolumab). Just recently, in October 2019, a combination of nivolumab with ipilimumab was first reimbursed, which presents another treatment option for this group of patients.[36]

Lastly, in Slovenia there is still a lot of space for improvement in the area of melanoma systemic treatment. The priorities should be including our patients in clinical trials and a better organisation of supportive facilities. The lack of focus on these priorities is possibly reflected in data showing an increase in the mortality-to-incidence ratios in Eastern European countries compared to Western Europe.[37]

Conclusions

The results from our retrospective analysis of treatment-naive patients with metastatic melanoma treated with PD-1 inhibitor pembrolizumab showed inferior median OS and similar median PFS and ORR compared to reported data from clinical studies. However, the patients with normal serum levels of LDH and a small number of organs

with metastatic involvement had comparable survival outcomes. The treatment resulted in a low toxicity rate and no treatment-related deaths. A lot still needs to be done in melanoma patient community so that the patients with bad prognostic factors can also achieve higher survival rates. This type of retrospective analysis gives us an insight into real-life patient care and represents an important contribution for oncological community and, most importantly, enables a better care for our patients.

Acknowledgements

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research article

Effect of radiotherapy on coronary arteries and heart in breast-conserving surgery: a dosimetric analysis

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Background. There are certain risks of radiotherapy (RT), especially patients with left-sided breast cancer have a higher tendency to develop cardiac complications than the right-sided cancers. This study aims to perform a dosimetric analysis the effect of RT on coronary arteries and heart in breast-conserving surgery.

Patients and methods. A total of 40 patients with early stage right and left-sided breast carcinomas (T1/T2 + N0) were randomly selected. RT was delivered to the entire breast, and tumor beds were boosted in these patients using tangential fields with computed tomography based planning. The doses for Left anterior descending coronary artery (LAD), left circumflex coronary artery (LCx), right ventricle (RV), left ventricle (LV), and heart were recorded and median values compared between groups.

Results. The highest mean of radiation dose in patients with left-sided breast cancer was to LAD 2402.48 ± 838.39 cGy, while the highest mean dose in right-sided breast cancer patients was to RV 130.18 ± 24.92. The highest maximum dose of radiotherapy was applied to heart at left-sided breast cancer patients as well as at right-sides prients. The mean V5 of the LV was 18.68% (6.89–31.69), mean V25 of the LV was 5.22% (0.45–16.54), mean V5 in bilateral ventricles was 23.73% (2.56–26.89), and mean V25 in bilateral ventricles 6.78% (0.63–13.63).

Conclusions. Especially in left-sided breast cancer, the most direct and best strategy to reduce and protect radiation-induced cardiac injury is to balance dose constraints between several high-dose regions of cardiac substructures and the mean heart dose.

Key words: breast cancer; radiation therapy; heart; coronary arteries

Introduction

Breast cancer is the most common cancer in women (excluding skin cancers) and is the second most common cause of death due to cancer.[1] The average risk of developing breast cancer in women is 12%. Age is the most important risk factor for breast cancer development. In addition, gender, a previous history of breast cancer or benign breast diseases, family history, race, menstrual history (early menarche and late menopause), first gestational age, no history of breastfeeding, alcohol consumption, diet rich in fat and calories, use of oral contraceptives, and postmenopausal hormone replacement therapy are the other risk factors for the occurrence of breast cancer.

Surgery is the main treatment modality. While surgery is performed in the early stage, locally advanced cases are operated after neoadjuvant chemotherapy. Surgery is performed as mastectomy (radical mastectomy, modified radical mastectomy, or total mastectomy) or breast-sparing surgery (wide excision, quadrantectomy, or lumpectomy). Adjuvant radiotherapy (RT) is administered to all the patients who underwent breast-sparing surgical therapy for breast cancer and those with 4 or more positive lymph nodes who underwent mastectomy.[2]

Long-term survival-related toxicities have become important to be studied, as longer-term survival rates have been achieved by the improvements in diagnosis and treatment of breast cancers. RT reduces the local recurrence rate of breast cancer[3], however, it is not clear that the survival advantage offered in addition to surgical treatment is not related to systemic therapy. Studies have shown the survival benefit in high-risk patients with RT.[4] However, there are certain risks of RT, such as skin reactions, cosmetic problems, edema in the upper extremity, pneumonia, and the cardiac toxicity, especially important in left-sided breast RT. In particular, the left anterior descending coronary artery (LAD) receives significant radiation, being in or near the target volume.[5,6] Moreover, patients with left-sided breast cancer have a higher tendency to develop cardiac complications than the right-sided ones.[7] In this study, we aimed to determine the RT doses to the LAD, left circumflex coronary artery (LCx), right ventricle (RV), left ventricle (LV), and heart in patients who underwent right and left-sided breast conservative surgery and determine whether these doses constituted a risk for ischemic heart disease.

Patients and methods

This study was conducted with an approval from the Ethics Committee at the Health Sciences University, Dr. Sadi Konuk Education and Research Hospital (2018-254), ethically in accordance with the World Medical Association Declaration of Helsinki.

A total of 40 patients with early stage right and left-sided breast carcinomas (T1/T2 + N0, according to 8th edition of American Joint Committee on Cancer staging, 2017) were randomly selected, from January 2017 to December 2018. Patient contouring was performed by two independent radiation specialists. RT was delivered to the entire breast and tumor beds were boosted in these patients using tangential fields with computed tomography (CT) based planning. Patients' age, tumor localization, stage, chemotherapy protocol and number, RT dose, and the doses for LAD, LCx, RV, LV, and heart were recorded.

We based on the handbook and quantec to dose limits for critical organs in our clinic. Handbook recommendation for breast RT is as follows: LV and combined bilateral ventricle limits: V5 ≤ 10 % and V25 ≤ 5 %. Contralateral breast Dmax ≤ 3%, ipsilateral lung V30 < 15%, contralateral lung V5 < 15%, heart V5 < 5% for R-sided tumors, and < 40% for L-sided tumors.

Treatment

Forty patients who underwent RT for right and left-sided breast cancers at the Radiation Oncology Department were included in the study. All patients underwent breast-conserving surgery. Patients were usually treated in supine positions with customized immobilization device. As a part of radiation planning, contrast-enhanced CT scans were obtained in 3-mm slices. During simulation, each patient was immobilized, with the ipsilateral arm above her head. Radiopaque catheters were placed to delineate the breast areas and incision scar on the CT scan. Each patient's CT data was transferred to an in-house 3-dimensional treatment planning system. The clinical target volumes (CTVs) were contoured and reviewed by two radiation oncologists.

The whole breast was considered as the CTV. The opposite breast, heart, LAD, LCx, RV, and LV were contoured. For RT planning, two tangential beams were used with matched posterior border to avoid divergence. Physical wedges of 15°–30° were used. Only whole breast irradiation plans were included for plan comparison in this study. The total dose prescribed was 60 Gy, with 2.0 Gy per fraction per day (50 Gy in 2 Gy/fraction to whole breast, 10 Gy in 2 Gy/5 fractions to tumor bed). The aim of the treatment plan was to achieve at least 95% of the planning target volume receiving 47.5 Gy (95% of 50.0 Gy), and the ipsilateral lung volume receiving 20 Gy or more (V20) ⊚ 10%, while keeping the contralateral lung below a mean dose of 5 Gy. In all the patients, the breast was treated with 6-MV photon beam. Boost was applied with electron energy. Electron energy was selected to allow the 85–90% isodose line to encompass the target. Dose volume histograms (DVH) were reviewed for all the patients. Maximum, minimum, and mean doses (Dmax, Dmin, and Dmean) to heart, LAD, LCx, RV, and LV were calculated from the cumulative DVH.

Statistical analysis

The analyses were performed with the NCSS 11 (Number Cruncher Statistical System, 2017, Statistical Software) Program and MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org;

TABLE 1. Patients' characteristics

		Right-sided breast cancer n (%)	Left-sided breast cancer n (%)
Breast conservation surgery		20 (100%)	20 (100%)
Age		57.33 ± 10.21 (40–80)	61.35 ± 9.91 (42–76)
T stage	T1	15 (75%)	12 (60%)
	T2	5 (25%)	8 (40%)
N stage	N0	20 (100%)	20 (100%)
M stage	M0	20 (100%)	20 (100%)
Tumor location	inner quadrant	6 (30%)	10 (50%)
	outer quadrant	14 (70%)	10 (50%)
Tumor size	≤ 2 cm	15 (75%)	12 (60%)
	2–5 cm	5 (25%)	8 (40%)
Chemotherapy	Yes	7 (35%)	11 (55%)
	No	13 (65%)	9 (45%)
Number of chemotherapy cycles	4	4 (20%)	6 (30%)
	6	3 (15%)	5 (25%)

TABLE 2. The dosimetric parameters of left anterior descending coronary artery (LAD), left circumflex coronary artery (LCx), right ventricle (RV), left ventricle (LV) and heart depending on the side of the tumor

Dosimetric parameters		Right-sided breast cancer	Left-sided breast cancer	*P* value
LAD	D_{mean}	97.57 ± 10.26 (73.8–114)	2402.48 ± 838.39 (1020–3783)	< 0.0001
	D_{max}	111.87 ± 16.15 (78.8–145)	4752.83 ± 498.46 (3700–5703.8)	< 0.0001
	D_{min}	83.12 ± 9.31 (63.7–100.1)	222.59 ± 76.42 (95–471)	< 0.0001
LCx	D_{mean}	84.99 ± 9.09 (63.8–100.8)	170.55 ± 45.36 (85.8–260.2)	< 0.0001
	D_{max}	96.27 ± 11.33 (75.6–116.3)	203.49 ± 55.9 (112–353.9)	< 0.0001
	D_{min}	74.59 ± 8.57 (54.5–92.1)	137.63 ± 38.59 (69.7–235.5)	< 0.0001
RV	D_{mean}	130.18 ± 24.92 (110.8–224.2)	563.65 ± 221.78 (140.9–875.6)	< 0.0001
	D_{max}	464.76 ± 517.55 (221.2–2619)	4576.55±1077.66 (460.1–6149.3)	< 0.0001
	D_{min}	75.51 ± 7.81 (58.8–90.5)	103.41 ± 28.67 (49.3–184.9)	< 0.0001
LV	D_{mean}	81.71 ± 7.9 (60.9–96.5)	536.8 ± 193.24 (230–1018.1)	< 0.0001
	D_{max}	149.13 ± 43.75 (85.1–264.2)	4822.6 ± 362.4 (3964.4–5835.4)	< 0.0001
	D_{min}	65.75 ± 7.02 (47.1–80.2)	105.49 ± 26.64 (52.2–184.2)	< 0.0001
Heart	D_{mean}	120.33 ± 19.05 (97.3 – 189.2)	396.56 ± 131.73 (158.5–588.7)	< 0.0001
	D_{max}	634.48 ± 751.55 (277–3759.5)	5032.44 ± 331.02 (4134.3–5992.9)	< 0.0001

All values are given as mean + standard deviation (range).

LAD = Left anterior descending coronary artery; LCx = left circumflex coronary artery; RV = right ventricle; LV = left ventricle

2018). Frequency and percentage values were given for categorical variables. Mean, standard deviation, median, minimum, and maximum values were given for continuous variables. Distribution analysis of the continuous variables was performed with the Kolmogorov–Smirnov test. The Mann–Whitney U-test was used for the independent two groups in the variables that did not realize the normal distribution assumption. A P value < 0.05 was considered statistically significant.

Results

A total of 40 patients undergoing breast-conserving surgery for early stage breast cancer were included in the study. Twenty patients had right-sided breast cancer and 20 had a left-sided one.

In patients with right-sided breast cancer, the median age was 57 years (40–80 years). In 15 of the patients (75%), the tumor size was ≤ 2 cm (T1), and in 5 of them (25%), it was 2–5 cm (T2). In 6 of the patients (30%), the tumor was in the inner quadrant, and in 14 of them (70%), in the external quadrant (Table 1). None of the patients had lymph node involvement or distant metastasis. Moreover, 7 of the patients (35%) received chemotherapy (4 cycles in 4 patients, 6 cycles in 3 patients).

In the patients with left-sided breast cancer, the median age was 61 years (42–76 years). In 12 of the patients (60%), the tumor size was ≤ 2 cm (T1), and in 8 of them (40%), it was 2–5 cm (T2). In 10 of the patients (50%), the tumor was located in the inner quadrant, and in other 10 of them (50%), in the external quadrant. None of the patients had lymph node involvement or distant metastasis. Moreover, 11 patients (55%) were treated with chemotherapy (4 cycles in 6 patients and 6 cycles in 5 patients) (Table 1).

The doses calculated after RT planning in patients with right- and left sided breast cancer are presented in Table 2. All Dmean, Dmax and Dmin values on LAD, LCx, RV, LV and heart of the patients with left-sided breast cancer were significantly higher than the values of the patients with right-sided cancer (P < 0.0001).

The mean V5 of the LV was 18.68% (6.89–31.69). It was 10% or less in 3 patients and high in 17 patients. The mean V25 of the LV was 5.22% (0.45–16.54). The mean V5 in the bilateral ventricles was 23.73% (2.56–26.89). The mean V25 in the bilateral ventricles was 6.78% (0.63–13.63). It was ≤ 5% in 7 patients and > 5% in 13 patients.

Discussion

The most important risk factor for breast cancer development is age. General treatment modalities are surgery (radical mastectomy or breast-sparing surgery or lumpectomy), KT (neoadjuvant or adjuvant) and RT (neoadjuvant or adjuvant). Adjuvant radiotherapy for breast cancer can cause late cardiac complications. Latest update for Early Breast Cancer Trial Writers Cooperation Group demonstrates radiation therapy was associated with excessive cardiac mortality disease. However, many of the studies included in a review involved older treatment techniques, which probably delivered a higher dose to the heart than seen in modern radiotherapy clinics. The issue of cardiac morbidity and mortality after breast cancer treatment is still relevant, as demonstrated by recent publications on the topic. The heart's dose distribution is not homogeneous, and the highest doses are probably to be delivered to the anterior heart which include the LAD. This is a concern since new research suggests that arteries are particularly susceptible to radiation, and LAD is a typical site of origin for ischemic heart disease.[8] Multiple follow-up studies have shown that delivery of radiation to chest wall or breast results in delayed cardiac morbidities ranging from ischemic heart disease (IHD) to acute coronary syndromes and finally congestive cardiac failure.[9] In our clinic, we used the regimens that were routinely used in breast cancer patients and determined our cardiac dose rates, according to the dose and plan used for right and left-side breast RT, and tried to evaluate the effect of these doses on coronary disease risk.

Aznar MC *et al.*, in their study, included women in the age ranging from 36 to 76 years, with median age 58.5 years, at the time of treatment.[8] Chung *et al.*, in their study, included women with a median age of 50 (25–74) years.[10] In our study, the median age of the patients with right-sided breast cancer was 57 (40–80) years and of those with the left-sided breast cancer was 61 (42–76) years.

Long-term survival-related toxicities have become important to be studied, as long-term survival rates have been achieved by the improvements in diagnosis and treatment of breast cancer. Cardiac toxicity is especially important to be checked in left-sided breast RT. In particular, the LAD receives significant radiation, as it is in or near the target volume. Moreover, studies have claimed the cardiovascular mortality to increase after RT.[5,6] Population-based analyses have proven that high-dose exposure to heart results in a cardiac morbidity and death[11], however, no cardiac morbidity was observed if heart exposure was negligible or low for the treatment technique used.[12] In our study, we postulated that the heart entered into the field of RT due to breast cancer should be examined as well as for the doses taken by the subgroups of heart but the cardiac morbidity or the cardiovascular mortality was not examined since it was not a follow-up study.

According to the Quantitative Analyses of Normal Tissue Effects in the Clinic, National Surgical Adjuvant Breast and Bowel Project, and Radiation Therapy Oncology protocols, the heart mean dose is < 26 Gy, < 4 Gy, and < 32 Gy, respectively. The RACE collaboration reported a dose-response relationship between the heart disease risk and mean dose to the heart.[2] Compared with the women with estimated heart doses < 5 Gy, the relative risks of heart disease in women with estimated doses 5 to 14 Gy and > 15 Gy were 15% and 108% higher, respectively. Although there was a 4% increase in the risk for each 1-Gy increase in the estimated average heart dose, it is not clear whether this linear relationship exists after very low cardiac doses. The Danish Breast Cancer Cooperative Group recommends that the volume of heart receiving more than 40 Gy be kept below 5%, as well as the volume receiving more than 20 Gy be kept under 10%.[13] Although it is generally accepted that a dose of 40 Gy or more of radiation can lead to heart disease, McGale and Darby have shown evidence of an increased risk of radiation-induced heart disease at doses below 5 Gy.[14] In our study, the mean doses for heart of patients with a left-sided breast cancer was 3.97 ± 1.32 Gy, and maximum dose was 50.32 ± 3.31 Gy. For hearts of patients with a right-sided breast cancer, the mean of dose was 1.20 ± 0.19 Gy, and maximum dose was 6.34 ± 7.52 Gy, suggesting a reasonable estimated lower dose on heart compared with the heart of patients with left-sided breast cancer for the relative risk of heart.

In one study, clinically significant perfusion defects after RT were not found when the mean heart rate was kept below < 5 Gy, using 3-dimensional conformal radiation therapy (3D-CRT) or intensity-modulated radiation therapy techniques, and precise measurements of cardiac function. In addition, there was no correlation between heart perfusion or function of low doses, according to the dose ratios of the heart. Although the long-term clinical significance of perfusion defects and heart disease is not known, the lack of worsening defects 1 year after a low-dose exposure is encouraging.[10] As a

limitation in our study, the long-term effects of RT should be investigated for the perfusion defects and heart disorders in future.

Although the survival advantage provided by adjuvant radiation has been demonstrated[15,16], the increased rates of cardiac morbidity and mortality have been well described.[17-20] To adequately predict the cardiac risks of modern radiation techniques, the determination of a relationship between cardiac doses and long-term morbidity and mortality is necessary. Previous reports have attempted to correlate the risk of cardiac injury with doses received by the heart.[19,21] Hooning *et al.*, retrospectively reviewed the incidence of cardiovascular disease in 10-year survivors of breast cancer, treated from 1970 through 1986.[21] Although the risk of cardiovascular disease increased with increasing estimated mean heart doses, the risk was decreased with more modern treatment techniques. More recently, Nilsson *et al.*, demonstrated with coronary angiography that the location and severity of coronary artery stenosis correlates with the expected regions of high-dose radiation, especially for left-sided radiation or inclusion of the internal mammary nodes.[19] Correa *et al*., conducted a study on 14 patients, 13 with left and one with right-sided breast cancer, who had stress tests and underwent cardiac catheterization. In this study, in 11 of the 13 patients, the LAD was affected. In 8 of these 11 patients, a single vessel was affected, in one patient, both LADs and the LCx were diseased, and in one other patient, three main coronary vessels were diseased. On the other hand, 1 patient had LCx and right coronary stenosis without evidence of LAD disease.[22] In our study, not only the mean and maximum doses of RT on heart of left-sided breast cancer were higher than the right-sided cancer, but also the mean, maximum and minimum doses on LAD, LCx, and both ventricles of left-sided cancer were also significantly higher than left-sided cancers, suggesting a possibility of tendency for heart diseases as well as LAD and LCx diseases.

Many different RT regimens were used from 1970 to 2000, and it was not possible to calculate the dose of heart and coronary arteries in most patients. Taylor *et al.* did a study to describe hot-spot areas for radiation and classify RT as high or low-risk.[23] In their study, the mean doses received by Swedish women treated for left-sided breast cancer in the 1990s were 3.0 ± 0.5 Gy to the heart and 12.0 ± 2.3 Gy to the LAD (including 1 cm margin).[24] Aznar *et al*. found that the mean doses to cardiac structures were 2.9 ± 2.2 Gy to the heart and 17.8 ± 14 Gy to the whole LAD (f1).[8] In our study, the mean doses to heart were calculated as 1.20 ± 0.19 Gy and 3.97 ± 1.32 Gy in the right- and left-sided breast cancer, respectively. Moreover, the mean doses to LAD were calculated as 0.97 ± 0.10 Gy and 24.03 ± 8.38 Gy in the right- and left-sided breast cancer, respectively. These differences with the literature could be caused by differences in treatment techniques or more likely in contouring strategy: in the present study, the irradiation technique uses tangential fields with CT based planning. These results also indicate that a very low dose to LAD seems to be associated with a very low dose to the heart for breast tumors in different sides. To provide additional information, we suggest that LAD should be contoured as a risk organ along with the whole heart and used prospectively for optimization of RT plan. If it is not possible to contour both structures owing to limited time, the LAD should be preferred as an organ at risk.

In RT to the breast cancer, the anterior part of the heart, including the LAD, receives the highest radiation doses. The LAD dose was generally greater than the dose administered to the whole heart or to the other two coronary arteries.[23] In another study, for irradiated left- *versus* right-sided breast cancers, the odds ratio (OR) for grade 3 to 5 stenosis in mid and distal (md) LAD + dD was 4.38 (95% confidence interval [CI], 1.64 to 11.7) and for grade 4 to 5 stenosis, the OR was 7.22 (95% CI, 1.64 to 31.8). For high-risk RT *versus* low-risk or no RT, the OR for grade 3 to 5 stenosis in hot-spot areas was 1.90 (95% CI, 1.11 to 3.24). An increase of stenosis in mdLAD + dD in irradiated left-sided breast cancer and an association between high-risk RT and stenosis in hot-spot areas for radiation indicate a direct link between radiation and location of coronary stenosis.[19] In our study, the highest radiation dose in patients with left-sided breast cancer was to LAD (mean 24.03 ± 8.39 Gy), while in right-sided breast cancer patients, it was to the RV (mean 1.30 ± 0.25 Gy), suggesting that it is not sufficient to calculate the dose to one organ as an estimate of the dose to all cardiac structures, and recommending that both ventricles and the LAD be contoured and their respective dose burdens be considered in RT of breast.

Handbook recommendation for breast RT is as follows: LV and combined bilateral ventricle limits: V5 ≤ 10% and V25 ≤ 5%. The American Society for Radiation Oncology (ASTRO) Consensus Statement dose constraints for 3D-CRT Accelerated Partial Breast Irradiation (IJROBP 2009) reports as follows: heart V5 < 5% for right-sided tumors and < 40% for left-sided tumors.[25] In our study, the

mean V5 of the LV was 18.68%, and 10% or less in 3 patients and higher in 17 patients compared with the recommendation of ASTRO Consensus Statement. However, the mean V25 of the LV was 5.22% and consistent with the recommendations. The mean V5 in the bilateral ventricles was 23.73% (2.56–26.89). The mean V25 in the bilateral ventricles was 6.78%, and ≤ 5% in 7 patients and > 5% in 13 patients. These limits are also higher for more patients than the recommendations, suggesting that might be linked to an increased risk of cardiac complications which has not been taken into account in the current study.

According to the guidelines, to minimize cardiac side effects, the left ventricular dose should be V5 ≤ 10%, the bilateral ventricular dose V25 ≤ 5%, and the whole heart dose < 4 Gy. For this purpose, in a dosimetric analysis, free and breath hold technique were planned with both forward and inverse IMRT presenting a significant decrease of radiation exposure to the contralateral breast, left and RVs, as well as proximal and especially distal LAD by breath hold with forward IMRT.[26] Another study reported that deep inspiration breath hold plans proved large reductions of dose to the heart. V20 of the heart is reduced from 7.8% to 2.3%, V40 from 3.4% to 0.3% and mean dose from 5.2 to 2.7 Gy.[27] This may be another goal of future studies to reduce the risks of heart diseases in RT of breast cancer.

During the past few decades, the wide variation in the cardiac doses worldwide are likely to have resulted from the diversity in RT practice. Our estimates including coronary artery and heart doses in right and left-sided breast cancers will be useful when deriving dose-response relationships. These results emphasize the importance of using treatment techniques in the left-sided breast cancer that minimize cardiac irradiation, consistent with the "as low as reasonably achievable" concept, and the need for further study of the long-term clinical impact after low-dose exposure.

Conclusions

Almost all patients with breast cancers are exposed to cardiotoxic chemotherapy and potentially cardiotoxic RT to the heart vessels. In the background of modern techniques, especially in left-sided breast cancer, the most direct and best strategy to reduce and protect radiation-induced cardiac injury is to balance dose constraints between several high-dose regions of cardiac substructures and the mean heart dose. If necessary, deep inspiration breath hold respiratory gating, prone positioning or MLC blocking may be used to minimize dose to heart. Additional work is needed to better understand the dose/volume/effect relationships for cardiac injury, and to find authoritative approaches, which can be the guidelines for detection and management of radiation-related heart diseases.

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Socialno-ekonomske neenakosti v incidenci raka v Evropi. Sistematični pregled populacijskih epidemioloških raziskav

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Izhodišča. V zadnjih dvajsetih letih ni bilo obsežnega pregleda raziskav, ki so proučevale socialno-ekonomske neenakosti v incidenci raka v Evropi. V tem času je prišlo do napredka v razvoju tako metod za povezovanje podatkov med podatkovnimi bazami kot analitičnih metod za raziskovanje socialno-ekonomskih neenakosti. Želeli smo na sistematični način posodobiti vedenje o povezanosti med incidenco raka pri odraslih prebivalcih Evrope po posameznih lokacijah in socialno-ekonomskim statusom, merjenim na individualnem ali območnem nivoju.

Materiali in metode. V treh podatkovnih bazah (*PubMed, Scopus in Web of Science*) smo na sistematični način sledeč smernicam PRISMA iskali raziskave, ki so proučevale incidenco raka po lokacijah pri odraslih Evropejcih v povezavi s socialno-ekonomskim statusom. V pregled literature smo vključili 91 člankov, objavljenih v angleškem jeziku v obdobju 2000–2020, ki so se v svoji zasnovi opirali na podatke o evropskih prebivalcih iz registrov raka in so o povezavi med rakom in socialno-ekonomskim statusom poročali z uporabo mer relativnega tveganja. Ugotovitve smo povzeli s kvalitativnim pristopom.

Rezultati. Odrasli Evropejci z nizkim socialno-ekonomskim statusom imajo povečano tveganje za raka glave in vratu, požiralnika in želodca, jeter in žolčnika, trebušne slinavke, pljuč, ledvic, sečnega mehurja, penisa in materničnega vratu. Najvišje relativno tveganje ugotavljajo za pljuča, glavo in vrat, želodec ter maternični vrat. Nasprotno je visok socialno-ekonomski status povezan z večjim tveganjem za raka ščitnice, dojk, prostate in kože. Tumorji osrednjega živčevja in krvni raki niso povezani s socialno-ekonomskim statusom. Razlika v tveganju za raka mod, ki je bilo predhodno večje pri skupinah z višjim socialno-ekonomskim statusom, se je v zadnjih desetletjih večinoma zmanjšala, medtem ko je smer povezanosti med rakom debelega črevesa in danke in socialno-ekonomskim statusom različna od države do države; na severu Evrope je praviloma negativna (večje tveganje pri nizkem socialno-ekonomskim statusom), drugje pa ponekod (še) pozitivna (večje tveganje pri visokem socialno-ekonomskem statusu). Pri rakih, kjer je povezanost s socialno-ekonomskim statusom negativna, so razlike glede na socialno-ekonomski status v glavnem bolj izrazite pri moških v primerjavi z ženskami.

Zaključki. V Evropi je incidenca raka za večino preiskovanih (običajno najpogostejših) lokacij tako ali drugače povezana s socialno-ekonomskim statusom. Socialno-ekonomske neenakosti v incidenci raka je mogoče v večji ali manjši meri (posredno) pojasniti z znanimi dejavniki tveganja, predvsem kajenjem. Zaznati je mogoče tudi neodvisne učinke različnih individualnih in območnih kazalcev socialno-ekonomskega statusa, ki odsevajo različne dimenzije družbene neenakosti, od izobrazbene do ekonomske in materialne.

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Perioperativna radioterapija ali samo kirurgija pri retroperitonealnih sarkomih. Sistematični pregled in meta-analiza

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Izhodišča. Jasnih dokazov ali radioterapija izboljša rezultate zdravljenja pri bolnikih z retroperitonealnimi sarkomi nimamo.

Materijal in metode. Naredili smo sistematičen pregled literature z uporabo podatkovnih baz *PubMed*, *Scopus* in *CENTRAL*. Podatke smo pridobili iz objavljenih primerjalnih raziskav pri bolnikih z retroperitonealnimi sarkomi, ki so bili samo operirani ali pa operirani in obsevani. Primarna cilja analize sta bila 5-letno celokupno preživetje in srednje preživetje. Sekundarna cilja sta bila preživetje brez ponovitve bolezni in delež resekcij R0. Zvezne izide smo računali s povprečji obteženih povprečnih razlik.

Rezultati. Analizirali smo 10 izmed 374 zbranih publikacij. Srednje celokupno preživetje in 5-letno preživetje sta bili značilno večji pri bolnikih zdravljenih z radioterapijo in kirurgijo v primerjavi z bolniki, ki so bili samo operirani ($p < 0,00001$, $p < 0,001$). Srednje preživetje brez ponovitve bolezni je bilo značilno višje pri bolnikih, ki so bili zdravljeni bodisi s preoperativno ($p < 0,001$) ali pooperativno ($p = 0,001$) radioterapijo v primerjavi s samo operiranimi bolniki. Srednji delež resekcij R0 je bil med obema skupinama podoben ($p = 0,56$).

Zaključki. Skupaj z radikalno kirurgijo bi lahko bila radioterapija standardno zdravljenje vsaj pri podskupini bolnikov z retroperitonealnimi sarkomi.

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Vrste kirurškega zdravljenja bolnikov s primarnim hiperparatiroidizmom

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Izhodišča. Primarni hiperparatiroidizem je tretja najpogostejša endokrina motnja, ki jo v večini primerov dokončno ozdravimo z operativnim posegom imenovanim paratiroidektomija. Kirurgija obščitnic je od svojih začetkov v prvi polovici 20. stoletja močno napredovala. Standardni obojestranski eksplorativni pristop so – predvsem zaradi napredka v razvoju slikovne diagnostike in vse boljšega poznavanja bolezni – danes zamenjali minimalno invazivni kirurški pristopi. Usmerjena paratiroidektomija je trenutno poseg izbora za zdravljenje primarnega hiperparatiroidizma po vsem svetu.

Zaključki. Operativno zdravljenje je edina metoda zdravljenja primarnega hiperparatiroidizma, s katero lahko dosežemo popolno ozdravitev. Najprimernejši kirurški pristop je odvisen od števila in mesta hiperaktivnih obščitničnih žlez, razpoložljivosti modernih slikovnih tehnik, omejitev posamezne vrste posega in strokovnega znanja.

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Sekvenčna intraarterijska infuzija mitomicina C po radioembolizaciji z mikrosferernim ^{90}Y pri jetrnih metastazah raka dojk, ki so bile neodzivne na kemoterapijo. Pilotna raziskava posamičnega centra

Aarts BM, Klompenhouwer EG, Dresen RC, Deroose CM, Beets-Tan RHG, Punie K, Neven P, Wildiers H, Maleux G

Izhodišča. Namen raziskave je bil oceniti varnost in izvedljivost intraarterijske infuzije mitomicina C po radioembolizaciji z uporabo smolnatih mikrosfer z itrijem 90 (^{90}Y) pri bolnicah z rakom dojk in jetrnimi metastazami.

Material in metode. V prospektivno pilotno raziskavo smo vključili bolnice z rakom dojke in jetrnimi metastazami, ki smo jih zdravili v obdobju 2012–2018. Bolnice so najprej prejele smolnate mikrosfere z ^{90}Y. Po 6–8 tednih smo odziv na zdravljenje ocenili z MRI, ^{18}F-FDG PET/CT in laboratorijskimi preiskavami. Po izključitvi napredujoče bolezni so 8 tednov kasneje bolnice dobile infuzijo mitomicina C v štirih različnih skupinah z odmerki, kot sledi; A: 6 mg v enem krogu, B: 12 mg v dveh krogih, C: 24 mg v dveh krogih in D: največ 72 mg v 6 krogih. V skupini bolnic, ki so dobile največji odmerek (skupina D), smo odziv najprej ocenili po dveh krogih in nato nadaljevali z infuzijami po izključitvi napredujoče bolezni. O neželenih dogodkih smo poročali v skladu s splošnimi terminološkimi merili za neželene dogodke (*angl. Common Terminology Criteria for Adverse Events*; CTCAE) različica 5.0.

Rezultati. Z ^{90}Y smo zdravili šestnajst bolnic. Štiri bolnice smo izključili iz raziskave, ker niso prejele infuzije mitomicina C zaradi dodatnega napredovanja jetrne bolezni (n = 3) in klinične in biokemične nestabilnosti (n = 1). Zato je bilo število bolnic v posameznih skupinah: v skupini A: 2, v skupini B: 1, v skupini C: 3 in v skupini D: 6 bolnic. Pri 4 od 12 bolnic (vse iz skupine D) je bil največji odmerek mitomicina C zaradi biokemične toksičnosti (n = 2) in napredujoče bolezni (n = 2) prilagojen. Neželeni učinek zdravljenja z ^{90}Y tretje stopnje je bila pri enem bolniku razjeda na sluznici želodca in posledica le-te dolgotrajnejša hospitalizacija.

Zaključki. Sekvenčno zdravljenje z intraarterijsko infuzijo mitomicina C po selektivni terapiji z notranjim sevanjem z ^{90}Y je bila izvedljiva pri 75 % bolnic z rakom dojke in jetrnimi metastazami, vendar je potrebna previdnost, da preprečimo refluks.

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Scintigrafsko slikanje kosti ocenjeno s programsko opremo DASciS kot napovedni dejavnik preživetja pri bolnikih, ki so imeli na kastracijo odporen rak prostate in smo jih zdravili z 223RaCl

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Izhodišča. Namen raziskave je bil oceniti scintigrafsko slikanje kosti kot napovedni dejavnik celokupnega preživetja, na začetku in med zdravljenjem z Ra-223, pri bolnikih z metastatskim rakom prostate odpornim na kastracijo (mCRPC). Indeks skeniranja kosti smo opredelili kot odstotek celotne količine metastaz kosti na scintigrafskih slikah celega telesa. Predstavljamo posebno programsko opremo (DASciS), ki jo je za izračun indeksa skeniranja kosti razvila inženirska ekipa Univerze „Sapienza" v Rimu.

Bolniki in metode. Podatke skeniranih kosti 127 bolnikov z mCRPC smo obdelali s programsko opremo DASciS. Kot napovedni dejavnik celokupnega preživetja smo proučili indeks skeniranja kosti.

Rezultati. Analiza skenov 546 kosti je pokazala, da je obseg scintigrafsko dokazane razširjenosti bolezni napovedni dejavnik celokupnega preživetja (0–3 % = 28 mesecev srednjega preživetja; 3–5 % = 11 mesecev srednjega preživetja; > 5 % = 5 mesecev srednjega preživetja). Ko smo indeks skeniranja kosti analizirali kot samostojen dejavnik za napoved celokupnega preživetja je bila AUC 88 %. Kombinacija dveh dejavnikov, indeksa skeniranja kosti in 3-variabilne napovedne ocene (3-PS) pa je izboljšala AUC na 91 %. Tako sta se ta dva dejavnika izkazala kot najboljša napovedna dejavnika celokupnega preživetja bolnikov z mCRPC.

Zaključki. Celokupno preživetje pri bolnikih z mCRPC zdravljenih z Ra-223 je v obratni korelaciji s spremembami na scintigrafskih slikah kosti. Programska oprema DASciS se zdi obetavno orodje za prepoznavanje bolnikov z mCRPC, ki bodo bolje odgovorili na zdravljenje z Ra-223. Indeks skeniranja kosti bi lahko bil dodatni napovedni dejavnik za celokupno preživetje, saj omogoča še bolj smotrno izbiro bolnikov za učinkovito zdravljenje in s tem tudi optimizira stroške.

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Tri-dimenzionalna magnetnoresonančna ocena vpliva polnjenosti mehurja na pomik in deformacijo prostate

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Izhodišča. Omejitvi pri natančnosti radioterapetskega zdravljenja sta pomik in deformacija tarčnega organa. Polnjenost mehurja je eden izmed znanih dejavnikov vpliva na pomik in deformacijo prostate. Namen raziskave je bil oceniti vpliv polnjenosti mehurja na pomik in deformacijo prostate s podrobnim tri-dimenzionalnim obrisom prostate na magnetnoresonančnih (MR) slikah.

Bolniki in metode. V prospektivno raziskavo z etičnim dovoljenjem smo vključili 15 zdravih prostovoljcev. Vsakega prostovoljca smo pripravili na MR preiskavo s 4 različnimi postopki pitja. Na posamezni MR preiskavi smo zajeli slike pred in po uriniranju. MR slike smo poravnali glede na kostne anatomske orientacijske točke. Naredili smo tri-dimenzionalno obrisovanje prostate, da bi ocenili pomik in deformacijo prostate. Glede na spremembo raztezanja mehurja in danke smo prostovoljce razdelili v dve skupini. Izključili pa smo MR preiskave, kjer smo ugotovili hkratno spremembo raztezanja mehurja in danke.

Rezultati. V skupino z raztezanjem mehurja smo vključili 40 MR preiskav, v skupino z raztezanjem danke pa 8 MR preiskav. Sprememba volumna danke je pomembnejše vplivala na pomik ($p < 0,01$) in deformacijo ($p = 0,02$) prostate kot sprememba volumna mehurja. Pomik prostate in polnjenost mehurja sta bila v zmerni odvisnosti ($r = 0,64$; $p < 0,01$). Pri spremembi volumna mehurja za manj kot dvakrat ni bilo pomembnega pomika prostate ($p < 0,01$). Deformacija prostate in polnjenost mehurja sta bila v zmerni odvisnosti ($r = 0,61$; $p < 0,01$).

Zaključki. Volumen mehurja minimalno vpliva na pomik prostate in ima zanemarljiv vpliv na deformacijo prostate. Pomik prostate lahko zmanjšamo, če je sprememba polnjenosti mehurja manjša kot dvakratni volumen mehurja.

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Ocena varnostnega robu po mikrovalovni ablaciji jetrnih tumorjev. Določitev inter- in intraindividualne variabilnosti

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Izhodišča. Namen raziskave je bil določiti inter- in intraindividualno variabilnost ocene varnostnega robu po mikrovalovni ablaciji jetrnih tumorjev z uporabo računalniške tomografije (CT) po ablativnem posegu ter določiti senzitivnost in specifičnost preiskave CT za identifikacijo nepopolne ablacije.

Bolniki in metode. Naredili smo retrospektivno analizo 58 bolnikov, pri katerih smo med septembrom 2017 in junijem 2019 opravili mikrovalovno ablacijo primarnega ali sekundarnega tumorja jeter (46 hepatocelularnih rakov, 9 zasevkov kolorektalnega raka, 3 zasevki raka pankreasa). Trije preiskovalci so ocenili uspešnost ablacije (popolna ali nepopolna) in minimalno širino varnostnega roba po ablaciji. Za oceno so uporabili primerjavo preiskave CT dan pred ablacijo in dan po ablaciji. Eden od preiskovalcev je isto oceno ponovil po 6 tednih. Magnetno resonančno (MRI) preiskavo jeter 6 tednov po posegu smo uporabili za zlati standard.

Rezultati. Medrazredni relacijski koeficient (ICC) ocene minimalne širine ablacijskega roba za vse tri preiskovalce je znašal 0,357 (95 %-interval zaupanja 0.194–0.522). ICC minimalne širine robu med dvema ocenama istega preiskovalca je znašal 0.774 (95 %-interval zaupanja 0.645–0.860). Vrednosti senzitivnosti in specifičnosti CT preiskave za oceno popolne ablacije tumorja (definirane kot odsotnost ostanka viabilnega tumorskega tkiva na MRI jeter 6 tednov po posegu) so bile 93 %/82 %/82 % in 33 %/17 %/83 %.

Zaključki. V klinični praksi širino varnostnega robu po ablaciji pogosto ocenimo z medsebojno primerjavo CT preiskav, ki jih opravimo pred in po ablativnem posegu. Dokazali smo, da je ta način ocene ablacijskega robu nezanesljiv (ICC 0,357). Menimo, da je potreben razvoj novih tehničnih rešitev za oceno širine ablacijskega robu.

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Perkutana mehanska trombektomija pri bolnikih s pljučno embolijo z visokim tveganjem in kontraindikacijami za zdravljenje s trombolizo

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Izhodišča. Pljučna embolija z visokim tveganjem je povezana z visoko zgodnjo umrljivostjo. Poročamo o lastnih izkušnjah s perkutano mehansko trombektomijo pri bolnikih s pljučno embolijo z visokim tveganjem in kontraindikacijami za zdravljenje s trombolizo.

Bolniki in metode. Raziskava je retrospektivna analiza zaporednih bolnikov s pljučno embolijo z visokim tveganjem in s kontraindikacijami za zdravljenje s trombolizo. Bolnike smo zdravili z metodo perkutane mehanske trombektomije, ki je obsegala trombektomijo in dodatno aspiracijo tromba, kadar je bilo to potrebno. Zbrali smo podatke o kliničnih parametrih, uspešnosti in preživetju do odpusta.

Rezultati. Od novembra 2005 do septembra 2015 smo zdravili 25 bolnikov s povprečno starostjo 62,6 ± 12,7 let; 64 % je bilo moških. Povprečni indeks stopnje pljučne embolije (*angl. simplified Pulmonary Embolism Severity Index*) je bil 2,9. Povprečna najvišja koncentracija laktata je bila 7,8 ± 6,6 mmol/L, vazopresorje smo uporabili v 77 %, mehanska ventilacija pa je bila potrebna v 59 %. Fragmentacijo z ali brez dodatne aspiracije tromba smo uporabili pri 14 bolnikih (56 %) in aspiracijo s katetrom Aspirex®S pri 11 bolnikih (44 %). Pri petih bolnikih (20 %) smo uporabili lokalno, pri treh (12 %) pa sistemsko trombolizo. Po posegu smo opazili neznačilno zvišanje sistemskega krvnega tlaka (100 ± 41 mm Hg proti 119 ± 34 mm Hg; p = 0,100) in srčne frekvence (99 ± 35 min^{-1} proti 87 ± 31 min^{-1}; p = 0,326). Ugotovili smo značilno znižanje tlačnega gradienta na trikuspidalni zaklopki po posegu (57 ± 14 mm Hg proti 31 ± 3 mm Hg; p = 0.018). Poseg je bil tehnično uspešen pri 20 bolnikih (80 %) in 17 bolnikov (68%) je preživelo do odpusta iz bolnišnice.

Zaključek. Pri bolnikih s pljučno embolijo z visokim tveganjem, ki imajo kontraindikacije za zdravljenje s trombolizo, je perkutana mehanska trombektomija obetajoča metoda za znižanje tlaka v pljučnem krvnem obtoku.

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Elektrokemoterapija pri zdravljenju pasjih oralnih malignih melanomov in napovedni dejavniki izida zdravljenja

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Izhodišča. Oralni maligni melanoma je zelo pogost in agresiven tumor v ustni votlini psov, ki ima zelo slabo napoved poteka bolezni. Te melanome lahko zdravimo z elektrokemoterapijo, ki je učinkovita lokalna terapija. Namen pričujoče prospektivne klinične raziskave je bil ugotoviti terapevtsko učinkovitost elektrokemoterapije, ki smo jo uporabili kot prvo zdravljenje, pri pasjem oralnem malignem melanoma. Analizirali smo tudi napovedne dejavnike izida zdravljenja.

Metode. V klinično študijo smo vključili 67 psov, ki niso bili primerni, da jih najprej zdravimo s kirurgijo. Zdravili smo jih z elektrokemoterapijo in sledili še dve leti za tem.

Rezultati. Z elektrokemoterapijo smo dosegli 100 %; 89,5 %; 57,7 %; in 36,4 % objektivnih odgovorov pri stadijih bolezni I; II; III ali IV. Kvaliteta življenja se je izboljšala le pri psih s stadiji bolezni I, II in III. Srednji čas do napredovanja bolezni je bil 11, 7, 4 in 4 mesece, in srednji čas preživetja zdravljenih psov 16,5; 9,0; 7,5 in 4,5 mesece glede na stadij bolezni I – IV. Statistično značilno boljši je bil odgovor na zdravljenje z elektrokemoterapijo pri stadiju I in II ($p = 0,0013$) in pri bolnikih brez invazije melanoma v kosti ($p = 0.043$).

Zaključki. Elektrokemoterapija je učinkovito lokalno zdravljenje oralnega malignega melanoma pri psih, kjer nobeno drugo zdravljenje ni mogoče. Boljši je izid zdravljenja pri manj napredovali bolezni in pri psih brez invazije melanoma v kosti.

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Dolgoročna učinkovitost elektrokemoterapije z nižjim odmerkom bleomicina pri starejših bolnikih

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Izhodišča. Elektrokemoterapija je lokalni način zdravljenja, ki temelji na elektroporaciji. Le-ta s pomočjo zunanjega električnega polja povzroči povečano prepustnost membrane in s tem povečan vstop citotoksičnih učinkovin v celice. Za zdravljenje ne-melanomskega kožnega raka se pri elektrokemoterapiji uporablja standardni odmerek bleomicina 15 000 IU/m^2. Namen raziskave je bil ovrednotiti dolgoročno učinkovitost elektrokemoterapije z nižjim odmerkom bleomicina v primerjavi z učinkovitostjo elektrokemoterapije s standardnim odmerkom bleomicina, pri starejših bolnikih z ne-melanomskim kožnim rakom.

Bolniki in metode. V raziskavo smo zajeli 28 bolnikov s 52 lezijami, ki so imeli ne-melanomske lezije kožnega raka in so bili starejši od 65 let. Eksperimentalna skupina z 12 bolniki (24 lezij) je prejela nižji odmerek bleomicina (10 000 IU/m^2), medtem ko je kontrolna skupina s 16 bolniki (28 lezij) prejela standardni odmerek bleomicina (15 000 IU/m^2).

Rezultati. Med skupinama bolnikov nismo zaznali statistično značilnih razlik. V eksperimentalni skupini je ponovno izraslo 39,0 % zdravljenih lezij, mediana sledenje je trajala 28 mesecev. V kontrolni skupini pa je ponovno izraslo 15,4 % zdravljenih lezij, mediana sledenje je trajala 40 mesecev.

Zaključki. Elektrokemoterapija z nižjim odmerkom bleomicina je izvedljiva metoda zdravljenja, primerna za bolnike starejše od 65 let, ki ima enako učinkovitost kot elektrokemoterapija s standardnim odmerkom. O tej možnosti moramo razmisliti, ko zdravimo starejše bolnike, s pridruženimi boleznimi, ki imajo kratko pričakovano življenjsko dobo.

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Serumski topni z mezotelinom povezani peptidi in genetska variabilnost MSLN pri boleznih, povezanih z izpostavljenostjo azbestu

Goričar K, Kovač V, Dodič-Fikfak M, Dolžan V, Franko A

Izhodišča. Izpostavljenost azbestu je povezana s povečanim tveganjem za razvoj več bolezni, vključno z malignim mezoteliomom. Izražanje mezotelina, glikoproteina celične membrane, je povečano pri malignem mezoteliomu; serumske topne z mezotelinom povezane peptide (SMRP) pa so že proučevali z namenom, da bi jih uporabljali kot diagnostične in prognostične biološke označevalce pri malignem mezoteliomu. Vendar interindividualna variabilnost ravni serumskih SMRP omejuje njihovo klinično uporabnost. Primarni namen raziskave je bil preučiti vpliv *MSLN* rs1057147 na raven SMRP v serumu pri preiskovancih, izpostavljenih azbestu, in bolnikih z azbestnimi boleznimi, kot tudi na preživetje pri bolnikih z malignim mezoteliomom.

Metode. Med 782 preiskovanci, izpostavljenimi azbestu, in bolniki z azbestnimi boleznimi, jih je 154 imelo maligni mezoteliom. Serumske ravni SMRP smo določili z encimsko imunoadsorpcijsko preiskavo. Vse preiskovance smo genotipizirali za polimorfizem *MSLN* rs1057147 s kompetitivno alelno-specifično verižno reakcijo s polimerazo. Za primerjavo različnih skupin smo uporabili neparametrične teste, logistično in Coxovo regresijo.

Rezultati. Bolniki z malignim mezoteliomom so imeli statistično značilno višjo raven SMRP kot ostali preiskovanci ($p < 0,001$). V primerjavi z nosilci dveh normalnih alelov *MSLN* rs1057147 so imeli heterozigoti in nosilci dveh polimorfnih alelov statistično značilno višjo raven SMRP pri preiskovancih brez malignega mezotelioma ($p < 0,001$), vendar ne pri bolnikih z malignim mezoteliomom ($p = 0,424$). Če smo v analizi upoštevali podatke o genotipu, se je specifičnost SMRP za optimalno mejno vrednost povečala s 88,5% na 92,7%. Celokupno preživetje je bilo statistično značilno nižje pri bolnikih z malignim mezoteliomom, ki so imeli vsaj en polimorfen alel rs1057147 (HR = 1,72; 95% CI = 1,15-2,55; p=0,008).

Zaključki. Genetska variabilnost *MSLN* vpliva na nivo SMRP in je bila povezana z nižjim preživetjem bolnikov z malignim mezoteliomom. Kombinacija genetskih in serumskih dejavnikov bi zato lahko služila kot boljši diagnostični ali prognostični biološki označevalec pri bolnikih z malignim mezoteliomom.

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Kinetika γ-H2AX med radioterapijo raka glave in vratu bi lahko napovedovala resnost mukozitisa

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Izhodišča. Cilj raziskave je bil oceniti spremembe v izražanju γ-H2AX v limfocitih periferne krvi glede na stopnjo z obsevanjem izzvanega mukozitisa.

Bolniki in metode. V raziskavo smo vključili 50 bolnikov z rakom glave in vratu, ki smo jih zdravili z radioterapijo ali kemoradioterapijo. Za določitev osnovne ravni γ-H2AX smo krvne vzorce zbrali pred zdravljenem. Drugi vzorec smo odvzeli 45 minut po prvi frakciji radioterapije in nato enkrat tedensko, prav tako 45 min po obsevanju. Pri bolnikih, ki smo jih zdravili s kemoradioterapijo, smo krvni vzorec odvzeli dan po kemoterapiji. Mukozitis smo ocenjevali enkrat tedensko in opredelili v skladu z lestvicama CTCAE v3 in 4 ter RTOG/EORTC. Analizo limfocitne periferne krvi smo opravili s pretočno citometrijo in v vseh časovnih točkah ocenili fosforilacijo γ-H2AX.

Rezultati. Blag do zmeren (1.–2. stopnje) mukozitis smo ugotovili pri 35 bolnikih, hud mukozitis (3. stopnje) pa se je razvil pri 15 bolnikih. Nismo ugotovili primer mukozitisa 4. stopnje. Razlika v izhodiščnih vrednostih γ-H2AX med skupinama z blagim in hudim mukozitisom ni bila statistično značilna ($p = 0,25$). Statistično značilno razliko v vrednosti γ-H2AX smo videli v 7. tednu zdravljenja ($p = 0,01$). Nobenih značilnih razlik v vrednosti γ-H2AX nismo našli niti med skupinama zdravljenima s sočasno kemoradioterapijo in samo radioterapijo niti med skupinama z in brez sočasnih pridruženih bolezni. V analizi kinetike γ-H2AX med zdravljenjem smo ugotovili statistično pomembno razliko ($p = 0,009$) med skupinama z blagim in z hudim mukozitisom. Po 4. tednu zdravljenja so se vrednosti γ-H2AX pomembno znižale v skupini s hudim mukozitisom in narasle pri bolnikih z blagimi stranskimi učinki. Ugotovljena razlika ni bila povezana s padcem števila limfocitov periferne krvi, ki je bilo v obeh skupinah podobno.

Zaključki. Predstavljeni rezultati kažejo da intenzivnost z obsevanjem povzročenega mukozitisa neposredno ne sovpada z vrednostmi γ-H2AX, ki smo jih izmerili *in vivo* v limfocitih periferne krvi. Napovedovanje stopnje mukozitisa na osnovi vrednosti γ-H2AX še ni mogoče, niti pred zdravljenjem niti med zdravljenjem, vendar preliminarni rezultati kažejo na pomembne razlike v kinetiki γ-H2AX med skupinama, kar spodbuja nadaljnje raziskave.

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Molekularna heterogenost celic raka dojke s povečano invazivnostjo

Negro G, Aschenbrenner B, Kranjc Brezar S, Čemažar M, Coer A, Gašljević G, Savić D, Sorokin M, Buzdin A, Callari M, Kvitsaridze I, Jewett A, Vasileva-Slaveva M, Ganswindt U, Skvortsova I, Skvortsov S

Izhodišča. V klinični onkologiji je še vedno izziv, kako obvladati napredovanje metastatske bolezni raka dojke. Zato je potrebno ugotoviti, kako celice različnih podtipov raka dojke razvijejo sposobnost metastaziranja. Cilj te raziskave je bil razjasniti podobnost med aktiviranimi molekularnimi potmi, ki so vpletene v povečanje invazivnosti različnih podtipov celic raka dojke.

Materiali in metode. V ta namen smo v raziskavi s pomočjo proteomike primerjali molekularne fenotipe starševskih in invazivnih (INV) celic raka dojke, MDA-MB-231 (trojno negativne), T47D (za hormonske receptorje pozitivne) in Au565 (Her2-pozitivne).

Rezultati. Invazivne celice so bile neodvisno od podtipov raka dojke morfološko podobne fibroblastom, imele so povečano invazivno in migracijsko zmogljivost, povečano ekspresijo označevalcev matičnih rakavih celic in upočasnjeno rast tumorjev na *in vivo* živalskih modelih. Obširna proteomska analiza je pokazala različno izražanje proteinov pri INV celicah in starševskih celicah, najbolj izrazite molekularne razlike so bile pri Her2-pozitivnih Au565-INV celicah v primerjavi s trojno negativnimi MDA-MB-231-INV in za hormonske receptorje pozitivnimi T47D-INV celicami. Čeprav je bilo pri celicah Au565-INV največje število dereguliranih proteinov, so le te imele najmanjše prekrivanje izraženih proteinov, ki so običajno izraženi v celicah MDA-MB-231-INV in T47D-INV.

Zaključki. Ugotovili smo, da invazivne celice, ki so pozitivne za hormonske receptorje pridobijo molekularne značilnosti trojno negativnih celic raka dojke. Her2-pozitivne invazine celice pa specifično spremenijo molekularni fenotip, ki je zelo omejeno vpleten v ključne molekularne poti prisotne v celicah MDA-MB -231-INV in T47D-INV. Ker invazivne celice, pozitivne za hormonske receptorje, delijo svoje molekularne lastnosti s trojno negativnimi celicami raka dojke, predvidevamo, da bi lahko te vrste metastatskih bolezni zdravili podobno, skupaj s hormonskim zdravljenjem. Nadalje, Her2-pozitivne metastaze je potrebno skrbno ovrednotiti za izbiro učinkovitejšega načina zdravljenja, ki se razlikuje od zdravljenja trojno negativnega in hormonsko pozitivnega metastatskega raka dojke.

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Retrospektivna analiza zdravljenja slovenskih bolnikov z metastatskim melanomom s pembrolizumabom v prvi liniji. Izkušnje iz klinične prakse

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Izhodišča. Iz do sedaj objavljenih kliničnih raziskav vemo, da zdravljenje z zaviralcem imunskih kontrolnih točk pembrolizumabom pri bolnikih z napredovalim malignim melanomom podaljša preživetja in ima zmerne stranske učinke. Ali ti rezultati sovpadajo z vsakodnevno klinično prakso, še ni popolnoma jasno. Z retrospektivno analizo smo želeli oceniti učinkovitost in toksičnost zdravljenja s pembrolizumabom pri predhodno nezdravljenih bolnikih z metastatskim malignim melanomom v vsakdanji klinični praksi v Sloveniji in te rezultate primerjati z rezultati objavljenih kliničnih raziskav.

Bolniki in metode. V opazovalno retrospektivno kohortno raziskavo smo vključili 138 bolnikov s predhodno nezdravljenim metastatskim malignim melanomom, ki smo jih zdravili s pembrolizumabom na Onkološkem inštitutu Ljubljana, Slovenija, v obdobju od januarja 2016 do decembra 2018. Podatke o značilnosti bolnikov in njihovem zdravljenju smo retrospektivno zbrali iz podatkovne bolnišnične baze. Statistično analizo smo naredili z uporabo programske opreme SPSS različice 22. Preživetje smo izračunali po metodi Kaplan-Meier. Bolnike smo sledili od januarja 2016 do junija 2019.

Rezultati. Ocenjeno srednje celokupno preživetje je bilo 25,1 mesecev (95 % interval zaupanja [IZ] 14,6–35,6), srednje preživetje brez napredovanja bolezni pa 10,7 mesecev (95 % IZ, 5,9–15,4). Pri 29 (21,0 %) bolnikih smo zabeležili popolni odgovor na zdravljenje, pri 31 (22,5 %) bolnikih delni odgovor in pri 23 (16,7 %) bolnikih stabilno bolezen. Število metastatskih lokalizacij in izhodiščni nivo laktatne dehidrogenaze (LDH) sta pomembno vplivala na preživetje zdravljenih bolnikov. Imunsko pogojene neželene učinke smo ugotovili pri 88 (63 %) bolnikih, od tega stopnjo 3 in 4 pri 12 (8,7 %) bolnikih. Zaradi toksičnosti je 16 (11,6 %) bolnikov zdravljenje prekinilo.

Zaključki. Podatki opisane raziskave kažejo slabše srednje celokupno preživetje in primerljivo srednje preživetje brez ponovitve bolezni pri bolnikih z razsejanim malignim melanomom, kje je bilo prvo zdravljenje s pembrolizumabom, v primerjavi s podatki ostalih objavljenih kliničnih raziskav. Bolniki z normalnimi vrednostmi LDH v serumu in bolniki z manjšim številom metastatskih lokalizacij so imeli primerljivo srednje celokupno preživetje. Pojavnost imunsko pogojenih neželenih učinkov pembrolizumaba je bila primerljiva.

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Vpliv radioterapije na koronarne arterije in srce pri operacijah raka z ohranitvijo dojk. Dozimetrična analiza

Soydemir Gocer GP, Ozer EE

Izhodišča. Radioterapija predstavlja določeno tveganje, zlasti pri bolnicah z rakom leve dojke, saj se pri njih srčni zapleti pojavljajo pogosteje kot pri raku na desni dojki. Cilj raziskave je bil z dozimetrično analizo preveriti učinek radioterapije na koronarne arterije in srce po operacijah, pri katerih je bila dojka ohranjena.

Bolnice in metode. V raziskavo smo vključili 40 naključno izbranih bolnic z rakom bodisi na levi ali desni dojki (stadij T1 / T2, N0). Bolnicam smo obsevali celotno dojko in dodatno tumorsko ležišče, uporabili tangencialna polja in načrtovali obsevanje s pomočjo računalniške tomografije. Analizirali smo vrednosti doze na levo sprednjo descendentno koronarno arterijo, levo cikumfleksno koronarno arterijo, desni prekat, levi prekat in srce. Povprečne vrednosti doz smo primerjali med skupinami bolnic z rakom na levi ali desni dojki.

Rezultati. Najvišja povprečna doza sevanja pri bolnicah z levostranskim rakom dojke je bila na descendentno koronarno arterijo 2402,48 ± 838,39 cGy, medtem ko je bila povprečna doza pri bolnicah z rakom na desni dojki nižja 130,18 ± 24,92 cGy. Največja povprečna doza sevanja je bil pri bolnicah z rakom na desni dojki ugotovljena na srcu, podobno tudi pri bolnicah z rakom na levi dojki. Povprečna vrednost V5 v levem prekatu je bila 18,68 % (6,89–31,69), povprečna vrednost V25 pa 5,22 % (0,45–16,54). Povprečna vrednost V5 v obeh prekatih je bila 23,73 % (2,56–26,89), povprečna vrednost V25 v obeh prekatih pa 6,78 % (0,63–13,63).

Zaključki. Posebno pri rakih leve dojke je najbolj neposredna in najboljša strategija za zmanjšanje in zaščito poškodb srca, tako načrtovati obsevanje, kjer zmanjšujemo sevalno dozo med različnimi regijami srca, zlasti tistimi, ki prejmejo visoke oz. srednje doze.

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Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – a report for the first quarter of 2020

Doc. Dr. Josip Cholewa Foundation for cancer research and education continues with its planned activities in the first quarter of 2020 and is commencing to prepare for the activities the whole year. Its primary focus remains the provision of grants and scholarships and other forms of financial assistance for basic, clinical and public health research in the field of oncology. An analysis of the ongoing activities in the last year was made in order to make an assessment of the impact of Foundation's activities, thus providing a basis for developing new strategies and approaches in its scope of fight against cancer.

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

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

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

Borut Štabuc, M.D., Ph.D.
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INDIKACIJE: Zdravilo Imfinzi je kot samostojno zdravljenje indicirano za zdravljenje lokalno napredovalega, neoperabilnega nedrobnoceličnega raka pljuč (NSCLC – "non small cell lung cancer") pri odraslih, ki imajo tumorje z ≥ 1 % izraženostjo PD-L1 na tumorskih celic in pri katerih bolezen ni napredovala po kemoradioterapiji na osnovi platine.
ODMERJANJE IN NAČIN UPORABE: Zdravljenje mora uvesti in nadzorovati zdravnik, ki ima izkušnje na področju zdravljenja raka. Bolnike z lokalno napredovalim nedrobnoceličnim rakom pljuč je treba za zdravljenje izbrati na podlagi izraženosti PD-L1, ugotovljene z validirano testno metodo. Odmerjanje: Priporočeni odmerek zdravila Imfinzi je 10 mg/kg v intravenski infuziji v 60 minutah vsaka 2 tedna do napredovanja bolezni ali nesprejemljive toksičnosti oziroma največ 12 mesecev. Pri klinično stabilnih bolnikih z začetnimi znaki napredovanja bolezni je priporočeno z zdravljenjem nadaljevati, dokler napredovanje bolezni ni potrjeno. Povečevanje ali zmanjševanje odmerka ni priporočljivo. Glede na individualno varnost in prenašanje je lahko potrebna odložitev odmerka ali prenehanje uporabe zdravila.
V primeru domnevnih imunsko pogojenih neželenih učinkov je treba opraviti ustrezno ovrednotenje za potrditev etiologije ozioma izključitev druge etiologije. Če se stanje ne izboljša ali se poslabša, pride v poštev povečanje odmerka kortikosteroidov in/ali dodatna uporaba sistemskih imunosupresivov. Po izboljšanju na ≤ 1. stopnjo je treba začeti s postopnim zmanjševanjem kortikosteroida in ga zmanjševati v obdobju vsaj 1 meseca. Po odložitvi uporabe je mogoče zdravilo Imfinzi znova začeti uporabljati v času 12 tednov, če se neželeni učinki izboljšajo na ≤ 1. stopnjo in je odmerek kortikosteroida zmanjšan na ≤ 10 mg prednizona ali ekvivalenta na dan. Če se imunsko pogojeni neželeni učinki 3. ali 4. stopnje (močno izraženi ali življenjsko nevarni) ponavljajo, je treba zdravilo Imfinzi dokončno ukiniti.
Pri ne imunsko pogojenih neželenih učinkih večjs v primeru 2. ali 3. stopnje izraženosti razmisliti o odložitvi uporabe zdravila Imfinzi do izboljšanja neželenih učinkov na ≤ 1. stopnjo ali na izhodiščno raven. Z uporabo zdravila Imfinzi je treba prenehati v primeru neželenih učinkov 4. stopnje (razen v primeru laboratorijskih nepravilnosti 4. stopnje, pri katerih naj odločitev o prekinitvi uporabe zdravila temelji na spremljajočih kliničnih znakih oziroma simptomih in na klinični presoji zdravnika).
Zdravilo Imfinzi je namenjeno za intravensko uporabo. Dati ga je treba kot raztopino za intravensko infundiranje v obdobju 60 minut.
KONTRAINDIKACIJE: Preobčutljivost na učinkovino (učinkovine) ali katero koli pomožno snov.
OPOZORILA IN PREVIDNOSTNI UKREPI: Za izboljšanje slednjivosti bioloških zdravil je treba jasno zabeležiti zastrškno ime in številko serije uporabljenega zdravila. Imunsko pogojeni pnevmonitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojeni pnevmonitis ali intersticijska bolezen pljuč, opredeljeno kot potreba po uporabi sistemskih kortikosteroidov in brez jasne druge etiologije. Pri bolnikih, zdravljenih z radioterapijo pljuč, je pogost radiacijski pnevmonitis in klinična slika pnevmonitisa in radiacijskega pnevmonitisa je zelo podobna. V študiji PACIFIC sta se pri bolnikih, ki so opravili zdravljenje z najmanj 2 ciklema sočasne kemoradioterapije od 1 do 42 dni pred začetkom preizkušanja, pnevmonitis ali radiacijski pnevmonitis pojavila pri 161 (33,9 %) bolnikih v skupini z zdravilom Imfinzi in pri 58 (24,8 %) bolnikih v skupini s placebom, vključno s 3. stopnjo (3,4 % in 3,0 %) in 5. stopnjo (1,1 % in 1,7 %). Bolnike je treba spremljati glede znakov in simptomov pnevmonitisa ali radiacijskega pnevmonitisa. Imunsko pogojeni hepatitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavil imunsko pogojeni hepatitis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojeni kolitis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojeni kolitis ali driska, opredeljeno kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojene endokrinopatije: Hipotiroidizem in hipertiroidizem: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojeni hipotiroidizem in hipertiroidizem (vključno s tiroiditisom); hipertiroidizmu lahko sledi hipotiroidizem. Bolnike je treba spremljati glede nenormalnih izvidov delovanje ščitnice pred zdravljenjem in redno med zdravljenjem ter kot je potrebno glede na klinično oceno. Imunsko pogojeni hipotiroidizem in hipertiroidizem (vključno s tiroiditisom) je treba obravnavati, in ukrepati, kot je priporočeno v povzetku glavnih značilnosti zdravila. Adrenalna insuficienca: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavila imunsko pogojena adrenalna insuficienca. Bolnike je treba spremljati glede kliničnih znakov in simptomov adrenalne insuficience. Sladkorna bolezen tipa 1: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavila imunsko pogojena sladkorna bolezen tipa 1. Bolnike je treba spremljati glede kliničnih znakov in simptomov sladkorne bolezni tipa 1. Hipofizitis/hipopituitarizem: Pri bolnikih, ki so prejemali zdravilo Imfinzi, sta se pojavila imunsko pogojena hipofizitis ali hipopituitarizem. Bolnike je treba spremljati glede kliničnih znakov in simptomov hipofizitisa ali hipopituitarizma. Imunsko pogojeni nefritis: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavil imunsko pogojeni nefritis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Imunsko pogojeni izpuščaj: Pri bolnikih, ki so prejemali zdravilo Imfinzi, se je pojavil imunsko pogojeni izpuščaj ali dermatitis, opredeljen kot potreba po sistemskih kortikosteroidih in brez jasne druge etiologije. Pri bolnikih, ki so bili zdravljeni z zaviralci PD-1, so poročali o pojavljanju Stevens Johnsonovega sindroma ali toksične epidermalne nekrolize. Drugi imunsko pogojeni neželeni učinki: Glede na mehanizem delovanja zdravila Imfinzi se lahko pojavijo še drugi potencialno imunsko pogojeni učinki. Naslednji imunski neželeni učinki so bili zabeleženi v kliničnih preizkušanjih (n = 1889) pri manj kot 1 % bolnikov, ki so prejemali samostojno zdravljenje z zdravilom Imfinzi: miokarditis, mioziti, polimiozitis. V programu kliničnih študij so pri bolnikih poročali o primerih pankreatitisa. Bolnike je treba spremljati glede znakov in simptomov in ukrepati, kot je priporočeno za imunsko pogojene neželene učinke.Z infundiranjem povezane reakcije: Bolnike je treba spremljati glede znakov in simptomov z infundiranjem povezanih reakcij. Pri bolnikih, ki so prejemali zdravilo Imfinzi, so bile opisane hude z infundiranjem povezane reakcije. Bolniki, ki niso bili vključeni v klinična preizkušanja: V študiji PACIFIC niso bili vključeni bolniki z naslednjimi značilnostmi: izhodiščno oceno zmogljivosti ECOG ≥ 2; aktivno ali predhodno dokumentirano avtoimunsko boleznjo v 2 letih pred začetkom študije; anamneze imunske pomanjkljivosti; anamneze hudih imunsko pogojenih neželenih učinkov; boleznmi, ki so zahtevale sistemsko imunosupresijo, razen fiziološkega odmerka sistemskih kortikosteroidov (≤ 10 mg na dan prednizona ali ekvivalenta); aktivno tuberkulozo ali okužbo s hepatitisom B ali C ali HIV; bolniki, ki so prejeli živo oslabljeno cepivo v 30 dneh pred začetkom zdravljenja z zdravilom Imfinzi ali v 30 dneh po začetku. Dokler takšnih podatkov ni, je treba durvalumab v teh skupinah bolnikov uporabljati previdno ter po skrbnem individualnem pretehtanju možnih koristi in tveganj za posameznega bolnika.
MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI: Razen fizioloških odmerkov sistemskih kortikosteroidov (≤ 10 mg na dan prednizona ali ekvivalenta) pred uvedbo durvalumaba ni priporočljivo uporabljati sistemskih kortikosteroidov ali imunosupresivov, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost durvalumaba. Vendar pa je mogoče kortikosteroide ali druge imunosupresive uporabiti po začetku zdravljenja z durvalumabom za zdravljenje imunsko pogojenih neželenih učinkov. Z durvalumabom niso izvedli formalnih farmakokinetičnih (PK) študij medsebojnega delovanja zdravil. Primarni poti odstranjevanja durvalumaba sta katabolizem beljakovin preko retikuloendotelijskega sistema oziroma tarčno posredovano odstranjevanje, zato ni pričakovati presenečnih medsebojnih delovanj med zdravili.
PLODNOST, NOSEČNOST IN DOJENJE: Ženske v rodni dobi morajo med zdravljenjem z durvalumabom in vsaj še 3 mesece po zadnjem odmerku durvalumaba uporabljati učinkovito kontracepcijo. Podatkov o uporabi durvalumaba pri nosečnicah ni. Glede na mehanizem delovanja durvalumab lahko vpliva na vzdrževanje nosečnosti; v skogerskem modelu nosečnosti pri miših je bilo ugotovljeno, da moteno signaliziranje PD-L1 poveča izgubo plodov. Pri nosečnici uporabljeni durvalumab lahko škoduje plodu in ga ni priporočljivo uporabljati med nosečnostjo in pri ženskah v rodni dobi, ki ne uporabljajo učinkovite kontracepcije med zdravljenjem in vsaj še 3 mesece po zadnjem odmerku.
Ni znano, ali se durvalumab pri človeku izloča v materinem mleku. Pri človeku protiteles lahko prehajajo v materino mleko, a možnost absorpcije in škode za novorojenčka ni znana. Toda možnega tveganja za dojenega otroka ni mogoče izključiti. Odločiti se je treba, ali naj ženska prekine z dojenjem ali naj prekine zdravljenje z durvalumabom oziroma sploh ne začne zdravljenja z njim, pri čemer je treba upoštevati koristi dojenja za otroka in koristi zdravljenja za žensko. Podatkov o možnih vplivih durvalumaba na plodnost pri človeku ni.
NEŽELENI UČINKI: Varnost zdravila Imfinzi (10 mg/kg) so ovrednotili v študiji PACIFIC (n = 476) pri bolnikih z lokalno napredovalim neoperabilnim NSCLC, ki so opravili zdravljenje z najmanj 2 ciklema sočasne kemoradioterapije od 1 do 42 dni pred vključitvijo v študijo. V tej populaciji bolnikov so bili najbolj pogosti neželeni učinki: kašelj (40,2 % v primerjavi s 30,3 % pri uporabi placebaj, okužbe zgornjih dihal (26,1 % v primerjavi z 11,5 % pri uporabi placeba) in izpuščaj (21,7 % v primerjavi z 12,0 % pri uporabi placeba). Najbolj pogost neželeni učinek 3. – 4. stopnje je bila pljučnica (6,5 % v primerjavi s 5,6 % pri uporabi placebaj. Pojavnost vseh neželenih učinkov 3. ali 4. stopnje je bila 12,8 % v skupini z zdravilom Imfinzi v primerjavi z 9,8 % v skupini s placebom. Zelo pogosti neželeni učinki: okužbe zgornjih dihal, pljučnica, hipotiroidizem, kašelj/produktiven kašelj, pnevmonitis, driska, bolečine v trebuhu, izpuščaj, srbenje, zvišana telesna temperatura. Pogosti neželeni učinki: zelene okužbe in okužbe ustnih mehkih tkiv, oralna kandidoza, gripa, hipertiroidizem, disfonija, kožne, zvišanje vrednosti aspartat aminotransferaze ali zvišanje vrednosti alanin aminotransferaze, nočno znojenje, dermatitis, mialgija, zvišanje vrednosti kreatinina v krvi, disurija, periferni edem. z infundiranjem povezane reakcije. Občasni neželeni učinki: adrenalna insuficienca, sladkorna bolezen tipa 1. intersticijska bolezen pljuč, hepatitis, mioziti, nefritis. Redki neželeni učinki: hipofizitis / hipopituitarizem, diabetes insipidus, polimiozitis.
VRSTA IN VSEBINA OVOJNINE: 2,4 ml koncentrata v stekleni viali iz stekla tipa 1 z elastomernim zamaškom in sivo snemno aluminijsko zaporo: viala vsebuje 120 mg durvalumaba. Pakiranje vsebuje 1 viało.
10 ml koncentrata v stekleni viali iz stekla tipe 1 z elastomernim zamaškom in belo snemno aluminijsko zaporo: viala vsebuje 500 mg durvalumaba. Pakiranje vsebuje 1 viało.
NAČIN IZDAJANJA ZDRAVILA: samo na recept **DATUM REVIZIJA BESEDILA:** september 2018 [SI-0378] **IMETNIK DOVOLJENJA ZA PROMET:** AstraZeneca AB, S-151 85, Sodertalje, Švedska

Pred predpisovanjem, prosimo, preberite celotem povzetek glavnih značilnosti zdravila.
Dodatne informacije so na voljo pri družbi AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, Ljubljana.
Samo za strokovno javnost. Informacija pripravljena: september 2019.

AstraZeneca

S-0985

KEYTRUDA®
(pembrolizumab, MSD)

Pomaga spreminjati pričakovanja o preživetju

- pri metastatskem NSCLC *,1,2
- in napredovalem melanomu[3]

*NSCLC – non-small cell lung cancer

Reference: 1. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et. al.; for the KEYNOTE-189 investigators. Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. N Engl J Med. 2018;378(22):2078–2092. **2.** Keytruda EU SmPC **3.** Hamid O, Robert C, Daud A, et. al. 5-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. Annals of Oncology 2019; 30: 582-588.

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila!

▼ Za to zdravilo se izvaja dodatno spremljanje varnosti. **Ime zdravila:** KEYTRUDA 25 mg/ml koncentrat za raztopino za infundiranje vsebuje pembrolizumab. **Terapevtske indikacije:** Zdravilo KEYTRUDA je kot samostojno zdravljenje indicirano za zdravljenje: napredovalega (neoperabilnega ali metastatskega) melanoma pri odraslih; za adjuvantno zdravljenje odraslih z melanomom v stadiju III, ki se je razširil na bezgavke, po popolni kirurški odstranitvi; metastatskega nedrobnoceličnega pljučnega raka (NSCLC) v prvi liniji zdravljenja pri odraslih, ki imajo tumorje z ≥ 50 % izraženostjo PD-L1 (TPS) in brez pozitivnih tumorskih mutacij EGFR ali ALK; lokalno napredovalega ali metastatskega NSCLC pri odraslih, ki imajo tumorje z ≥ 1 % izraženostjo PD-L1 (TPS) in so bili predhodno zdravljeni z vsaj eno shemo kemoterapije, bolniki s pozitivnimi tumorskimi mutacijami EGFR ali ALK so pred prejemom zdravila KEYTRUDA morali prejeti tudi tarčno zdravljenje; odraslih bolnikov s ponovljenim ali neodzivnim klasičnim Hodgkinovim limfomom (cHL), pri katerih avtologna presaditev matičnih celic (ASCT) in zdravljenje z brentuksimabom vedotinom (BV) nista bila uspešna, in odraslih bolnikov, ki za presaditev niso primerni, zdravljenje z BV pa pri njih ni bilo uspešno; lokalno napredovalega ali metastatskega urotelijskega raka pri odraslih, predhodno zdravljenih s kemoterapijo, ki je vključevala platino; lokalno napredovalega ali metastatskega urotelijskega raka pri odraslih, ki niso primerni za zdravljenje s kemoterapijo, ki vsebuje cisplatin in imajo tumorje z izraženostjo PD-L1 ≥ 10, ocenjeno s kombinirano pozitivno oceno (CPS); ponovljenega ali metastatskega ploščatoceličnega raka glave in vratu (HNSCC) pri odraslih, ki imajo tumorje z ≥ 50 % izraženostjo PD-L1 (TPS), in pri katerih je bolezen napredovala med zdravljenjem ali po zdravljenju s kemoterapijo, ki je vključevala platino. Zdravilo KEYTRUDA je kot samostojno zdravljenje ali v kombinaciji s kemoterapijo s platino in 5-fluorouracilom (5-FU) indicirano za prvo linijo zdravljenja metastatskega ali neoperabilnega ponovljenega ploščatoceličnega raka glave in vratu pri odraslih, ki imajo tumorje z izraženostjo PD-L1 s CPS ≥ 1. Zdravilo KEYTRUDA je v kombinaciji s pemetreksedom in kemoterapijo na osnovi platine indicirano za prvo linijo zdravljenja metastatskega neploščatoceličnega NSCLC pri odraslih, pri katerih tumorji nimajo pozitivnih mutacij EGFR ali ALK; v kombinaciji s karboplatinom in bodisi paklitakselom bodisi nab-paklitakselom je indicirano za prvo linijo zdravljenja metastatskega ploščatoceličnega NSCLC pri odraslih; v kombinaciji z aksitinibom je indicirano za prvo linijo zdravljenja napredovalega raka ledvičnih celic (RCC) pri odraslih. **Odmerjanje in način uporabe:** Testiranje PD-L1 pri bolnikih z NSCLC, urotelijskim rakom ali HNSCC: Pri bolnikih z NSCLC je priporočljivo opraviti testiranje izraženosti PD-L1 tumorja z validirano preiskavo. Bolnike s HNSCC ali predhodno nezdravljenim urotelijskim rakom je treba za zdravljenje izbrati na podlagi izraženosti PD-L1, potrjene z validirano preiskavo. Odmerjanje: Priporočeni odmerek zdravila KEYTRUDA za samostojno zdravljenje je bodisi 200 mg na 3 tedne ali 400 mg na 6 tednov, apliciran z intravensko infuzijo v 30 minutah. Priporočeni odmerek za kombinirano zdravljenje je 200 mg na 3 tedne, apliciran z intravensko infuzijo v 30 minutah. Za uporabo v kombinaciji glejte povzetke glavnih značilnosti sočasno uporabljenih zdravil. Če se uporablja kot del kombiniranega zdravljenja skupaj z intravensko kemoterapijo, je treba zdravilo KEYTRUDA aplicirati prvo. Bolnike je treba zdraviti do napredovanja bolezni ali nesprejemljivih toksičnih učinkov. Pri adjuvantnem zdravljenju melanoma je treba zdravilo uporabljati do ponovitve bolezni, pojava nesprejemljivih toksičnih učinkov oziroma mora zdravljenje trajati do enega leta. Če je aksitinib uporabljen v kombinaciji s pembrolizumabom, se lahko razmisli o povečanju odmerka aksitiniba nad začetnih 5 mg v presledkih šest tednov ali več. Pri bolnikih starih ≥ 65 let, bolnikih z blago do zmerno okvaro ledvic, bolnikih z blago okvaro jeter prilagoditev odmerka ni potrebna. ***Odložitev odmerka ali ukinitev zdravljenja:*** Za primere, kjer je treba zdravljenje zadržati, dokler se neželeni učinki ne zmanjšajo na stopnjo 0-1 in kadar je treba zdravilo KEYTRUDA trajno ukiniti, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. **Kontraindikacije:** Preobčutljivost na učinkovino ali katero koli pomožno snov. **Povzetek posebnih opozoril, previdnostnih ukrepov, interakcij in neželenih učinkov:** Imunsko pogojeni neželeni učinki (pnevmonitis, kolitis, hepatitis, nefritis, endokrinopatije, neželeni učinki na kožo in drugi): Pri bolnikih, ki so prejemali pembrolizumab, so se pojavili imunsko pogojeni neželeni učinki, vključno s hudimi in smrtnimi primeri. Večina imunsko pogojenih neželenih učinkov, ki so se pojavili med zdravljenjem s pembrolizumabom, je bila reverzibilnih in so jih obvladali s prekinitvami uporabe pembrolizumaba, uporabo kortikosteroidov in/ali podporno oskrbo. Pojavijo se lahko tudi po zadnjem odmerku pembrolizumaba in hkrati prizadanejo več organskih sistemov. V primeru suma na imunsko pogojene neželene učinke je treba poskrbeti za ustrezno oceno za potrditev etiologije oziroma izključitev drugih vzrokov. Glede na izraženost neželenega učinka je treba zadržati uporabo pembrolizumaba in uporabiti kortikosteroide – za natančna navodila, prosimo, glejte Povzetek glavnih značilnosti zdravila Keytruda. Zdravljenje s pembrolizumabom lahko poveča tveganje za zavrnitev pri prejemnikih presadkov čvrstih organov. Pri bolnikih, ki so prejemali pembrolizumab, so poročali o hudih z infuzijo povezanih reakcijah, vključno s preobčutljivostjo in anafilaksijo. Pembrolizumab se iz obtoka odstrani s katabolizmom, zato presnovnih medsebojnih delovanj zdravil ni pričakovati. Uporabi sistemskih kortikosteroidov ali imunosupresivov pred uvedbo pembrolizumaba se je treba izogibati, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost pembrolizumaba. Vendar pa je kortikosteroide ali druge imunosupresive mogoče uporabiti za zdravljenje imunsko pogojenih neželenih učinkov. Kortikosteroide je mogoče uporabiti tudi kot premedikacijo, če je pembrolizumab uporabljen v kombinaciji s kemoterapijo, kot antiemetično profilakso in/ali za ublažitev neželenih učinkov, povezanih s kemoterapijo. Ženske v rodni dobi morajo med zdravljenjem s pembrolizumabom in vsaj še 4 mesece po zadnjem odmerku pembrolizumaba uporabljati učinkovito kontracepcijo, med nosečnostjo in dojenjem se ga ne sme uporabljati. Varnost pembrolizumaba pri samostojnem zdravljenju so v kliničnih študijah ocenili pri 5.884 bolnikih z napredovalim melanomom, kirurško odstranjenim melanomom v stadiju III (adjuvantno zdravljenje), NSCLC, cHL, urotelijskim rakom ali HNSCC s štirimi odmerki (2 mg/kg na 3 tedne, 200 mg na 3 tedne in 10 mg/kg na 2 ali 3 tedne). V tej populaciji bolnikov je mediani čas opazovanja znašal 7,3 mesece (v razponu od 1 dneva do 31 mesecev), najpogostejši neželeni učinki zdravljenja s pembrolizumabom so bili utrujenost (32 %), navzea (20 %) in diareja (20 %). Večina poročanih neželenih učinkov pri samostojnem zdravljenju je bila po izraženosti 1. ali 2. stopnje. Najresnejši neželeni učinki so bili imunsko pogojeni neželeni učinki in hude z infuzijo povezane reakcije. Varnost pembrolizumaba pri kombiniranem zdravljenju s kemoterapijo so ocenili pri 1.067 bolnikih NSCLC ali HNSCC, ki so v kliničnih študijah prejemali pembrolizumab v odmerkih 200 mg, 2 mg/kg ali 10 mg/kg na vsake 3 tedne. V tej populaciji bolnikov so bili najpogostejši neželeni učinki naslednji: anemija (50 %), navzea (50 %), utrujenost (37 %), zaprtost (35%), diareja (30 %), nevtropenija (30 %), zmanjšanje apetita (28 %) in bruhanje (25 %). Pri kombiniranem zdravljenju s pembrolizumabom je pri bolnikih z NSCLC pojavnost neželenih učinkov 3. do 5. stopnje znašala 67 %, pri zdravljenju samo s kemoterapijo pa 66 %, pri kombiniranem zdravljenju s pembrolizumabom pri bolnikih s HNSCC 85 % in pri zdravljenju s kemoterapijo v kombinaciji s cetuksimabom 84 %. Varnost pembrolizumaba v kombinaciji z aksitinibom so ocenili v klinični študiji pri 429 bolnikih z napredovalim rakom ledvičnih celic, ki so prejemali 200 mg pembrolizumaba na 3 tedne in 5 mg aksitiniba dvakrat na dan. V tej populaciji bolnikov so bili najpogostejši neželeni učinki diareja (54 %), hipertenzija (45 %), utrujenost (38 %), hipotiroidizem (35 %), zmanjšan apetit (30 %), sindrom palmarno-plantarne eritrodisestezije (28 %), navzea (28 %), zvišanje vrednosti ALT (27 %), zvišanje vrednosti AST (26 %), disfonija (25 %), kašelj (21 %) in zaprtost (21 %). Pojavnost neželenih učinkov 3. do 5. stopnje je bila med kombiniranim zdravljenjem s pembrolizumabom 76 % in pri zdravljenju s sunitinibom samim 71 %. Za celoten seznam neželenih učinkov, prosimo, glejte celoten Povzetek glavnih značilnosti zdravila. **Način in režim izdaje zdravila:** H – Predpisovanje in izdaja zdravila je samo na recept, zdravilo se uporablja samo v bolnišnicah. **Imetnik dovoljenja za promet z zdravilom:** Merck Sharp & Dohme B.V., Waarderweg 39, 2031 BN Haarlem, Nizozemska. **Datum zadnje revizije besedila**: 14. november 2019.

MSD

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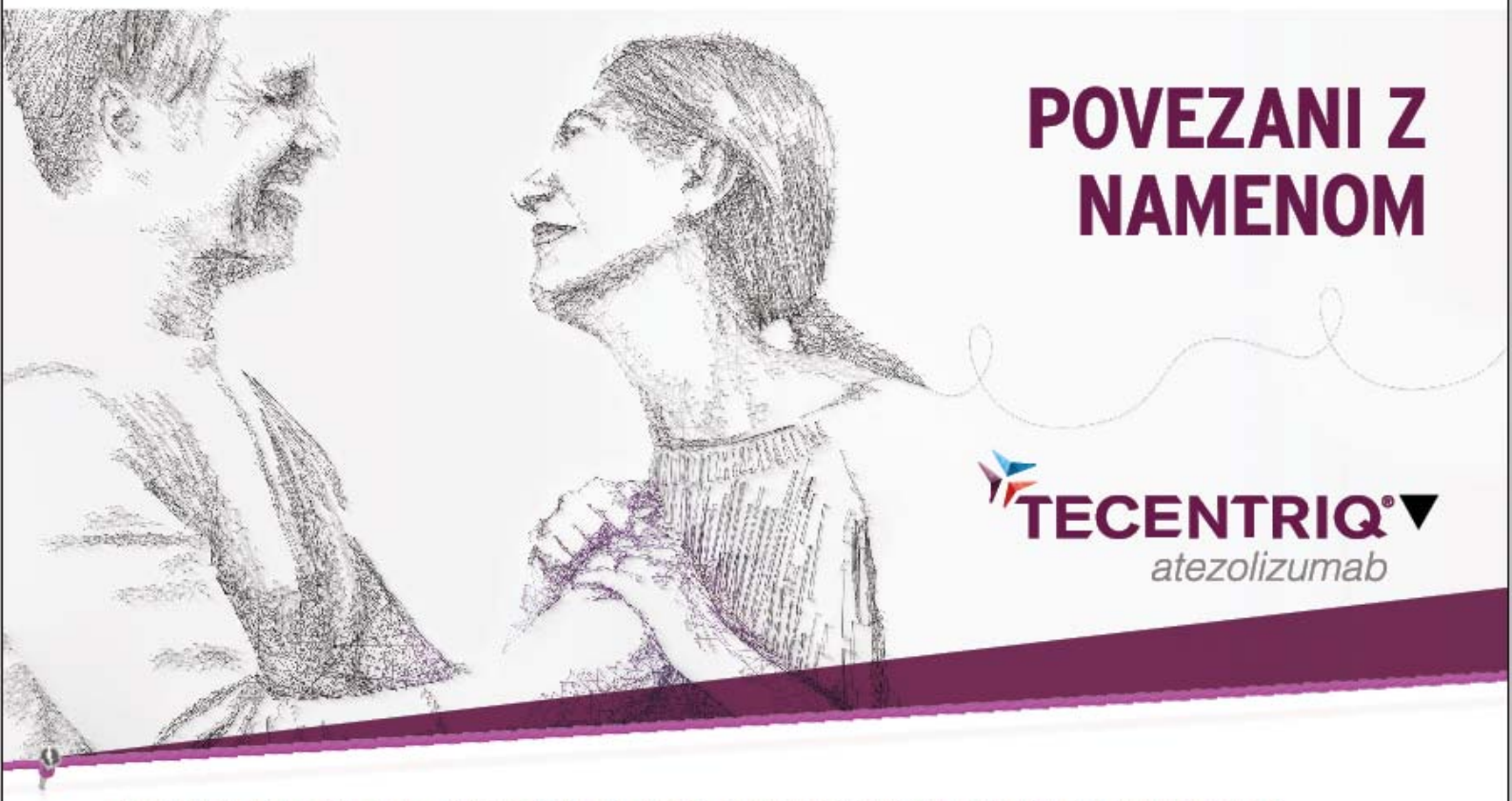
Samo za strokovno javnost. H - Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila Keytruda, ki je na voljo pri naših strokovnih sodelavcih ali na lokalnem sedežu družbe.



POVEZANI Z NAMENOM

TECENTRIQ® ▼
atezolizumab

ZDRAVILO TECENTRIQ JE INDICIRANO ZA ZDRAVLJENJE RAZLIČNIH VRST RAKA:

- NEDROBNOCELIČNI RAK PLJUČ
- DROBNOCELIČNI RAK PLJUČ
- TROJNO NEGATIVNI RAK DOJK
- UROTELIJSKI KARCINOM

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA TECENTRIQ

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

Ime zdravila: Tecentriq 840 mg/1200 mg koncentrat za raztopino za infundiranje. **Kakovostna in količinska sestava:** 840 mg: ena 14-ml viala s koncentratom vsebuje 840 mg atezolizumaba. Po razredčenju 1 ml raztopine vsebuje približno 3,2 mg atezolizumaba v 840-mg odmerku in približno 6 mg atezolizumaba v 1680-mg odmerku. 1200 mg: ena 20-ml viala s koncentratom vsebuje 1200 mg atezolizumaba. Po razredčenju 1 ml raztopine vsebuje približno 4,4 mg atezolizumaba. Atezolizumab je humanizirano monoklonsko protitelo IgG1 z inženirsko obdelano domeno Fc, ki je pridobljeno iz celic jajčnika kitajskega hrčka s tehnologijo rekombinantne DNA in deluje na ligando programirano celično smrt 1 (PD-L1). **Terapevtske indikacije:** Urotelijski karcinom (840 mg in 1200 mg): Zdravilo Tecentriq je kot monoterapija indicirano za zdravljenje odraslih bolnikov z lokalno napredovalim ali razsejanim urotelijskim karcinomom, ki: so bili predhodno zdravljeni s kemoterapijo na osnovi platine ali niso primerni za zdravljenje s cisplatinom in katerih tumorji izražajo PD-L1 v ≥ 5 %. Nedrobnocelični rak pljuč (le za 1200 mg): Zdravilo Tecentriq je v kombinaciji z bevacizumabom, paklitakselom in karboplatinom indicirano kot prva linija zdravljenja odraslih bolnikov z razsejanim neploščatoceličnim nedrobnoceličnim rakom pljuč (NDRP). Pri bolnikih z EGFR mutiranim ali ALK pozitivnim NDRP je zdravilo Tecentriq v kombinaciji z bevacizumabom, paklitakselom in karboplatinom indicirano le, ko so ic črpana ustrezna tarčna zdravljenja. Nedrobnocelični rak pljuč (840 mg in 1200 mg): Zdravilo Tecentriq je kot monoterapija indicirano za zdravljenje odraslih bolnikov z lokalno napredovalim ali razsejanim NDRP, ki so bili predhodno zdravljeni s kemoterapijo. Bolnikiz EGFR mutiranim ali ALK pozitivnim NDRP morajo pred uvedbo zdravila Tecentriq prejeti tudi tarčno zdravljenja. Nedrobnocelični rak pljuč (le za 1200 mg): Zdravilo Tecentriq je v kombinaciji s karboplatinom in etoposidom indicirano kot prva linija zdravljenja odraslih bolnikov z razsejanim drobnoceličnim rakom pljuč (DRP). Rak dojk (le za 840 mg): Zdravilo Tecentriq je v kombinaciji z nab-paklitakselom indicirano za zdravljenje odraslih bolnikov z inoperabilnim lokalno napredovalim ali razsejanim trojno negativnim rakom dojk (TNRD), katerih tumorji izražajo PD-L1 v ≥ 1 % in predhodno še niso prejemali kemoterapije zaradi razsejane bolezni. **Odmerjanje in način uporabe:** Zdravilo Tecentriq moraja uvesti in nadzorovati zdravnik z izkušnjami pri zdravljenju raka. Testiranje PD-L1 pri bolnikih z urotelijskim karcinomom in TNRB: bolnike s predhodno neodstranljivim urotelijskim karcinomom in TNRD je treba za zdravljenje izbrati na podlagi tumorskega izražanja PD-L1, pohranjenega z validirano preiskavo. Odmerjanje: *Zdravilo Tecentriq v monoterapiji:* 840 mg: priporočeni odmerek zdravila Tecentriq je 840 mg intravensko na vsaka dva tedna ali 1680 mg intravensko na vsake štiri tedne. 1200 mg: priporočeni odmerek zdravila Tecentriq je 1200 mg intravensko na vsake tri tedne. *Zdravilo Tecentriq v kombinaciji: Prva linija zdravljenja neploščatoceličnega NDRP: Zdravilo Tecentriq v kombinaciji z bevacizumabom, paklitakselom in karboplatinom:* Med uvodno fazo je priporočeni odmerek zdravila Tecentriq 1200 mg v intravenski infuziji, čemur sledijo bevacizumab, paklitaksel in nato karboplatin na tri tedne, skupno štiri ali šest ciklov. Uvodni fazi zdravljenja sledi faza vzdrževanja brez kemoterapije, med katero se na tri tedne uporabi zdravila Tecentriq odmerku 1200 mg v intravenski infuziji aplicira na tri tedne. *Zdravilo Tecentriq v kombinaciji z nab-paklitakselom in karboplatinom:* Med uvodno fazo je priporočeni odmerek zdravila Tecentriq 1200 mg v intravenski infuziji, čemur sledita nab-paklitaksel in nato karboplatin na vsake tri tedne, skupna štiri ali šest ciklov. Uvodni fazi zdravljenja sledi faza vzdrževanja brez kemoterapije, med katero se zdravilo Tecentriq v odmerku 1200 mg v intravenski infuziji aplicira na tri tedne. *Prva linija zdravljenja razsejanega DRP: Zdravilo Tecentriq v kombinaciji z karboplatinom in etoposidom:* Med uvodno faze je 1. dan cikla priporočeni odmerek zdravila Tecentriq 1200 mg v intravenski infuziji, čemur sledita najprej karboplatin in nato etoposid v intravenski infuziji. Etoposid v intravenski infuziji se aplicira tudi 2. in 3. dan. Ta shema se aplicira na vsake tri tedne v skupno štirih ciklih. Uvodni fazi sledi faza vzdrževanja brez kemoterapije, med katero se zdravilo Tecentriq odmerka 1200 mg v intravenski infuziji aplicira na tri tedne. *Zdravilo Tecentriq v kombinaciji z nab-paklitakselom v 1. liniji razsejanega TNRD:* Priporočeni odmerek zdravila Tecentriq je 840 mg v intravenski infuziji, ki ji sledi 100 mg/m² nab-paklitaksela. V vsakem 28-dnevnem ciklu se zdravilo Tecentriq uporabi 1., in 15. dan, nab-paklitaksel pa 1., 8. in 15. dan. *Trajanje zdravljenja:* zdravljenje z zdravilom Tecentriq je priporočljivo nadaljevati, dokler je klinično koristno ali dokler se ne pojavijo nesprejemljivi toksični učinki. *Zapuščeni ali opuščeni odmerki:* v primeru izpuščenega načrtovanega odmerka zdravila Tecentriq je treba odmerek dati čim prej. Umik dajanja zdravila je treba nato prilagoditi ustreznemu presledku med odmerki. *Prilagoditev odmerka med zdravljenjem:* odmerkov zdravila Tecentriq ni priporočljivo zmanjševati. *Zapustitev odmerka ali prenehanje uporabe* glede na neželeni učinek je opisano v SmPC. Posebne populacije bolnikov: Starejši: glede na populacijsko farmakokinetično analizo bolnikom v starosti ≥ 65 let odmerka zdravila Tecentriq ni treba prilagoditi. *Način uporabe:* zdravilo Tecentriq je namenjeno za intravensko uporabo. Infuzij ne se sme dajati kot hiter intravenski odmerek ali bolus. Začetni odmerek zdravila Tecentriq je treba dotiv 60 minutah. Če bolnik prvo infuzijo dobro prenese, je mogoče vse nadaljnje infuzije dati v 30 minutah. **Kontraindikacije:** Preobčutljivost na atezolizumab ali katero koli pomožno snov. **Posebna opozorila in previdnostni ukrepi:** Sledljivost: Za izboljšanje sledljivosti bioloških zdravil je treba tastniško ime in številko serije aporabljenega zdravila jasno zabeležiti v bolnikovi dokumentaciji. Imunsko pogojeni neželeni učinki: Večina imunsko pogojenih neželenih učinkov, ki so se pojavili med zdravljenjem z atezolizumabom, je bila po prekinitvi atezolizumaba in uvedbi kortikosteroidov in/ali podpornega zdravljenja reverzibilna. Opazili s imunsko pogojene neželene učinke, ki vplivajo na več kot en organski sistem. Imunsko pogojeni neželeni učinki, povezani z atezolizumabom, se lahko pojavijo po zadnjem odmerku atezolizumaba. Pri sumu na imunsko pogojene neželene učinke je treba opraviti temeljito oceno za potrditev etiologije s izrome izključitev drugih vzrokov. Glede na izrazitost neželenega učinka je treba uporabo atezolizumaba odložiti in uvesti kortikosteroide. Atezolizumab je treba trajno prenehati uporabljati pri vseh imunsko pogojenih neželenih učinkih 3. stopnje, ki se ponovijo, in pri vseh imunsko pogojenih neželenih učinkih 4. stopnje, z izjemo endokrinopatij, ki jih je mogoče nadzorovati z nadomestnimi hormoni. Imunsko pogojeni pnevmonitis: v kliničnih preskušanjih atezolizumaba so opažali primere pnevmonitisa, vključno s smrtnimi primeri. Bolnike je treba spremljati glede znakov in simptomov pnevmonitisa. Imunsko pogojeni hepatitis: v kliničnih preskušanjih atezolizumaba so opažali primere hepatitisa, nekatere s smrtnim izidom. Bolnike je treba spremljati glede znakov in simptomov hepatitisa. Vrednosti AST, ALT in bilirubina je treba spremljati pred začetkom zdravljenja z atezolizumabom, tedno med zdravljenjem in kot je potrebno glede na klinično oceno. Imunsko pogojeni kolitis: v kliničnih preskušanjih atezolizumaba so opažali primere diareje ali kolitisa. Bolnike je treba spremljati glede znakov in simptomov kolitisa. Imunsko pogojene endokrinopatije: v kliničnih preskušanjih atezolizumaba so opažali hipotiroidizem, hipertiroidizem, insuficienco nadledvičnih žlez, hipofizitis in sladkorna bolezen tipa 1, vključno z diabetično ketoacidozo. Bolnike je treba spremljati glede znakov in simptomov endokrinopatij. Imunska pogojeni meningoencefalitis: v kliničnih preskušanjih atezolizumabom so spažali meningoencefalitis. Bolnike je treba spremljati glede znakov in simptomov meningitisa ali encefalitisa. V primeru meningitisa ali encefalitisa je treba zdravljenje z atezolizumabom trajno ukiniti ne glede na njuno stopnjo. Imunska pogojene nevropatije: pri bolnikih, zdravljenihz atezolizumabom, so spažali miastenijski sindrom/miastenijo gravis ali Guillain-Barréjev sindrom, ki je lahkoživljenje ogrožujoč. Bolnike je treba spremljati glede znakov in simptomov motorične in senzorične nevropatije. V primeru miastenijskega sindroma/miastenije gravis ali Guillain-Barréjevega sindroma je treba zdravljenje z atezolizumabom trajno prekiniti ne glede na njihova stopnja. Imunsko pogojeni pankreatitis: v kliničnih preskušanjih z atezolizumabom so spažili pankreatitis, vključno z zvišanjem amilaze in lipaze v serumu. Bolnike je treba nadzorovati glede znakov in simptomov, ki kažejo na akutni pankreatitis. Imunsko pogojeni miokarditis: v kliničnih preskušanjih atezolizumabom so spažili miokarditis. Bolnike je treba nadzorovati glede znakov in simptomov, ki kažejo na miokarditis. Imunsko pogojeni nefritis: v kliničnih preskušanjih z atezolizumabom so opažili nefritis. Bolnike je treba nadzorovati glede sprememb v delovanju ledvic. Imunsko pogojeni miozitis: V kliničnih preskušanjih atezolizumabom so opažili primere miozitisa, vključno s smrtnimi primeri. Bolnike je treba nadzorovati glede znakov in simptomov, ki kažejo na miozitis. Z infundiranjem povezane reakcije: pri zdravljenju z atezolizumabom so spažali z infundiranjem povezane reakcije. Pri bolnikih, ki imajo z infundiranjem povezane reakcije 1. ali 2. stopnje, je treba hitrost infundiranja zmanjšati ali zdravljenje prekiniti. Pri bolnikih, ki imajo z infundiranjem povezane reakcije 3. ali 4. stopnje, je treba zdravljenje z atezolizumabom trajno ukiniti. Bolniki, ki imajo z infundiranjem povezane reakcije 1. ali 2. stopnje, lahko še naprej prejemajo atezolizumab pod natančnim nadzorom; v poštev pride premedikacija z antipiretikom in antihistaminikom. Bolniki, ki niso bili vključeni v klinična preskušanja: v klinična preskušanja niso bili vključeni bolniki z naslednjimi stanji: z anamnezo avtoimunske bolezni, anamnezo pnevmonitisa, simptomatskimi možganskimi zasevki, okužbo z virusom HIV, s hepatitisom B ali hepatitisom C, pomembnimi sočasnimi boleznimi ter bolniki z neradostnim hematološkimi delovanjem in delovanjem končnih organov. Prav tako v klinična preskušanjanso bili vključeni bolniki, ki so bili obdobju 28 dni predvključitvijo v študijo cepljeni z živim oslabljenim cepivom, ki so v obdobju 4 tednov predvključitvijo v študijo dobili sistemsko imunostimulacijska sredstva ali v obdobju 2 tednov sistemska imunosupresivna zdravila. Opozorilna kartica za bolnika: Vsi zdravniki, ki predpisujejo zdravilo Tecentriq, morajo biti dobro seznanjeni z informacijami za zdravnika in Smernicami za vodenje bolnikov. Zdravnik, ki predpiše zdravilo, se mora z bolnikom pogovoriti o tveganjih zdravljenja z zdravilom Tecentriq. Bolnika je treba dati Opozorilno karticoza bolnika in mu naročiti, naj jo ima ednо pri sebi. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Formalnih študij farmakokinetičnega medsebojnega delovanja zdravila z atezolizumabom nisa uvedli. Ker se atezolizumab odstrani iz obtoka s katabolizmom, ni pričakovati presnovnih medsebojnih delovanj med zdravili. Uporabi sistemskih kortikosteroidov ali imunosupresiw se je pred uvedbo atezolizumaba treba izogibati, ker lahko vplivajo na farmakodinamično aktivnost in učinkovitost atezolizumaba. Vendar pa se sistemske kortikosteroide ali druge imunosupresivne lahko uporabi poz začetku zdravljenja z atezolizumabom za zdravljenje imunsko pogojenih neželenih učinkov. **Neželeni učinki:** povzetek neželenih učinkov, ki so se v kliničnih preskušanjih pojavili bolnikom, zdravljenim z atezolizumabom. *Zelo pogosti:* okužba sečil, okužba pljuč, anemija, trombocitopenija, nevtropenija, levkopenija, hipotiroidizem, zmanjšan apetit, periferna nevropatija, omotica, glavobol, kašelj, dispneja, navzea, bruhanje, diareja, zaprtost, izpuščaj, srbenje, artralgija, bolečina v hrbtu, mišično-skeletna bolečina, zvišana telesna temperatura, utrujenost in astenija. *Pogosti:* trombocitopenija, zmanjšano število limfocitov, z infundiranjem povezane reakcije, hipotiroidizem, hipokaliemija, hiponatriemija, hiperglikemija, hipomagneziemija, sinkopa, hipotenzija, pnevmonitis, hipoksija, zamašenost nosu, nazofaringitis, distonija, bolečine v trebuhu, kolitis, disfagija, orofaringealna bolečina, stomatitis, dingevzija, zvišanje AST, zvišanje ALT, hepatitis, proteinurija, gripi podobna bolezen in mrzlica. Poročanje o domnevnih neželenih učinkih: Poročanje o domnevnih neželenih učinkih zdravila po izdeji dovoljenja za promet je pomembno. Omogoča nenreč stalno spremljanje razmerja med koristmi in tveganj zdravila. Od zdravstvenih delavcev se zahteva, da poročajo o katerem koli domnevnem neželenem učinku zdravila na: Javna agencija Republike Slovenije za zdravila in medicinske pripomočke, Sektor za farmakovigilanco, Nacionalni center za farmakovigilanco, Slovenčeva ulica 22, SI-1000 Ljubljana, Tel: +386 (0)8 2000 500, Faks: +386 (0)8 2000 510, e-pošta: h-farmakovigilanca@jazmp.si, spletna stran: **www.jazmp.si.** Za zagotavljanje sledlji osti zdravila je pomembno, da pri izpolnjevanju obrazca o domnevnih neželenih učinkih zdravila navedete število serije biološkega zdravila. **Režim izdaje zdravila:** R. **Imetnik dovoljenja za promet:** Roche Registration GmbH, Emil-Barell-Strasse 1, 79639 Grenzach-Wyhlen, Nemčija. **Verzija:** 1.0/19. **Informacija pripravljena:** januar 2020.

PM-014-2020-HCH

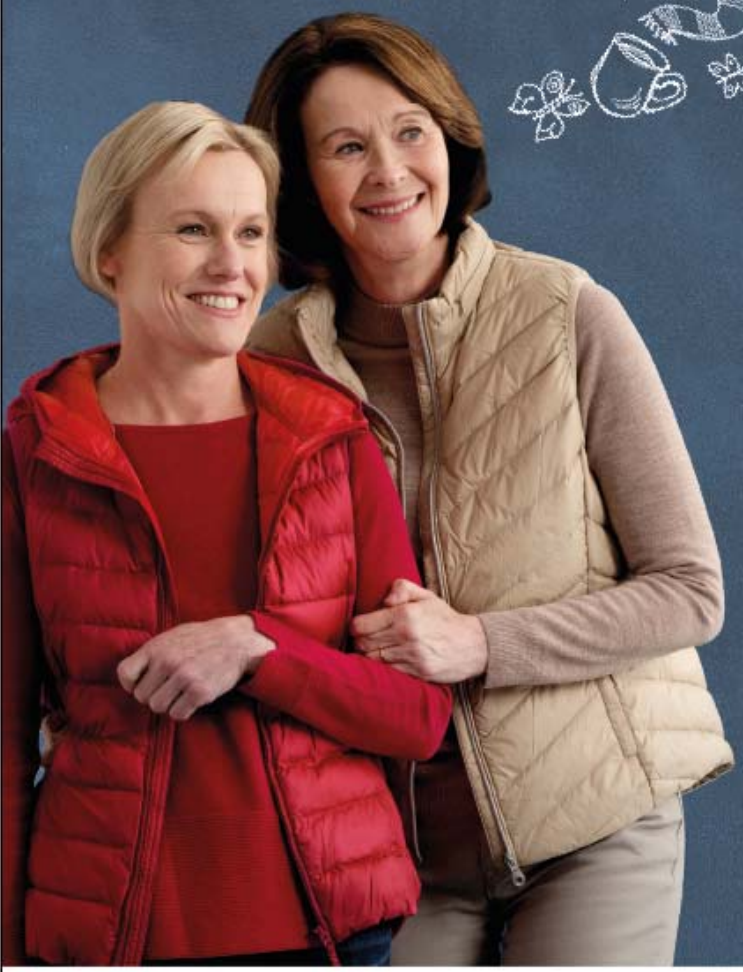
DODATNE INFORMACIJE SO NA VOLJO PRI: Roche farmacevtska družba d.o.o., Stegne 13g, 1000 Ljubljana

Samo za strokovno javnost.

Lonsurf®

trifluridin/tipiracil

Več časa za trenutke, ki štejejo



Kolorektalni rak

Zdravilo Lonsurf je indicirano v monoterapiji za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom (KRR), ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenje z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF – Vascular Endothelial Growth Factor) in zaviralci receptorjev za epidermalni rastni dejavnik (EGFR – Epidermal Growth Factor Receptor).

Rak želodca

Zdravilo Lonsurf je indicirano v monoterapiji za zdravljenje odraslih bolnikov z metastatskim rakom želodca vključno z adenokarcinomom gastro-ezofagealnega prehoda, ki so bili predhodno že zdravljeni z najmanj dvema sistemskima režimoma zdravljenja za napredovalo bolezen

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SERVIER

Skrajšan povzetek glavnih značilnosti zdravila: Lonsurf 15 mg/6,14 mg filmsko obložene tablete in Lonsurf 20 mg/8,19 mg filmsko obložene tablete

▼Za to zdravilo se izvaja dodatno spremljanje varnosti. Tako bodo hitreje na voljo nove informacije o njegovi varnosti. Zdravstvene delavce naprošamo, da poročajo o katerem koli domnevnem neželenem učinku zdravila. **SESTAVA*: Lonsurf 15 mg/6,14 mg:** Ena filmsko obložena tableta vsebuje 15 mg trifluridina in 6,14 mg tipiracila (v obliki klorida). **Lonsurf 20 mg/8,19 mg:** Ena filmsko obložena tableta vsebuje 20 mg trifluridina in 8,19 mg tipiracila (v obliki klorida). **TERAPEVTSKE INDIKACIJE*:** Kolorektalni rak – zdravilo Lonsurf je indicirano v monoterapiji za zdravljenje odraslih bolnikov z metastatskim kolorektalnim rakom, ki so bili predhodno že zdravljeni ali niso primerni za zdravljenja, ki so na voljo. Ta vključujejo kemoterapijo na osnovi fluoropirimidina, oksaliplatina in irinotekana, zdravljenja z zaviralci žilnega endotelijskega rastnega dejavnika (VEGF – Vascular Endothelial Growth Factor) in zaviralci receptorjev za epidermalni rastni dejavnik (EGFR – Epidermal Growth Factor Receptor). Rak želodca – zdravilo Lonsurf je indicirano v monoterapiji za zdravljenje odraslih bolnikov z metastatskim rakom želodca vključno z adenokarcinomom gastro-ezofagealnega prehoda, ki so bili predhodno že zdravljeni z najmanj dvema sistemskima režimoma zdravljenja za napredovalo bolezen. **ODMERJANJE IN NAČIN UPORABE*:** Priporočeni začetni odmerek zdravila Lonsurf pri odraslih je 35 mg/m²/odmerek peroralno dvakrat dnevno na 1. do 5. dan in 8. do 12. dan vsakega 28-dnevnega cikla zdravljenja, najpozneje 1 uro po zaključku jutranjega in večernega obroka. Odmerjanje, izračunano glede na telesno površino, ne sme preseči 80 mg/odmerek. Možne prilagoditve odmerka glede na varnost in prenašanje zdravila: dovoljena so največ 3 zmanjšanja odmerka na najmanjši odmerek 20 mg/m² dvakrat dnevno. Potem ko je bil odmerek zmanjšan, povečanje ni dovoljeno. **KONTRAINDIKACIJE*:** Preobčutljivost na zdravilni učinkovini ali katero koli pomožno snov. **OPOZORILA IN PREVIDNOSTNI UKREPI*:** Supresija kostnega mozga: Pred uvedbo zdravljenja in po potrebi za spremljanje toksičnosti zdravila, najmanj pred vsakim ciklom zdravljenja, je treba pregledati celotno krvno sliko. Zdravljenja ne smete začeti, če je absolutno število nevtrofilcev < 1,5 x 10⁹/l, če je število trombocitov < 75 x 10⁹/l ali če se je pri bolniku zaradi predhodnih zdravljenj pojavila klinično pomembna nehematološka toksičnost 3. ali 4. stopnje, ki še traja. Bolnike je treba skrbno spremljati zaradi morebitnih okužb, uvesti je treba ustrezne ukrepe, kot je klinično indicirano. Toksičnost za prebavila: Potrebna je uporaba antiemetikov, antidiaroikov ter drugih ukrepov, kot je klinično indicirano. Če je potrebno, prilagodite odmerke. Ledvična okvara: Zdravilo Lonsurf ni primerno za uporabo pri bolnikih s hudo ledvično okvaro ali končno stopnjo ledvične okvare. Bolnike z ledvično okvaro je potrebno med zdravljenjem skrbno spremljati; bolnika z zmerno ledvično okvaro je treba zaradi hematološke toksičnosti bolj pogosto spremljati. Jetrna okvara: Uporaba zdravila Lonsurf pri bolnikih z obstoječo zmerno ali hudo jetrno okvaro ni priporočljiva. Proteinurija: Pred začetkom zdravljenja in med njim je priporočljivo spremljanje proteinurije z urinskimi testnimi lističi. Pomožne snovi: Zdravilo vsebuje laktozo. **INTERAKCIJE*:** Zdravila, ki medsebojno delujejo z nukleozidnimi prenašalci CNT1, ENT1 in ENT2, zaviralci OCT2 ali MATE1, substrati humane timidin-kinaze (npr. zidovudinom), hormonskimi kontraceptivi. **PLODNOST*, NOSEČNOST IN DOJENJE*:** Ni priporočljivo. **KONTRACEPCIJA*:** Ženske in moški morajo uporabljati učinkovito metodo kontracepcije med zdravljenjem in do 6 mesecev po zaključku zdravljenja. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA STROJEV*:** Med zdravljenjem se lahko pojavijo utrujenost, omotica ali splošno slabo počutje. **NEŽELENI UČINKI*:** *Zelo pogosti:* nevtropenija, levkopenija, anemija, trombocitopenija, zmanjšan apetit, diareja, navzea, bruhanje, utrujenost. *Pogosti:* okužba spodnjih dihal, febrilna nevtropenija, limfopenija, hipoalbuminemija, disgevzija, periferna nevropatija, dispneja, bolečina v trebuhu, zaprtja, stomatitis, bolezni ustne votline, hiperbilirubinemija, sindrom palmarne plantarne eritrodisestezije, izpuščaj, alopecija, pruritus, suha koža, proteinurija, pireksija, edem, vnetje sluznice, splošno slabo počutje, zvišanje jetrnih encimov, zvišanje alkalne fosfataze v krvi, zmanjšanje telesne mase. *Občasni:* septični šok, infekcijski enteritis, pljučnica, okužba žolčevoda, gripa, okužba sečil, gingivitis, herpes zoster, tinea pedis, okužba s kandido, bakterijska okužba, okužba, nevtropenični sepsa, okužba zgornjih dihal, konjunktivitis, bolečina zaradi raka, pancitopenija, granulocitopenija, monocitopenija, eritropenija, levkocitoza, monocitoza, dehidracija, hiperglikemija, hiperkaliemija, hipokaliemija, hipofosfatemija, hipernatriemija, hiponatriemija, hipokalciemija, protin, anksioznost, nespečnost, nevrotoksičnost, disestezija, hiperestezija, hipoestezija, sinkopa, parestezija, pekoč občutek, letargija, omotica, glavobol, zmanjšana ostrina vida, zamegljen vid, diplopija, katarakta, suho oko, vrtoglavica, neugodje v ušesu, angina pektoris, aritmija, palpitacije, embolija, hipertenzija, hipotenzija, vročinski oblivi, pljučna embolija, plevralni izliv, izcedek iz nosu, disfonija, orofaringealna bolečina, epistaksa, kašelj, hemoragični enterokolitis, kvavitev v prebavilih, akutni pankreatitis, ascites, ileus, subileus, kolitis, gastritis, refluksni gastritis, ezofagitis, moteno praznjenje želodca, abdominalna distenzija, analno vnetje, razjede v ustih, dispepsija, gastroezofagealna refluksna bolezen, proktalgija, bukalni polip, kvavitev dlesni, glositis, parodontalna bolezen, bolezen zob, siljenje na bruhanje, flatulenca, slab zadah, hepatotoksičnost, razširitev žolčnih vodov, luščenje kože, urtikarija, preobčutljivostne reakcije na svetlobo, eritem, akne, hiperhidroza, žulj, bolezni nohtov, otekanje sklepov, artralgija, bolečina v kosteh, mialgija, mišično-skeletna bolečina, mišična oslabelost, mišični krči, bolečina v okončinah, ledvična odpoved, neinfektivni cistitis, motnje mikcije, hematurija, levkociturija, motnje menstruacije, poslabšanje splošnega zdravstvenega stanja, bolečina, občutek spremembe telesne temperature, kseroza, nelagodje, zvišanje kreatinina v krvi, podaljšanje intervala QT na elektrokardiogramu, povečanje mednarodnega umerjenega razmerja (INR), podaljšanje aktiviranega parcialnega tromboplastinskega časa (aPTČ), zvišanje sečnine v krvi, zvišanje laktatne dehidrogenaze v krvi, znižanje celokupnih proteinov, zvišanje C-reaktivnega proteina, zmanjšan hematokrit. *Post-marketinške izkušnje:* intersticijska bolezen pljuč. **PREVELIKO ODMERJANJE*:** Neželeni učinki, o katerih so poročali v povezavi s prevelikim odmerjanjem, so bili v skladu z uveljavljenim varnostnim profilom. Glavni pričakovani zaplet prevelikega odmerjanja je supresija kostnega mozga. **FARMAKODINAMIČNE LASTNOSTI*:** *Farmakoterapevtska skupina:* zdravila z delovanjem na novotvorbe, antimetaboliti, oznaka ATC: L01BC59. Zdravilo Lonsurf sestavljata antineoplastični timidinski nukleozidni analog, trifluridin, in zaviralec timidin-fosforilaze (TPaze), tipiracilijev klorid. Po privzemu v rakave celice timidin-kinaza fosforilira trifluridin. Ta se v celicah nato presnovi v substrat deoksiribonukleinske kisline (DNA), ki sevgradi neposredno v DNA ter tako preprečuje celično proliferacijo. TPaza hitro razgradi trifluridin in njegova presnova po peroralni uporabi je hitra zaradi učinka prvega prehoda, zato je v zdravilo vključen zaviralec TPaze, tipiracilijev klorid. **PAKIRANJE*:** 20 filmsko obloženih tablet. **NAČIN PREDPISOVANJA IN IZDAJE ZDRAVILA:** Rp/Spec. **Imetnik dovoljenja za promet:** Les Laboratoires Servier, 50, rue Carnot, 92284 Suresnes cedex, Francija. **Številka dovoljenja za promet z zdravilom:** EU/1/16/1096/001 (Lonsurf 15 mg/6,14 mg), EU/1/16/1096/004 (Lonsurf 20 mg/8,19 mg). **Datum zadnje revizije besedila: september 2019. *Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila. Celoten povzetek glavnih značilnosti zdravila in podrobnejše informacije so na voljo pri: Servier Pharma d.o.o., Podmilščakova ulica 24, 1000 Ljubljana, tel: 01 563 48 11, www.servier.si.**

Datum priprave informacije: november 2019.
LNF AD1 C1 19/20 Samo za strokovno javnost.

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Dent RAG, Cole P. In vitro maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95. doi: 10.1038/bjc.1981.71

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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XALKORI 200 mg, 250 mg trda kapsula

Sestava in oblika zdravila: Ena kapsula vsebuje 200 mg ali 250 mg krizotiniba. Indikacije: Monoterapija za: - prvo linijo zdravljenja odraslih bolnikov z napredovalim nedrobnoceličnim pljučnim rakom (NSCLC – *Non-Small Cell Lung Cancer*), ki je ALK (anaplastična limfomska kinaza) pozitiven; - zdravljenje odraslih bolnikov s predhodno zdravljenim, napredovalim NSCLC, ki je ALK pozitiven; - zdravljenje odraslih bolnikov z napredovalim NSCLC, ki je ROS1 pozitiven. Odmerjanje in način uporabe: Zdravljenje mora uvesti in nadzorovati zdravnik z izkušnjami z uporabo zdravil za zdravljenje rakavih bolezni. Preverjanje prisotnosti ALK in ROS1: Pri izbiri bolnikov za zdravljenje je treba pred zdravljenjem opraviti točno in validirano preverjanje prisotnosti ALK ali ROS1. Odmerjanje: Priporočeni odmerek je 250 mg dvakrat na dan (500 mg na dan), bolniki pa morajo zdravilo jemati brez prekinitev. Če bolnik pozabi vzeti odmerek, ga mora vzeti takoj, ko se spomni, razen če do naslednjega odmerka manjka manj kot 6 ur. V tem primeru bolnik pozabljenega odmerka ne sme vzeti. *Prilagajanje odmerkov:* Glede na varnost uporabe zdravila pri posameznem bolniku in kako bolnik zdravljenje prenaša, utegne biti potrebna prekinitev in/ali zmanjšanje odmerka pri bolnikih, ki se zdravijo s krizotinibom 250 mg peroralno dvakrat na dan (za režim zmanjševanja odmerka glejte poglavje 4.2 v povzetku glavnih značilnosti zdravila). Za prilagajanje odmerkov pri *hematoloških in nehematoloških toksičnosti* (povečanje vrednosti AST, ALT, bilirubina; ILD/pnevmonitis; podaljšanje intervala QTc, bradikardija, bolezni oči) glejte preglednici 1 in 2 v poglavju 4.2 povzetka glavnih značilnosti zdravila. *Okvara jeter:* Pri zdravljenju pri bolnikih z okvaro jeter je potrebna previdnost. Pri blagi okvari jeter prilagajanje začetnega odmerka ni priporočeno, pri zmerni okvari jeter je priporočeni začetni odmerek 200 mg dvakrat na dan, pri hudi okvari jeter pa 250 mg enkrat na dan (za merila glede klasifikacije okvare jeter glejte poglavje 4.2 v povzetku glavnih značilnosti zdravila). *Okvara ledvic:* Pri blagi in zmerni okvari prilagajanje začetnega odmerka ni priporočeno. Pri hudi okvari ledvic (ki ne zahteva peritonealne dialize ali hemodialize) je začetni odmerek 250 mg peroralno enkrat na dan; po vsaj 4 tednih zdravljenja se lahko poveča na 200 mg dvakrat na dan. *Starejši bolniki (≥ 65 let):* Prilagajanje začetnega odmerka ni potrebno. *Pediatrična populacija:* Varnost in učinkovitost nista bili dokazani. Način uporabe: Kapsule je treba pogoltniti cele, z nekaj vode, s hrano ali brez nje. Ne sme se jih zdrobiti, raztopiti ali odpreti. Izogibati se je treba uživanju grenivk, grenivkinega soka ter uporabi šentjanževke. Kontraindikacije: Preobčutljivost na krizotinib ali katerokoli pomožno snov. Posebna opozorila in previdnostni ukrepi: Določanje statusa ALK in ROS1: Pomembno je izbrati dobro validirano in robustno metodologijo, da se izognemo lažno negativnim ali lažno pozitivnim rezultatom. Hepatotoksičnost: V kliničnih študijah so poročali o hepatotoksičnosti, ki jo je povzročilo zdravilo (vključno s primeri s smrtnim izidom). Delovanje jeter, vključno z ALT, AST in skupnim bilirubinom, je treba preveriti enkrat na teden v prvih 2 mesecih zdravljenja, nato pa enkrat na mesec in kot je klinično indicirano. Ponovitve preverjanj morajo biti pogostejše pri povečanih vrednosti stopnje 2, 3 ali 4. Intersticijska bolezen pljuč (ILD)/pnevmonitis: Lahko se pojavi huda, življenjsko nevarna ali smrtna ILD/pnevmonitis. Bolnike s simptomi ILD/pnevmonitisa je treba spremljati, zdravljenje pa prekiniti ob sumu na ILD/pnevmonitis. Podaljšanje intervala QT: Opažali so podaljšanje intervala QTc. Pri bolnikih z obstoječo bradikardijo, podaljšanjem intervala QTc v anamnezi ali predispozicijo zanj, pri bolnikih, ki jemljejo antiaritmike ali druga zdravila, ki podaljšujejo interval QT, ter pri bolnikih s pomembno obstoječo srčno boleznijo in/ali motnjami elektrolitov je treba krizotinib uporabljati previdno; potrebno je redno spremljanje EKG, elektrolitov in delovanja ledvic; preiskavi EKG in elektrolitov je treba opraviti čimbližje uporabi prvega odmerka, potem se priporoča redno spremljanje. Če se interval QTc podaljša za 60 ms ali več, je treba zdravljenje s krizotinibom začasno prekiniti in se posvetovati s kardiologom. Bradikardija: Lahko se pojavi simptomatska bradikardija (lahko se razvije več tednov po začetku zdravljenja); izogibati se je treba uporabi krizotiniba v kombinaciji z drugimi zdravili, ki povzročajo bradikardijo; pri simptomatski bradikardiji je treba prilagoditi odmerek. Srčno popuščanje: Poročali so o hudih, življenjsko nevarnih ali smrtnih neželenih učinkih srčnega popuščanja. Bolnike je treba spremljati glede pojavov znakov in simptomov srčnega popuščanja in ob pojavu simptomov zmanjšati odmerjanje ali prekiniti zdravljenje. Nevtropenija in levkopenija: V kliničnih študijah so poročali o nevtropeniji, levkopeniji in febilni nevtropeniji; spremljati je treba popolno krvno sliko (pogosteje preiskave, če se opazijo abnormalnosti stopnje 3 ali 4 ali če se pojavi povišana telesna temperatura ali okužba). Perforacija v prebavilih: V kliničnih študijah so poročali o perforacijah v prebavilih, v obdobju trženja pa o smrtnih primerih perforacij v prebavilih. Krizotinib je treba pri bolnikih s tveganjem za nastanek perforacije v prebavilih uporabljati previdno; bolniki, pri katerih se razvije perforacija v prebavilih, se morajo prenehati zdravitis krizotinibom; bolnike je treba poučiti o prvih znakih perforacije in jim svetovati, naj se nemudoma posvetujejo z zdravnikom. Vpliv na ledvice: V kliničnih študijah so opazili zvišanje ravni kreatinina v krvi in zmanjšanje očistka kreatinina. V kliničnih študijah in v obdobju trženja so poročali tudi o odpovedi ledvic, akutni odpovedi ledvic, primerih s smrtnim izidom, primerih, ki so zahtevali hemodializo in hiperkaliemiji stopnje 4. Vpliv na vid: V kliničnih študijah so poročali o izpadu vidnega polja stopnje 4 z izgubo vida. Če se na novo pojavi huda izguba vida, je treba zdravljenje prekiniti in opraviti oftalmološki pregled. Če so motnje vida trdovratne ali se poslabšajo, je priporočljiv oftalmološki pregled. Histološka preiskava, ki ne nakazuje adenokarcinoma: Na voljo so le omejeni podatki pri NSCLC, ki je ALK in ROS1 pozitiven in ima histološke značilnosti, ki ne nakazujejo adenokarcinoma, vključno s ploščatoceličnim karcinomom (SCC). Medsebojno delovanje z drugimi zdravili in druge oblike interakcij: Izogibati se je treba sočasni uporabi z močnimi zaviralci CYP3A4, npr. atazanavir, ritonavir, kobicistat, itrakonazol, ketokonazol, posakonazol, vorikonazol, klaritromicin, telitromicin in eritromicin (razen če morebitna korist za bolnika odtehta tveganje, v tem primeru je treba bolnike skrbno spremljati glede neželenih učinkov krizotiniba), ter grenivko in grenivkinim sokom, saj lahko povečajo koncentracije krizotiniba v plazmi. Izogibati se je treba sočasni uporabi z močnimi induktorji CYP3A4, npr. karbamazepin, fenobarbital, fenitoin, rifampicin in šentjanževka, saj lahko zmanjšajo koncentracije krizotiniba v plazmi. Učinek zmernih induktorjev CYP3A4, npr. efavirenz in rifabutin, še ni jasen, zato se je treba sočasni uporabi s krizotinibom izogibati. Zdravila, katerih koncentracije v plazmi lahko krizotinib spremeni (midazolam, alfentanil, cisaprid, ciklosporin, derivati ergot alkaloidov, fentanil, pimozid, kinidin, sirolimus, takrolimus, digoksin, dabigatran, kolhicin, pravastatin; sočasni uporabi s temi zdravili se je treba izogibati oziroma izvajati skrben klinični nadzor; bupropion, efavirenz, peroralni kontraceptivi, raltegravir, irinotekan, morfin, nalokson, metformin, prokainamid). Zdravila, ki podaljšujejo interval QT ali ki lahko povzročijo Torsades de pointes (antiaritmiki skupine IA (kinidin, disopiramid), antiaritmiki skupine III (amiodaron, sotalol, dofetilid, ibutilid), metadon, cisaprid, moksifloksacin, antipsihotiki) – v primeru sočasne uporabe je potreben skrben nadzor intervala QT. Zdravila, ki povzročajo bradikardijo (nedihidropiridinski zaviralci kalcijevih kanalčkov (verapamil, diltiazem), antagonisti adrenergičnih receptorjev beta, klonidin, gvanfacin, digoksin, metfolkin, antiholinesteraze, pilokarpin) – krizotinib je treba uporabljati previdno. Plodnost, nosečnost in dojenje: Ženske v rodni dobi se morajo izogibati zanositvi. Med zdravljenjem in najmanj 90 dni po njem je treba uporabljati ustrezno kontracepcijo (velja tudi za moške). Zdravilo lahko škoduje plodu in se ga med nosečnostjo ne sme uporabljati, razen če klinično stanje matere ne zahteva talega zdravljenja. Matere naj se med jemanjem zdravila dojenju izogibajo. Zdravilo lahko zmanjša plodnost moških in žensk. Vpliv na sposobnost vožnje in upravljanja strojev: Lahko se pojavijo simptomatska bradikardija (npr. sinkopa, omotica, hipotenzija), motnje vida ali utrujenost; potrebna je previdnost. Neželeni učinki: Najresnejši neželeni učinki so bili hepatotoksičnost, ILD/pnevmonitis, nevtropenija in podaljšanje intervala QT. Najpogostejši neželeni učinki (≥ 25 %) so bili motnje vida, navzea, diareja, bruhanje, edem, zaprtje, povečane vrednosti transaminaz, utrujenost, pomanjkanje apetita, omotica in nevropatija. Ostali zelo pogosti (≥ 1/10 bolnikov) neželeni učinki so: nevtropenija, anemija, levkopenija, disgevzija, bradikardija, bolečina v trebuhu in izpuščaj. Način in režim izdaje: Predpisovanje in izdaja zdravila je le na recept, zdravilo pa se uporablja samo v bolnišnicah. Izjemoma se lahko uporablja pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. Imetnik dovoljenja za promet: Pfizer Europe MA EEIG, Boulevard de la Plaine 17, 1050 Bruxelles, Belgija. Datum zadnje revizije besedila: 31.10.2019.

Pred predpisovanjem se seznanite s celotnim povzetkom glavnih značilnosti zdravila.

Vir: 1. Povzetek glavnih značilnosti zdravila Xalkori, 31.10.2019

XALKORI®
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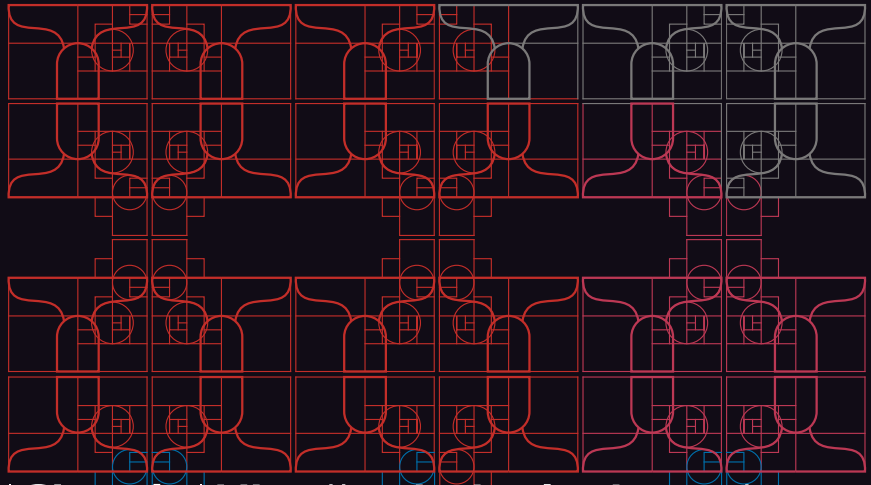
XAB-01-20 Samo za strokovno javnost



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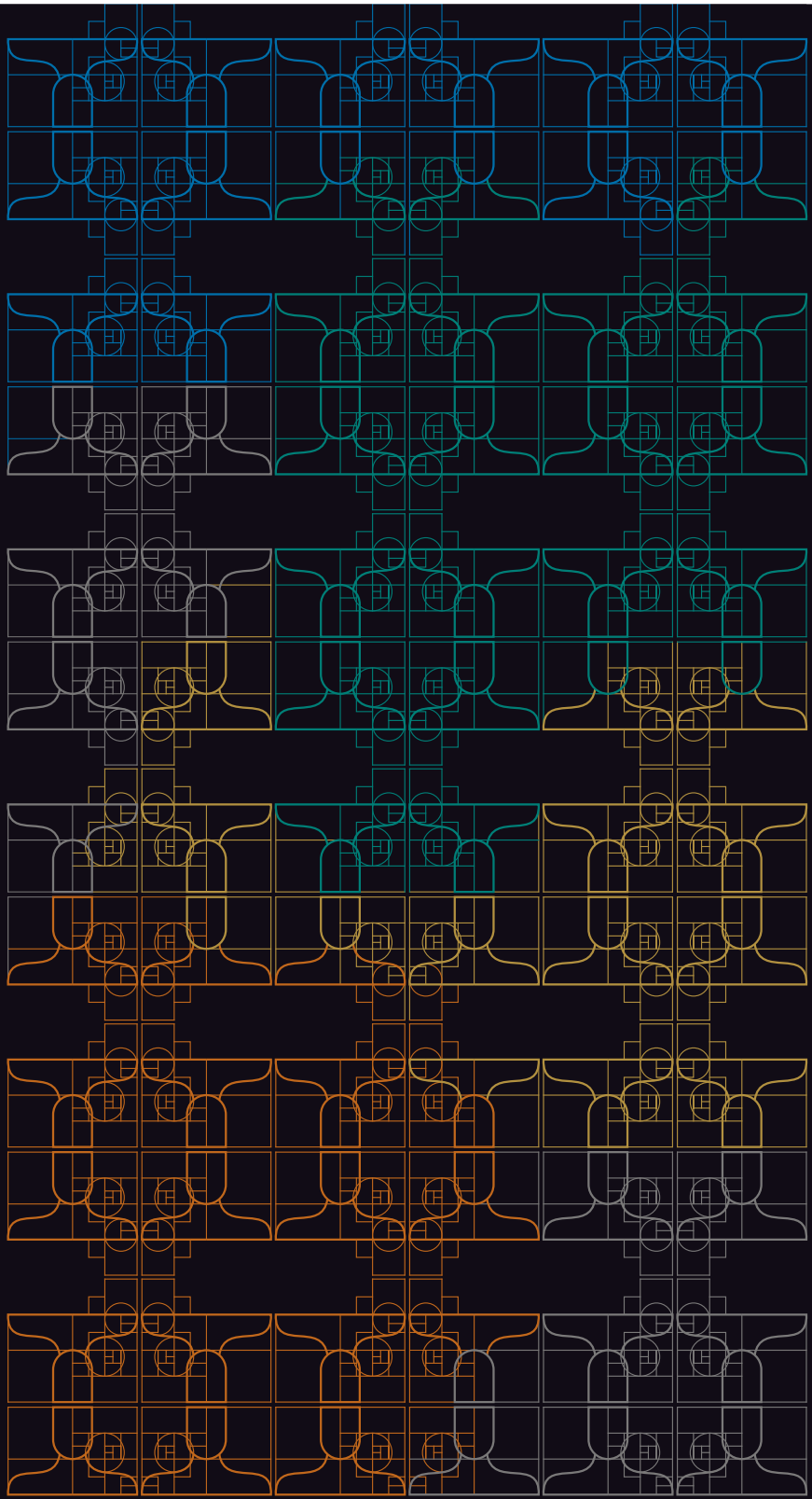

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