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ALIMTA®
pemetreksed

Pravi **Izbor**
Pravi **Bolnik**
Pravi **Čas**

Edina histološko usmerjena citostatična terapija

ALIMTA/cisplatin:
Zdravljenje prvega reda pri bolnikih z nedrobnoceličnim pljučnim karcinomom, ki nimajo pretežno luskaste histologije

Edina kombinirana terapija s signifikantno izboljšanim preživetjem:
12,6 meseca pri bolnikih z adenokarcinomom pljuč[1]

*vs. Gemcitabine/Cisplatin
1. Scagliotti GV et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26(21):3543-51.

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Ime zdravila ALIMTA 100 mg prašek za koncentrat za raztopino za infundiranje in ALIMTA 500 mg prašek za koncentrat za raztopino za infundiranje **Kakovostna in količinska sestava** ALIMTA 100 mg: vsaka viala vsebuje 100 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Po pripravi vsebuje vsaka viala 25 mg/ml pemetrekseda. Pomožne snovi: Vsaka viala vsebuje približno 11 mg natrija. Manitol, klorovodikova kislina, natrijev hidroksid. ALIMTA 500 mg: vsaka viala vsebuje 500 mg pemetrekseda (v obliki dinatrijevega pemetrekseda). Po pripravi vsebuje vsaka viala 25 mg/ml pemetrekseda. Pomožne snovi: Vsaka viala vsebuje približno 54 mg natrija. Manitol, klorovodikova kislina, natrijev hidroksid. **Terapevtske indikacije:** ALIMTA je v kombinaciji s cisplatinom indicirana za zdravljenje bolnikov z neresektabilnim malignim plevralnim mezoteliomom, ki jih še nismo zdravili s kemoterapijo. ALIMTA je v kombinaciji s cisplatinom indicirana kot zdravljenje prvega izbora za bolnike z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom, ki nima pretežno luskaste celične histologije. ALIMTA je indicirana kot monoterapija za zdravljenje lokalno napredovalega ali metastatskega nedrobnoceličnega pljučnega karcinoma, ki nima pretežno luskaste celične histologije pri bolnikih, pri katerih bolezen ni napredovala neposredno po kemoterapiji na osnovi platine. Zdravljenje prvega izbora naj bo platinasta dubleta z gemcitabinom, paklitakselom ali docetakselom. ALIMTA je indicirana kot monoterapija za zdravljenje drugega izbora bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim karcinomom, ki nima pretežno luskaste celične histologije. **Odmerjanje in način uporabe:** ALIMTO smemo dajati le pod nadzorom zdravnika, usposobljenega za uporabo kemoterapije za zdravljenje raka. ALIMTA v kombinaciji s cisplatinom Priporočeni odmerek ALIMTE je 500 mg/m2 telesne površine (TP), dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21-dnevnega ciklusa. Priporočeni odmerek cisplatina je 75 mg/m2 TP, infundiran v dveh urah približno 30 minut po zaključku infuzije pemetrekseda prvi dan vsakega 21 dnevnega ciklusa. Priporočeni odmerek cisplatina je 75 mg/m2 TP, infundiran v dveh urah približno 30 minut po zaključku infuzije pemetrekseda prvi dan vsakega 21 dnevnega ciklusa. Bolniki morajo prejeti zadostno antiemetično zdravljenje, pred in/ali po prejemanju cisplatina jih moramo tudi ustrezno hidrirati. ALIMTA kot samostojno zdravilo Priporočeni odmerek ALIMTE je 500 mg/m2 TP, dan kot intravenska infuzija v 10 minutah prvi dan vsakega 21 dnevnega ciklusa. Režim premedikacije Da zmanjšamo incidenco in resnost kožnih reakcij, dajemo kortikosteroid dan pred dajanjem pemetrekseda, na dan dajanja pemetrekseda in naslednji dan. Kortikosteroid naj ustreza 4 mg deksametazona, danega peroralno dvakrat dnevno. Za zmanjšanje toksičnosti morajo bolniki dnevno jemati tudi peroralno folno kislino ali multivitaminski pripravek, ki jo vsebuje (350 do 1000 mikrogramov). V sedmih dneh pred prvim odmerkom pemetrekseda morajo vzeti vsaj pet odmerkov folne kisline, odmerjanje pa morajo nadaljevati ves čas zdravljenja in še 21 dni po zadnjem odmerku pemetrekseda. Bolniki morajo prejeti tudi intramuskularno injekcijo vitamina B12 (1000 mikrogramov) v tednu pred prvim odmerkom pemetrekseda in enkrat vsake tri cikluse zatem. Kasnejše injekcije vitamina B12 lahko dajemo isti dan kot pemetreksed. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Dojenje. Sočasno cepljenje proti rumeni mrzlici. **Posebna opozorila in previdnostni ukrepi:** Pemetreksed lahko zavre delovanje kostnega mozga, kar se kaže kot nevtropenija, trombocitopenija in anemija (ali pancitopenija). Pri bolnikih, ki pred zdravljenjem niso prejemali kortikosteroidov, so poročali o kožnih reakcijah. Uporabe pemetrekseda pri bolnikih z očistkom kreatinina < 45 ml/min ne priporočamo. Bolniki z blagim do zmernim popuščanjem delovanja ledvic naj se izogibajo jemanju nesteroidnih protivnetnih zdravil (NSAID), denimo, ibuprofena in acetilsalicilne kisline 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Vsi bolniki, ki jih lahko zdravimo s pemetreksedom, naj se izogibajo jemanju NSAID-ov z dolgimi razpolovnimi časi izločanja vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda. Poročali so o resnih ledvičnih primerih, vključno z akutno ledvično odpovedjo, s pemetreksedom samim ali v povezavi z drugimi kemoterapevtiki. Pri bolnikih s klinično pomembno tekočino tretjega prostora moramo razmisliti o drenaži izliva pred dajanjem pemetrekseda. Kot posledico toksičnosti pemetrekseda v kombinaciji s cisplatinom za prebavila so opažali hudo dehidracijo, zato moramo bolnike pred prejemanjem terapije in/ali po njej ustrezno hidrirati, prejeti morajo zadostno antiemetično zdravljenje. Občasno so v kliničnih študijah pemetrekseda, običajno ob sočasnem dajanju z drugo citotoksično učinkovino, poročali o resnih srčnožilnih dogodkih, vključno z miokardnim infarktom in možganskožilnimi dogodki. Odsvetujemo uporabo živih oslabljenih cepiv. Spolno zrelim moškim odsvetujemo zaploditev otroka v času zdravljenja in še 6 mesecev zatem. Priporočamo ukrepe prosti zanositvi ali vzdržnost. Zaradi možnosti, da zdravljenje s pemetreksedom povzroči trajno neplodnost, naj se moški pred začetkom zdravljenja posvetujejo o shranjevanju semena. Ženske v rodni dobi morajo v času zdravljenja s pemetreksedom uporabljati učinkovito kontracepcijo. Poročali so o primerih radiacijske pljučnice pri bolnikih, ki so jih zdravili z radiacijo pred, med ali po zdravljenju s pemetreksedom. Poročali so o radiacijskem izpuščaju pri bolnikih, ki so se zdravili z radioterapijo pred tedni ali leti. Zdravilo Alimta 500 mg vsebuje približno 54 mg natrija na vialo. Pomembno za bolnike, ki so na dieti z nadzorovanim vnosom natrija. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Sočasno dajanje nefrotoksičnih zdravil (denimo, aminoglikozidov, diuretikov zanke, spojin platine, ciklosporina) lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Sočasno dajanje snovi, ki se tudi izločajo s tubulno sekrecijo (denimo, probenecid, penicilin), lahko potencialno povzroči zakasnjeni očistek pemetrekseda. Pri bolnikih z normalnim delovanjem ledvic lahko visoki odmerki nesteroidnih protivnetnih zdravil (NSAID-i; denimo, ibuprofen) in acetilsalicilna kislina v visokih odmerkih zmanjšajo eliminacijo pemetrekseda in tako lahko povečajo pojavnost neželenih učinkov pemetrekseda. Pri bolnikih z blagim do zmernim popuščanjem delovanja ledvic se moramo izogibati sočasnemu dajanju pemetrekseda z NSAID-i (denimo, ibuprofenom) ali acetilsalicilne kisline v visokih odmerkih 2 dni pred dajanjem pemetrekseda, na dan dajanja in še 2 dni po dajanju pemetrekseda. Sočasnemu dajanju NSAID-ov z daljšimi razpolovnimi časi s pemetreksedom se moramo izogibati vsaj 5 dni pred dajanjem pemetrekseda, na dan dajanja in še vsaj 2 dni po dajanju pemetrekseda.Velika različnost med posamezniki v koagulacijskem statusu v času bolezni ter možnost medsebojnega delovanja med perioralnimi antikoagulacijskimi učinkovinami ter kemoterapijo proti raku zahtevata povečano pogostnost spremljanja INR. **Kontraindicirana sočasna uporaba:** Cepivo proti rumeni mrzlici: tveganje za smrtno generalizirano bolezen po cepljenju. **Odsvetovana sočasna uporaba:** Živa oslabljena cepiva (razen proti rumeni mrzlici): tveganje za sistemsko, potencialno smrtno bolezen. **Neželeni učinki** Klinične študije malignega plevralnega mezotelioma *Zelo pogosti:* znižani nevtrofili/granulociti, znižani levkociti, znižan hemoglobin, znižani trombociti, nevropatija-senzorična, diareja, bruhanje, stomatitis/faringitis, slabost, anoreksija, zaprtje, izpuščaj, alopecija, povišan kreatinin, znižan očistek kreatinina, utrujenost. *Pogosti:* dehidracija, motnje okusa, konjuktivitis, dispepsija. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija, zdravljenje 2. izbora. *Zelo pogosti:* znižan nevtrofili/granulociti, znižani levkociti, znižan hemoglobin, diareja, bruhanje, stomatitis/faringitis, slabost, anoreksija, izpuščaj/luščenje, utrujenost. *Pogosti:* znižani trombociti, zaprtje, povišanje SGPT (ALT), povišanje SGOT (AST), srbenje, alopecija, povišana telesna temperatura. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA v kombinaciji s cisplatinom, zdravljenje 1. izbora. *Zelo pogosti:* znižan hemoglobin, znižani nevtrofili/granulociti, znižani levkociti, znižani trombociti, slabost, bruhanje, anoreksija, zaprtje, stomatitis/faringitis, diareja brez kolostomije, alopecija, izpuščaj/luščenje, povišan kreatinin. *Pogosti:* nevropatija-senzorična, motnje okusa, dispepsija/zgaga. Klinične študije nedrobnoceličnega pljučnega karcinoma - ALIMTA monoterapija, vzdrževalno zdravljenje: *Zelo pogosti:* znižan hemoglobin, slabost, anoreksija, utrujenost, izpuščaj/luščenje, utrujenost. *Pogosti:* infekcija, znižani levkociti, znižani nevtrofili, nevropatija-senzorična, bruhanje, mukozitis/stomatitis, diareja, povišanje ALT (SGPT), povišanje AST (SGOT). Občasno so v kliničnih študijah pemetrekseda poročali o primerih resnih srčnožilnih in možganskožilnih dogodkov, vključno z miokardnim infarktom, angino pektoris, cerebrovaskularnim insultom in prehodnimi ishemičnimi atakami; primerih kolitisa ter o primerih intersticijske pljučnice z respiratorno insuficience, primerih edema in o ezofagitisu/radiacijskem ezofagitisu. Redkeje pa o primerih potencialno resnega hepatitisa in pancitopenije. Po uvedbi zdravila na trg so poročali o primerih akutne odpovedi ledvic s pemetreksedom samim ali v povezavi z drugimi kemoterapevtiki, primerih radiacijske pljučnice pri bolnikih, ki so jih zdravili z radiacijo pred, med ali po njihovem zdravljenju s pemetreksedom, primerih radiacijskega izpuščaja pri bolnikih, ki so se v preteklosti zdravili z radioterapijo in o primerih periferne ishemije, ki je včasih vodila v nekrozo okončin. **Imetnik dovoljenja za promet** Eli Lilly Nederland B.V., Grootslag 1 5, NL 3991 RA, Houten, Nizozemska. Datum zadnje revizije besedila 21.09.2009. **Način izdaje zdravila:** H
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review

Radiotherapy in combination with vascular-targeted therapies

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Background. Given the critical role of tumor vasculature in tumor development, considerable efforts have been spent on developing therapeutic strategies targeting the tumor vascular network. A variety of agents have been developed, with two general approaches being pursued. Antiangiogenic agents (AAs) aim to interfere with the process of angiogenesis, preventing new tumor blood vessel formation. Vascular-disrupting agents (VDAs) target existing tumor vessels causing tumor ischemia and necrosis. Despite their great therapeutic potential, it has become clear that their greatest clinical utility may lie in combination with conventional anticancer therapies. Radiotherapy is a widely used treatment modality for cancer with its distinct therapeutic challenges. Thus, combining the two approaches seems reasonable.

Conclusions. Strong biological rationale exist for combining vascular-targeted therapies with radiation. AAs and VDAs were shown to alter the tumor microenvironment in such a way as to enhance responses to radiation. The results of preclinical and early clinical studies have confirmed the therapeutic potential of this new treatment strategy in the clinical setting. However, concerns about increased normal tissue toxicity, have been raised.

Key words: antiangiogenic agents; vascular-disrupting agents; radiotherapy

Introduction

Radiotherapy is an effective and widely used treatment modality for many tumors, with about half of all cancer patients undergoing radiation therapy as a part of their treatment.[1] Although widely used, tumor radioresistance remains a major problem and a need exists to improve the cure rate by radiation therapy alone. As the patient population treated with radiotherapy is so enormous, enhancing the therapeutic outcome for even a relatively small proportion of these has the potential to translate to a highly significant clinical benefit. Combinations of cytotoxic chemotherapeutic agents with radiation have a synergistic effect on tumor response and are firmly established in clinical practice for a wide spectrum of tumors.[2] In recent years, there has been increasing interest in combining vascular-targeted therapies with radiation.[3] The enhanced antitumor efficacy of combined treatment may be explained by the alteration of the tumor microenvironment by vascular-targeted agents resulting in increased radiosensitivity of the tumor. However, the mechanisms of interaction between the two treatment modalities are complex and involve interactions between tumor stroma, the vasculature and the tumor cells themselves, which are not currently well understood. Therefore, the ideal way to use this potentially powerful combination for tumor cure has yet to be determined.

Tumor angiogenesis

Angiogenesis is a critical step in tumor progression, as tumors are unable to grow beyond 2 mm^3 without a vascular supply, due to lack of oxygen and nutrients.[4] Formation of new blood vessels occurs from pre-existing vessels and allows the tumor to grow and expand rapidly.[5] Tumors switch in their development to an angiogenic phenotype. The

transition from dormant to the angiogenic state of the tumor is termed the "angiogenic switch" and is caused by a shift in the balance of anti- and pro-angiogenic factors.[6] It is regulated by environmental factors and by genetic alterations that act to either up-regulate pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) and/or down-regulate inhibitors of angiogenesis, such as angiostatin, endostatin, thrombospondin and interferons.[7]

The multistep process of tumor angiogenesis is characterized by degradation of the extracellular matrix, followed by proliferation and migration of the underlying endothelial cells into the tumor, with resultant vessel formation.[5] The initial step in the process is activation of quiescent endothelial cells by binding of tumor-produced or stromal-produced growth factors to endothelial receptors. VEGF is a potent and specific growth factor that plays a pivotal role in endothelial cell activation.[8] The main effects of VEGF are to increase vessel permeability and induce endothelial cell migration and proliferation, leading to the formation of endothelial sprouts, which then anastomose to form vascular loops and networks.[9,10] VEGF also acts as a survival factor for endothelial cells by inhibiting apoptosis.[11] It is therefore a pivotal driver of tumor angiogenesis, allowing tumor progression from in situ lesions to widespread disease, and providing the tumor with a route via which cells can get into the circulation and form distant metastases.[4,12] VEGF is secreted by almost all solid tumors.[13] Proliferating endothelial cells found in and around tumors produce multiple growth factors that not only promote endothelial cell growth but also tumor cell growth, invasion, and survival.[14,15] Angiogenesis therefore provides both a perfusion effect and a paracrine effect for a growing tumor and tumor cells and endothelial cells can drive each other with resultant perpetuation and amplification of the malignant phenotype.[16]

Newly formed tumor blood vessels are distinct from those of normal tissue. They are markedly disordered, often dilated, tortuous and characterized by a relative lack of pericytes and other supporting cells, impaired blood flow and increased vascular permeability.[17] Extravasation of macromolecules and pertinent development of high interstitial fluid pressure often results in vascular collapse, which leads to acidic and hypoxic areas heterogeneously distributed within the tumor mass.[18,19] Hypoxia resulting from such functional vessel abnormalities is termed "acute" or "perfusion-limited". The affected tumor cells are found much closer to blood vessels than would be expected from diffusion limitations and are exposed to oxygen concentrations that vary transiently between normal, anoxia and anywhere in-between. On the other hand, "chronic" or "diffusion-limited" hypoxia is found at an increased distance from blood vessels. In this type of hypoxia, individual perfused vessels are characterized by an oxygenation gradient surrounding them. Cells in this area exist at all possible oxygen concentrations ranging from anoxia at distant locations to normal values next to the vessels.[20]

Hypoxia, angiogenesis and radioresistance

Hypoxia is an important stimulus for angiogenesis.[21] Hypoxia inducible factor-1 (HIF-1) is a major mediator of the response to hypoxia. It is a transcriptional factor that regulates a number of processes, including VEGF transcription, apoptosis and cell cycle arrest.[22,23] HIF-1 is regulated mainly by hypoxia, but it can also be activated in response to radiation.[24] Both the hypoxic tumor microenvironment and external stresses such as ionizing radiation, lead to the up-regulation of many other pro-angiogenic factors, including VEGF, angiopoietin-2, nitric oxide synthase, platelet-derived growth factor (PDGF) and basic fibroblastic growth factors (bFGF).[25,26] It has been shown that radiotherapy alone can potentiate angiogenic processes.[27] Increased VEGF production in response to irradiation has been observed in various cancer cell lines.[28] This is a part of the overall cellular response to stress and it is associated with the induction of a variety of transcription factors that can activate transcription of cytokines, growth factors, and cell cycle-related genes.

Hypoxia in tumors is strongly associated with radiation resistance as oxygen is required to chemically modify free-radical damage to the target DNA. When radiation is absorbed by the tissue, it creates reactive oxygen species that react with and damage cellular DNA, thus triggering cell death by apoptosis and/or necrosis. Cells irradiated in the presence of air are about three times more sensitive than cells irradiated under conditions of severe hypoxia.[29] Pre-treatment measurements of tumor oxygenation have been shown to predict the response to radiotherapy and the likelihood of tumor recurrence, progression and metastatic disease in many human tumors.[30] A more moderate hypoxia than is needed for maximum resistance to radiation has al-

so been shown to have a negative impact on tumor control. This may be due to the fact that hypoxia influences a number of biological responses that affect tumor properties important for the treatment outcome, including angiogenesis.[20] Different levels of hypoxia in a tumor thus provide the conditions for existence of viable cells that are not only radioresistant but angiogenic as well.[31,32]

Vascular-targeted therapies

The importance of targeting tumor vasculature development and function first became apparent in the 1970s through the seminal studies of Judah Folkman, who demonstrated that angiogenesis is crucial for the growth and survival of tumor cells. His findings suggest that both tumor cells and their supporting endothelial cells are potential targets for cell killing and should be considered when planning cancer treatment.[4] Destroying the tumor vasculature deprives tumors of nutrients and oxygen necessary for their growth and should also inhibit metastatic spread, theoretically leading to tumor regression.

As a therapeutic group, vascular-targeting agents are unique as they have highly specific targets, while simultaneously having the potential to be effective against a broad range of tumor types. They are now divided into two classes; antiangiogenic agents (AAs), which inhibit the formation of new blood vessels, and vascular-disrupting agents (VDAs), which act against existing tumor vasculature. AAs are considered to be cytostatic in nature in contrast to VDAs, which are thought to be cytotoxic. Although there are differences between the two groups, including their administration schedules, individual agents might show both antiangiogenic and vascular-disrupting effects.[33]

Antiangiogenic agents

AAs aim to prevent the growth of new blood vessels in tumors. One of the most widely studied targets for angiogenesis being explored clinically is VEGF and its receptors. VEGF is a ligand with a central role in signaling pathways controlling tumor blood vessel development and survival. The binding of VEGF ligands activates receptor tyrosine kinases, designated VEGFR1, VEGFR2 and VEGFR3, which in turn activate a network of distinct downstream signaling pathways. Although the effects of VEGF receptors (VEGFR) signaling were initially thought to be specific for the vasculature, VEGF can also play a role in many other processes.[34] VEGFR1 expression by colon cancer cells contributes to colon cancer cell motility and invasiveness but has little direct effect on proliferation of these cells. VEGFR2 expression by lung cancer cells may play a role in tumor cell survival after cytotoxic stress.[35,36] Many different strategies for inhibiting VEGF activity have been evaluated, including the neutralization of the ligand or receptor by antibodies, blocking VEGFR signaling with tyrosine kinase inhibitors and even antiangiogenic gene therapy based on modulating the expression of VEGF pathway-related genes.[34,37]

Bevacizumab is a humanized monoclonal antibody that acts by binding and neutralizing VEGF. In a pivotal clinical trial conducted by Hurwtz *et al*., bevacizumab in combination with fluorouracil-based chemotherapy, significantly improved the overall survival for patients with metastatic colorectal cancer over chemotherapy alone.[38] Improved overall survival with combination therapy was also shown for patients with NSCLC and improved progression-free survival for patients with metastatic breast cancer and renal cell cancer was observed.[39-41]

Small molecule tyrosine kinase inhibitors (TKIs) present another class of antiangiogenic agents. They act by preventing activation of growth factor receptors, thus inhibiting downstream signaling pathways. They offer the theoretical advantage of being simultaneously active against receptors for different growth factors. Sunitinib, for example, targets VEGFRs, platelet-derived growth factor receptor (PDGFR) and c-kit and has shown significant efficacy in clinical trials for renal cancer.[42]

So far improvements in overall survival have only been seen in patients with colorectal and non-small cell lung cancers, when AAs were given in combination with chemotherapy. One possible reason why single-agent AAs ultimately fail is that there is up-regulation of other pro-angiogenic factors leading to angiogenesis and tumor resistance, hence the rationale for these drugs to be combined with chemotherapy or radiotherapy.[43]

Vascular-disrupting agents

VDAs cause a rapid shutdown of perfusion in the established tumor vasculature, leading to tumor cell ischemia and secondary tumor cell death. These agents have the potential to destroy existing

tumor masses and may be therefore particularly suitable for treating large tumors, which are typically resistant to conventional therapies.[33]

Two major classes of VDAs that selectively target tumor vessels are in clinical development; the ligand-directed VDAs and small molecule VDAs. Biological or ligand-directed VDAs work by using antibodies, peptides or growth factors which selectively bind to the endothelium. Coagulation and/or endothelial cell death is then achieved by coupling the vascular-targeting moiety with a toxin (*e.g.* ricin) or a pro-coagulant.[44] Small molecule VDAs are at a much more advanced stage of clinical development than ligand-based therapies. These agents work by inducing vascular collapse, leading to extensive necrosis in tumors and include flavonoids and tubulin-depolymerizing/binding agents.[33] Flavone acetic acid and its derivatives, particularly 5,6-dimethyl-xanthenone-4-acetic acid (DMXAA), have a complex mechanism of action and are believed to work by inducing the release of vasoactive agents and cytokines, such as tumor necrosis factor alpha (TNF-α), which leads to hemorrhagic necrosis.[45] The tubulin-binding agents (*e.g.* combretastatin A-4 disodium phosphate) are believed to work by selective disruption of the cytoskeleton in proliferating endothelial cells in tumors. The subsequent change in endothelial cell shape leads to vessel blockage, thrombus formation, rapid reduction in tumor blood flow, and secondary tumor necrosis.[46]

Recently, electrochemotherapy has been recognized to have a vascular-disrupting effect besides a direct cytotoxic effect on tumor cells.[47,48] Due to non-selective permeabilization of cells in the tumors exposed to electric pulses, endothelial cells also undergo apoptosis by uptake of bleomycin or cisplatin.[48,49] This leads to permanent blood flow abrogation of the affected vessels leading to tumor hypoxia and necrosis, similar as in other vascular-disrupting agents.[50] It has been estimated that the vascular-disrupting effect contributes 20-30% to the overall antitumor effectiveness of electrochemotherapy.[48,49]

The result of selective vascular destruction common to all of these strategies is extensive central tumor necrosis that leaves only a thin layer of viable cells at the tumor periphery. These cells are believed to obtain nutrients and oxygen from vessels of the surrounding normal tissue and their repopulation may be the cause of treatment failure when VDAs are used in monotherapy, therefore combining VDAs with other standard treatment is an obvious option.[33]

Combined treatments

As oxygen is crucial for maximal effectiveness of radiation, a logical concern when combining AAs and VDAs with radiation would be that compromising tumor vasculature by these agents would leave a tumor hypoxic and, thus, less radiosensitive. However, the mechanisms of interaction between the two treatment modalities have proved to be more complex and involve changes in the tumor microenvironment that may in fact result in an improved treatment outcome.[51]

Radiotherapy and antiangiogenic agents

The understanding that tumor micro environmental factors, such as hypoxia, promote up-regulation of angiogenic and survival pathways leading to increased radioresistance, and that radiotherapy itself has pro-angiogenic effects, has prompted studies combining AAs with radiation.

Teicher's group was the first to show that AAs increase the tumor response when combined with single dose radiotherapy.[52,53] A number of preclinical studies have since indicated that AAs can enhance the response to radiation (Table 1). The list of AAs evaluated in combination with radiation include non-specific antiangiogenic agent angiostatin, agents targeting the VEGF signaling pathway (anti-VEGF, anti-VEGFR antibodies and tyrosine kinase inhibitors), COX-2 inhibitors and epidermal growth factor receptor (EGFR) inhibitors which also target tumor cells. The antiangiogenic and antitumor effects have been reported to be additive as well as synergistic.[71] Lee *et al.* conducted important animal experiments using an anti-VEGF antibody in combination with radiotherapy, resulting in synergistic antitumor effects. The anti-VEGF antibody decreased tumor interstitial fluid pressure and increased tumor perfusion, probably due to an observed reduction of tumor vascular density with vessel reorganization.[59] In addition, AAs have been shown to increase oxygenation, thus increasing overall radiosensitivity. Jain tried to reconcile the paradoxical effects of AAs on oxygenation with the concept of "normalization" of the tumor vasculature.[72] He postulated that rather than obliterating all tumor blood vessels, AAs destroyed only immature vessels, reduced vascular permeability and interstitial fluid pressure, and increased pericyte recruitment to stabilize intact vessels. Such normalization of tumor vasculature resulted in a more stable, organized vasculature, which could

TABLE 1. Preclinical combination trials with antiangiogenic agents and radiotherapy

Antiangiogenic agent	Tumor model	Reference
TNP-470	Lewis lung carcinoma	52, 53
	C3H mammary carcinoma	54
	U87 glioblastoma	55
Angiostatin	Lewis lung carcinoma	56
	D54 human glioblastoma	56
Endostatin	SQ-20B squamous cell carcinoma	57
Anti-VEGF antibody	Lewis lung carcinoma	58
	SQ-20B squamous cell carcinoma	58
	Seg-1 esophageal adenocarcinoma	58
	U87 glioblastoma	58, 59
	LS1747 colon adenocarcinoma	59
	Seg-1 esophageal adenocarcinoma	60
	U87 glioblastoma	59
VEGFR-2 blockade		
SU5416	GL261 murine glioblastoma	61
DC101	54A small cell lung cancer	62
	U87 glioblastoma	62
	MCa4 mammary carcinoma	63
	MCa35 mammary carcinoma	63
VEGFR tyrosine kinase inhibitors		
PTK787/ZK222584	SW480 human colon adenocarcinoma	64
ZD6474	CaLu-6 non-small cell lung cancer	65
	HT49 colorectal carcinoma	66
AZD2171	H460 non-small cell lung cancer	67
	CaLu-6 non-small cell lung cancer	68
	LoVo colorectal carcinoma	69
Multi-kinase inhibitors		
SU11248 (sunitinib)	Lewis lung carcinoma	69
	GL261 murine glioblastoma	69
SU6668	Lewis lung carcinoma	70
	GL261 murine carcinoma	70

deliver oxygen and nutrients to the tumor more efficiently via well-functioning vessels, thereby decreasing hypoxia and hence radioresistance (Figure 1). However, continued antiangiogenic activity could cause vessel regression and impaired delivery leading to exacerbation of hypoxic conditions and radioresistance. Benefits of such a combination therapy may therefore be dependent upon a transient "normalization window" of opportunity when blood flow and tumor oxygenation are increased.[73]

Optimal timing for delivery of antiangiogenic therapy during the course of radiation to achieve the greatest enhancement of the radiation response, remains unknown and few studies have compared different sequences of radiation therapy and AAs.[74] Recently, ZD6474 (vandetanib), a small molecule inhibitor of VEGFR2 with additional activity against EGFR, was combined with radiation therapy in the treatment of tumor xenografts. Two combination schedules were examined with vandetanib administered before each dose of radiation (concurrent schedule) or 30 minutes after the last dose of radiotherapy (sequential schedule). The growth delay induced using the concurrent schedule was greater than that induced by vandetanib or radiation treatment alone but the sequential schedule maximally delayed tumor growth. The authors demonstrated that a less pronounced response in the concurrent schedule was due to reduced tumor vascular perfusion caused by administration of vandetanib, which impaired re-oxygenation between radiation fractions, thereby decreasing radiosensitivity. In addition, the enhanced effect of vandetanib and radiotherapy in the sequential schedule could be explained by abrogation of VEGF-dependent survival signaling, which is supposed to have an important role in tumor recovery after irradiation.[65]

The enhancement of the effect of radiation therapy by antiangiogenic therapy may be also influenced by the tumor microenvironment. This was shown in a study by Lund *et al.* who treated mice with glioblastoma xenografts implanted into the

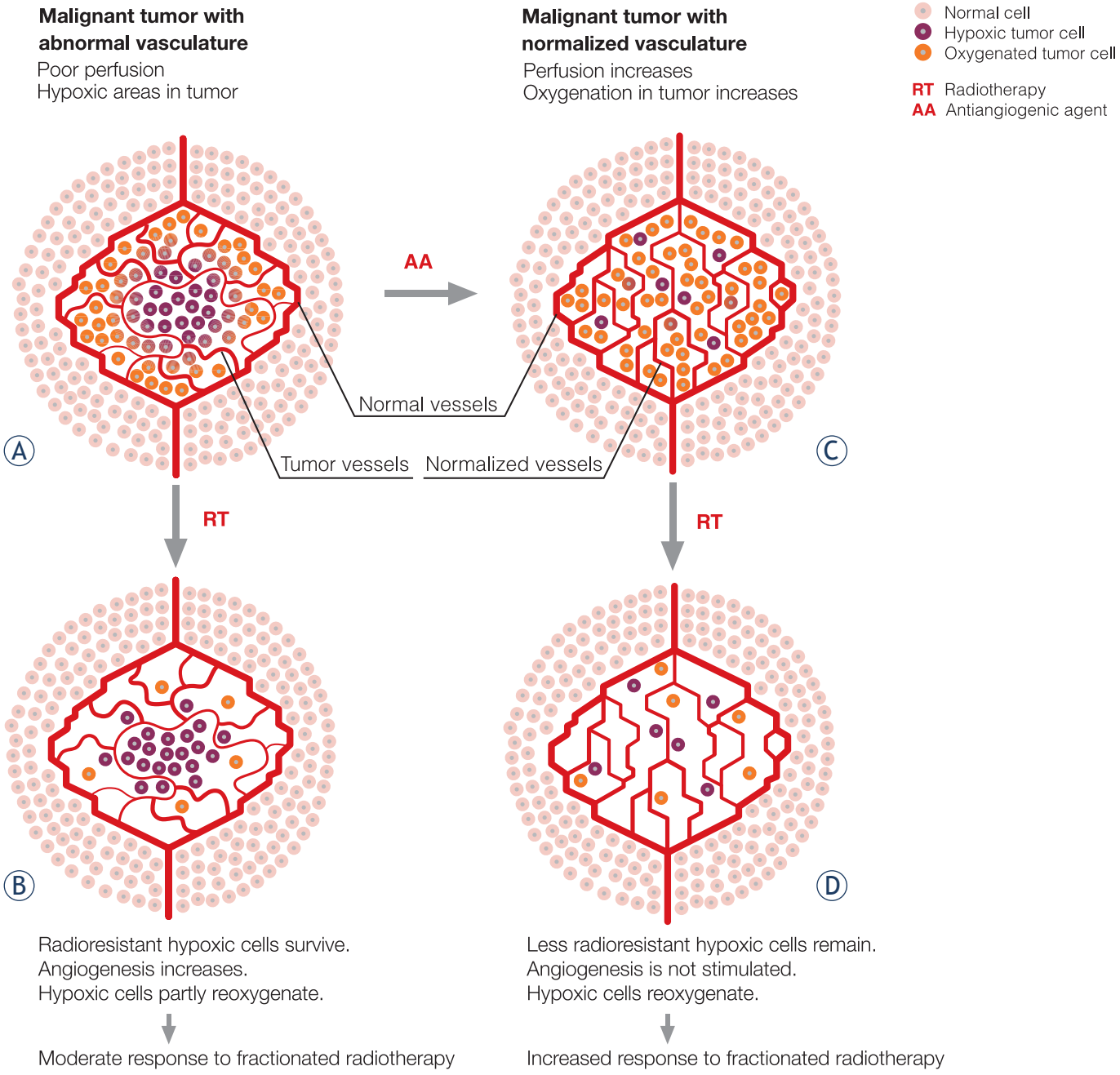

FIGURE 1. Theoretical model explaining the biological rationale for combining radiotherapy and AAs.

A) Abnormal tumor vasculature largely composed of immature, disordered, often dilated and tortuous blood vessels is characterized by increased vascular permeability and impaired blood flow which leads to functional vessel abnormalities resulting in hypoxic areas in the tumor. B) After irradiation, oxygenated cells are destroyed, leaving behind the radioresistant hypoxic cells which release proangiogenic factors and further promote angiogenesis. During the time between radiation fractions hypoxic cells partly reoxygenate and further stimulate tumor repopulation, ultimately resulting in a moderate response to fractionated radiation. C) Pretreatment with AA destroys immature, inefficient tumor vessels and cause vessel reorganization thus increasing tumor perfusion and oxygenation. D) With irradiation many radiosensitive oxygenated cells are killed. The few remaining hypoxic cells reoxygenate, without angiogenesis being increased. The result is a less pronounced tumor repopulation and better overall response to fractionated radiation.

TABLE 2. Preclinical combination trials with vascular-disrupting agents and radiotherapy

Vascular disrupting agent	Tumor model	Reference
Tumor necrosis factor	MCA-K mammary carcinoma	76
	MCA-K mammary carcinoma	77
Flavone acetic acid	C3H mammary carcinoma	78
DMXAA	RIF-1 fibrosarcoma	79
	MDAH-MCa4 mammary carcinoma	79
	C3H mammary carcinoma	80
	KHT sarcoma	80
Combretastatin A-4 disodium phosphate	KHT sarcoma	81
	Carcinoma NT	82
	C3H mammary carcinoma	83
	KHT sarcoma	83
	Kaposi's sarcoma	84
	Rhabdomyosarcoma	85
ZD6126	C3H mammary carcinoma	86
	A549 NSCLC	87
	U87 glioblastoma	88
	KHT sarcoma	89
MN-029	KHT sarcoma	90

thigh or intracranially with TNP-470 and/or radiation therapy.[55] Significant enhancement of the tumor response to TNP-470 and radiation was seen in the thigh tumors, but no additive effect was observed in intracranial tumors. The authors proposed that differences in the capillary beds and microenvironment of the brain and the subcutaneous tissues of the thigh may have contributed to the differences in response.

Radiotherapy and vascular-disrupting agents

The presence of a viable rim of tumor cells at the periphery after VDA treatment, as shown in preclinical studies, explains the modest tumor control seen in the single-agent phase I studies.[30] It has been suggested that increased blood flow in the adjacent normal tissue, together with probable rapid up-regulation of angiogenic factors, such as VEGF, directly facilitates growth and expansion of the remaining rim of viable cells.[75] These cells are believed to be well oxygenated and thus present an excellent target for conventional cytotoxic therapies. A logical rationale for combining VDAs with radiation would therefore be the interaction of the two treatments at the tumor microregional level; VDA reducing or eliminating the poorly oxygenated and hence radioresistant subpopulation of tumor cells and radiation killing the remaining well oxygenated peripheral cells (Figure 2). A number of pre-clinical studies performed on rodent tumor models over the past few years have reported enhanced tumor killing when VDAs were given in combination with radiotherapy (Table 2).

A study by Murata *et al*. showed the importance of scheduling.[83] In his study for the murine CH3 tumors no improvement in local control was seen when combretastatin A-4 disodium phosphate was given 60 minutes before radiation compared to improved results when given concurrently or after radiotherapy. A likely explanation for this finding is that the vascular shutdown induced by the VDAs may have rendered some tumor cells hypoxic at the time of irradiation and that these cells later re-oxygenated and survived. It was suggested that blood flow needs to be re-established in the remaining viable tissue to obtain maximum radiosensitization of the tumor. The greatest enhancement of the radiation response in fractionated dose regimens may be achieved when VDA is administered within a few hours after radiation. Under such conditions, antitumor effects may be greater than additive.[91] An interesting animal study conducted by Siemann and Rojiani using the tubulin-binding agent *N-acetyl*-colchinol (ZD6126) and radiation showed that enhanced killing was more likely in larger tumors than in smaller ones.[89] This observation may be explained by the fact that larger tumors are less radiosensitive due to increased hypoxic regions, which can be compensated by

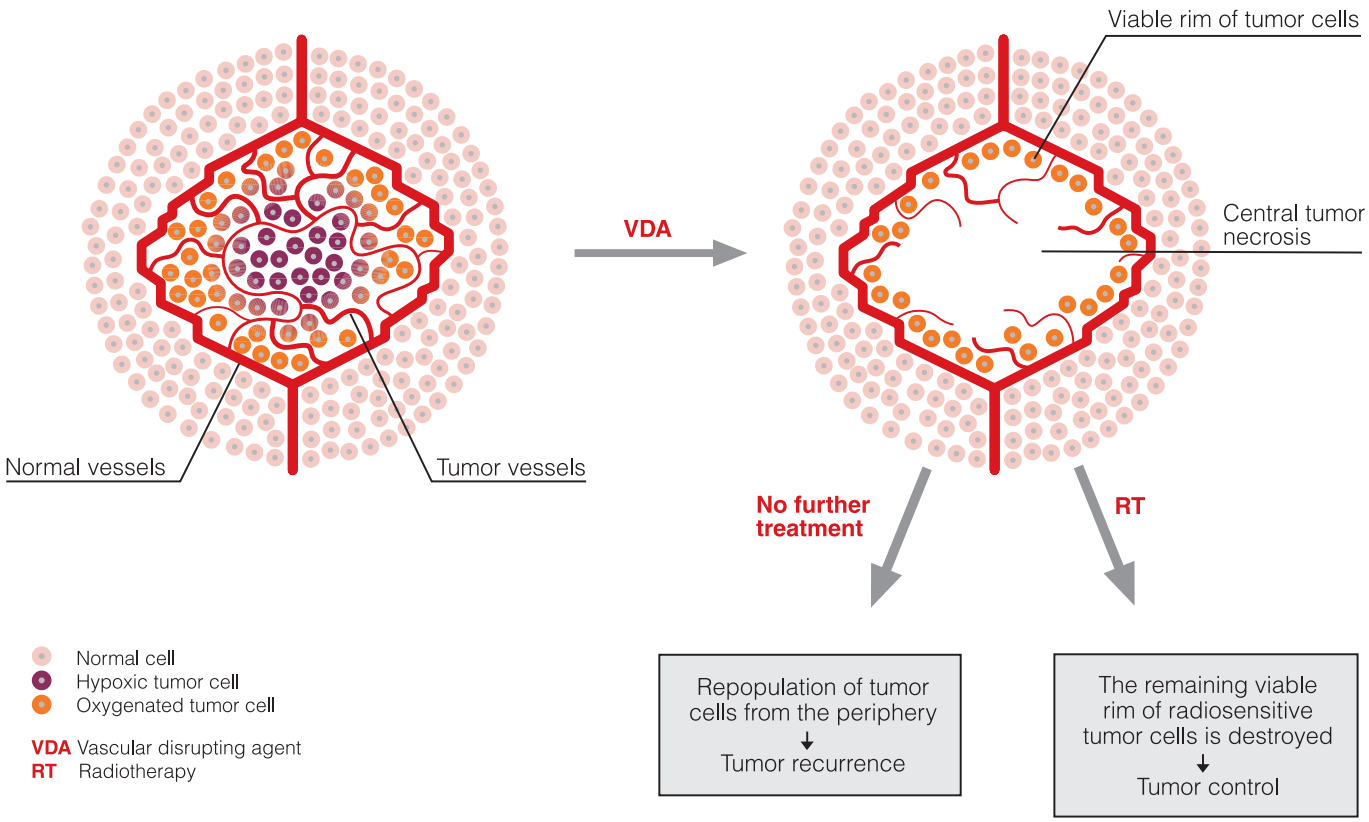

FIGURE 2. Schematic representation of the rationale for combining radiotherapy and VDAs.

The result of VDA treatment is selective destruction of tumor vessels which causes extensive central tumor necrosis leaving only a thin layer of viable cells at the tumor periphery. These cells are believed to obtain nutrients and oxygen from vessels of the surrounding normal tissue and their repopulation may be the cause of treatment failure when VDAs are used in monotherapy. Combined treatment of VDA with radiotherapy may be more successful as radiation can destroy the viable tumor rim of well oxygenated and thus radiosensitive peripheral tumor cells remaining after the use of VDA.

VDAs, whereas smaller tumors are more radiosensitive with fewer areas affected by VDAs.

Studies combining electrochemotherapy with tumor irradiation were also performed. The potentiation of the radiation response in experimental tumors was demonstrated with a single dose and fractionated radiation regime. A potentiating effect of 2.7 was observed with single dose irradiation and 4.6 with the fractionated regime.[92-95] The effect of combined treatment was also demonstrated on tubal dedifferentiated papillary adenocarcinoma skin metastases.[96] An enhanced radiation response with this treatment modality can be explained in part by radiosensitization of tumor cells that occurs in the process of electropermeabilization leading to increased uptake of radiosensitizing chemotherapeutic drugs, and in part by a vascular-disrupting effect, which is a result of electrochemotherapy as described in the previous section.

A therapeutic approach combining VDAs and radiotherapy may therefore be particularly suitable for treating larger tumors. The greatest antitumor effect may be achieved by administering VDA after radiation fractions. However, in order to determine the optimal treatment schedule in the course of fractionated radiation, further investigations are needed.

Clinical trials on radiation and vascular-targeted therapies

The agents most widely explored in clinical trials are AAs targeting VEGF and its receptors. Of many explored in clinical trials, three have been approved for clinical use; two small molecule TKIs in monotherapy (sorafenib, sunitinib) for meta-

TABLE 3. Clinical trials of vascular-targeted agents in combination with chemoradiation/radiation therapy

Vascular- targeted agent	Phase	Tumor	Treatment regiment	Reference
Bevacizumab (B)	I	poor-prognosis head and neck cancer	chemoradiotherapy + B	102
	II	glioblastoma multiforme after surgery	temozolomide + radiotherapy + B → temozolomide + B	103
	II	locally advanced rectal cancer	standard preoperative chemoradiotherapy + B	104
	I/II	locally advanced inoperable colorectal cancer	chemoradiotherapy + B	105
	II	locally advanced inoperable pancreatic cancer	chemoradiotherapy + B → maintenance chemotherapy + B	106
		NSCLC	chemoradiotherapy + B	107
Sunitinib (S)	I	oligometastatic cancer	IGRT + S → maintenance S	108
CA4P		advanced NSCLC	palliative radiotherapy + CA4P	109

NSCLC = non-small cell lung cancer, IGRT = image-guided radiotherapy, CA4P = combretastatin A-4 disodium phosphate

static renal and hepatocellular carcinoma and an anti-VEGF monoclonal antibody (bevacizumab) in combination with chemotherapy for metastatic colorectal cancer, NSCLC and breast cancer.[97-101] Today none of these agents is approved in combination with radiation therapy. However, several phase I and II clinical trials have been concluded and numerous are ongoing (Table 3). Many of the trials have showed a promising antitumor response. However, increased toxicity, such as fistula formation, wound healing problems and thrombosis, have been observed in some studies, especially when the VEGF inhibitor was combined with chemoradiotherapy protocols.[102,107]

VDAs are in a less advanced stage of clinical development, with only a few early trials concluded, mainly evaluating VDAs in monotherapy or chemotherapy combinations.[109-111] Currently, the most widely explored VDA in clinical trials is combretastatin A-4 disodium phosphate, which has already been evaluated in several Phase I trials evaluating dosage schedules and toxicity, and has recently entered Phase II trials in combination with chemotherapy, radiation and radioisotopes.[51]

Conclusion

Advances in the understanding of tumor biology have led to development of novel antitumor agents targeting tumor vasculature. Initial clinical trials testing these agents in monotherapy were somewhat disappointing and it has now become clear, that in most advanced malignancies, vasculature-targeting strategies will be most effective when used in combination with conventional anticancer therapies. Preclinical experiments on animal tumor models using different AAs and VDAs revealed possible mechanisms responsible for the synergistic antitumor effects of radiation and vascular-targeting strategies, based on AAs/VDAs altering the tumor microenvironment in such a way as to enhance responses to radiation therapy. The importance of treatment sequencing has been demonstrated in these preclinical studies.

Several early clinical trials combining AAs with radiation have showed the potential benefits of this treatment strategy in the clinical setting, warranting further investigations. However, the potential for higher rates of normal tissue toxicity has been documented, particularly in trials where AAs were combined with chemoradiotherapy. This indicates the need for careful design of future clinical trials with optimal radiotherapy planning and delivery in order to minimize damage to normal tissues. It might be prudent to first evaluate in early trials the combination of AAs/VDAs with radiotherapy alone. Further attention should be placed on the doses of AAs/VDAs, as currently there is little data suggesting that higher doses are necessarily better at enhancing the radiation response. Conventional strategies for monitoring anticancer therapies may not apply for vascular-targeted agents and clinical trials need to be designed not only to determine if the agents are safe and have evidence of efficacy,

but also to validate both invasive and noninvasive surrogates of response. This will enable optimal treatment scheduling and, perhaps more importantly, selection of the patients and tumor types that will respond best to this new treatment strategy.

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review

Hyperhomocysteinemia and the role of B vitamins in cancer

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Background. Patients suffering from malignancies have increased complications due to corresponding cardiovascular diseases and risk factor for the development of venous thromboembolism. Epidemiological studies have shown that increased homocysteine plasma concentration (hyperhomocysteinemia) is related to a higher risk of coronary heart disease, stroke, peripheral vascular disease and malignancies. Homocysteine (tHcy) is an intermediate sulfur-containing amino acid produced from methionine during processing of dietary proteins. The plasma homocysteine levels are strongly influenced by diet, as well as by genetic factors. Folic acid, vitamins B6 and B12 are dietary components which influence the plasma homocysteine levels the most. Several studies have found that high blood levels of B vitamins are related to the integrity and function of DNA, and, are at least related to lower concentration of homocysteine. Folate depletion has been found to change DNA methylation and DNA synthesis in both animal and human studies. Because of this critical role of folate, most studies including homocysteine have focused on these two actions.

Conclusions. Hyperhomocysteinemia proves to be the most common condition highly associated with both venous and arterial thrombosis in many cancer patients, while the associated pathophysiology has not been precisely established yet. Therefore, of current interest is the possible role of folate metabolism developing into a cancer initiating hyperhomocysteinemia. This review will discuss this possibility.

Key words: homocysteine; hyperhomocysteinemia; B vitamins; cancer

Introduction

It has been long postulated that the plasma homocysteine concentration is inversely related to the occurrence of cardiovascular and cerebrovascular diseases.[1] More recently, increased plasma homocysteine concentration has been postulated as a risk factor for cancer and even as a novel tumour marker.[2] This increased risk can be attributed to the high prevalence of classical factors in these patients, such as hypertension, diabetes, and dyslipidemia, but most certainly (also) to factors resulting from the malignant disease and the applied selected therapy. For example, back in 1865 Trousseau described hypercoagulability and increasing risk of »spontaneous coagulation« in patients with cancer.[3] Nowadays, it is established that breast, pancreas, and gastrointestinal cancers are associated with a higher incidence of thrombosis. With more advanced stages of cancer there is a lower overall survival rate[4], but, also a greater risk of venous thromboembolism[5], what can additionally influences on the survival of patients. A raised plasma homocysteine level is associated with serum B vitamins concentration, especially folate levels, since these are required in homocysteine metabolism. Adequate B vitamins intake is essential for nucleotide biosynthesis, DNA replication and methyl group supply and thus for cell growth and repair.[6] Evidence suggests that folate depletion fosters the development of cancer, particularly colorectal cance[6-10], whereas high doses of folic acid may enhance growth of cancer cells.[7] However, the complexity of the folate metabolic pathway may suggest that different metabolites of folate might induce multiple effects in normal, preneoplastic and malignant cells.

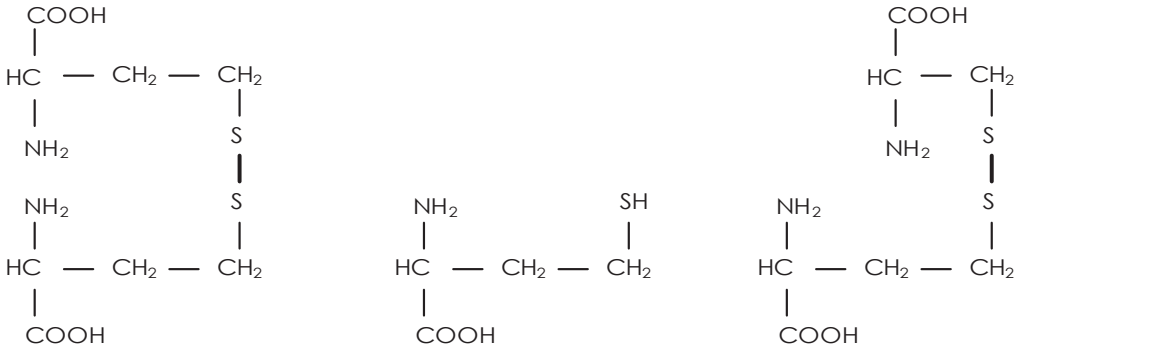

FIGURE 1. Structural formulae: Homocysteine, homocystine and mixed disulfide (cysteine-homocysteine).

Homocysteine, metabolism and cardiovascular complications

Proliferating cells secrete more homocysteine compared to non-proliferating cells. Homecysteine is a sulphur-containing intermediate in the normal metabolism of the essential amino acid methionine present in almost all body cells and mostly 5 to 10% of daily synthesized homocysteine (1.2 mmol/day)[11] is transferred into the blood through hepatocytes. The thyol group of homocysteine makes it readily available to be oxidized in the blood at physiological pH upon which it forms disulfide bonds with other thyols (Figure 1).[12]

In a healthy population the frequency of moderate hyperhomocysteinemia (12-30 μmol/L) is 5 to 7%, with higher values for men being attributed to gender differences like estrogen presence in women. This is confirmed by the fact that after menopause the blood levels of homocysteine of woman approximate those in men.[13] Another cause for moderate hyperhomocysteinemia is an unbalanced diet with suboptimal intake of vitamins (B6, B12 and folates), acting as coenzymes in the metabolism of homocysteine.[14,15] In the elderly, such a moderate hyperhomocysteinemia due to lack of vitamins B and folates is very common. A survey, carried out by Herrmann *et al*., showed that 32% of healthy elderly people aged 65 to 75, and 58% of those over 85 years of age suffer from hyperhomocysteinemia, indicating that hyperhomocysteinemia significantly increases with age[16], therefore it decreases in younger people as the incidence of malignancies.[17]

Via the trans-sulfuration pathway homocysteine is converted into cystathionine to form cysteine by cystathionine-ß-synthase, with vitamin B6 as a co-factor. Another pathway of homocysteine metabolism is the re-methylation pathway, which is connected with the folate metabolic pathway. It involves the transfer of a methyl group from 5-methyl-tetrahydrofolate to homocysteine to form methionine, and eventually S-adenosylmethionine. The methyl transfer from 5-methyl-tetrahydrofolate to homocysteine is catalyzed by methionine-synthase, and requires vitamin B12 as a cofactor (Figure 2). Important to notice is that S-adenosylmethionine is the universal methyl donor for methylation reactions. The resulting tetrahydrofolate transfers into the 5.10 methyltetrahydrofolate with the enzyme 5.10 methyltetrahydrofolate reductase (MTHFR) and then into the 5 methyltetrahydrofolate (5-MTHF).[18] Cellular availability of 5-MTHF may be of great importance in regulating cellular effects of homocysteine related to cell growth. Therefore, deficiencies of folate and vitamin B12 and reduced activity of the involved metabolic enzymes will inhibit the breakdown of homocysteine, which will lead to an increase of the intracellular homocysteine concentration.[19]

Moreover, hereditary causes of increased homocysteine blood concentrations exist (hyperhomocysteinemia). Most studies refer to changes in the genes for those enzymes that lead to severe hyperhomocysteinemia, such as the CBS gene (cistation-β syntases) or in the GCT gene (γ cistationase), both coding the trans-sulfuration pathway. Further, mutations do occur in genes coding for enzymes involved in the remethylation pathway and the related folate metabolic pathway. For a homozygous person with a mutation MTHFR 677C => T the enzyme activity is reduced to 35% of the normal.[20] A typical mutation in Europe occurs within the gene for MTHFR 677C => T, with different incidences between German (24.5%) and Italian (43.8%) popula-

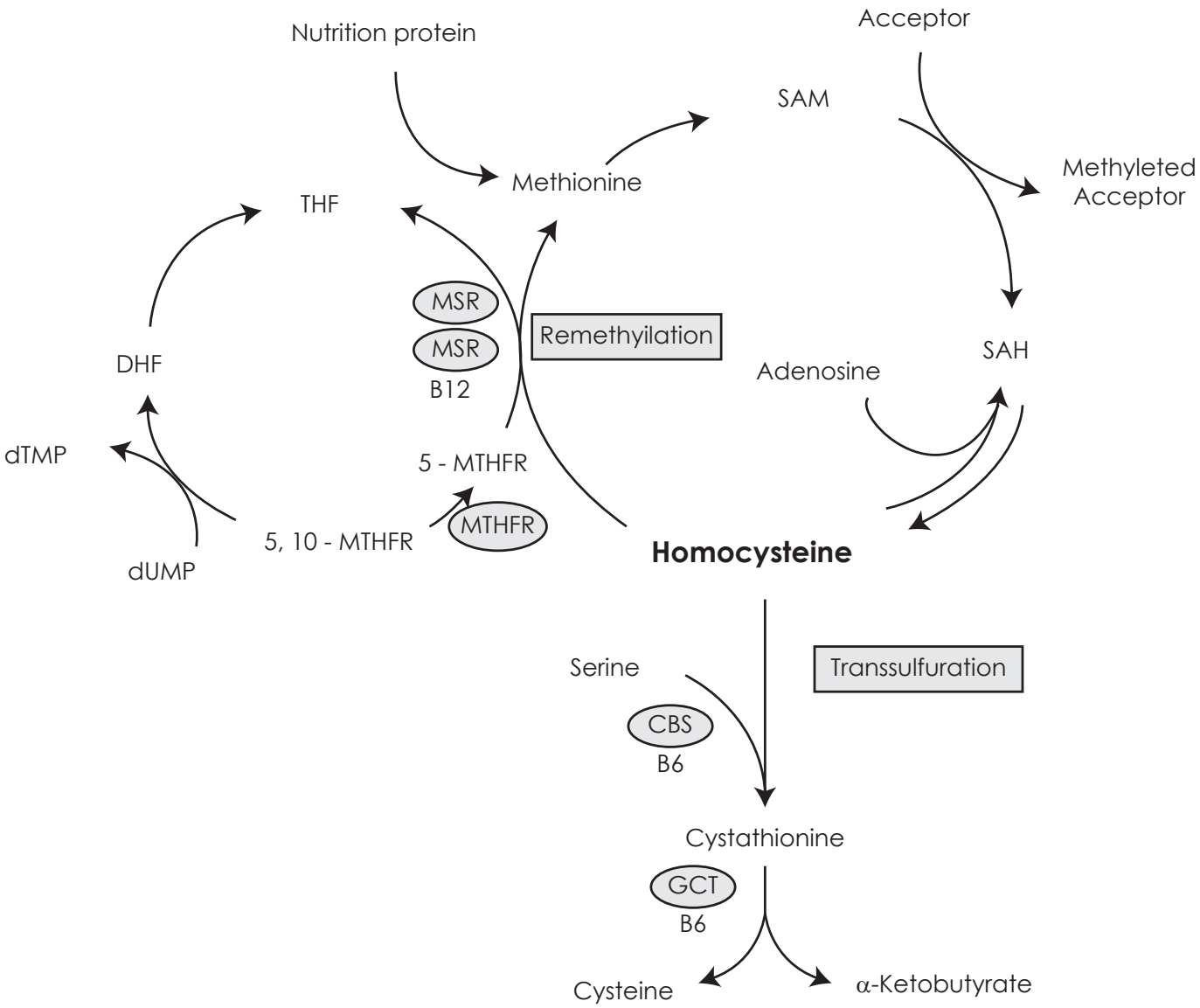

FIGURE 2. Metaboliolism of homocysteine. dUMP - desoxyuridine monophosphate, dTMP - desoxytimidine monophosphste, THF - tetrahydrofolate, DHF - dihydrofolate, 5-MTHF - 5-methyltetrahydrofolate, 5,10-MTHF - 5,10-methyltetrahydrofolate, 5,10 MTHFR - 5,10- methyltetrahydrofolate reductase, MS - metionin synthase, MSR - metionin synthase reductase, B12 - vitamin B12, SAM - S-adenosylmethionine, SAH - S-adenosylhomocysteine, CBS - cystathionine β-synthase, GCT - γ-cystathionase, B6 - vitamin B6.

tions.[20] Moreover, it seems that this mutation and the reduced activity of the enzyme MTHFR are not connected with hyperhomocysteinemia if persons have balanced diet with optimal intake of vitamins (B6, B12 and folates).[19]

Hyperhomocysteinemia is frequently associated with folate deficiency and it has been long postulated that the plasma homocysteine concentration is inversely related to the occurrence of cardiovascular disease and venous thrombosis.[21-24]

Disturbed homocysteine metabolism, hyperhomocysteinemia and cancer

Hyperhomocysteinemia is commonly occurring in a wide range of unrelated diseases. For example, in patients with renal failure a strong, positive correlation was observed between homocysteine (tHcy) levels, serum creatinine, and the renal

glomerular filtration rate. Rheumatoid arthritis impaired gastric and other disturbances results often in elevated blood tHcy.[25] The disease among which elevated tHcy are observed are: Systemic lupus erythematosus, non-insulin-dependent and insulin-dependent diabetes mellitus, hypothyroidism, cognitive impairment and neuropsychiatric disorders (dementia, depression, schizophrenia), fibromyalgia and chronic fatigue syndrome, Parkinson's disease, cerebrovascular disorders, and aseptic meningitis.[26]

Increased tHcy levels are often found in patients with neoplastic diseases.[27] *In vitro* it was shown that some cancer cell lines are incapable of remethylating tHcy and it was recently shown that ovarian cancer cells from patients with elevated tHcy have impaired capacity to remethylate tHcy.[28,29] Tempting to conclude that hyperhomocysteinemia in cancer patients could be secondary to the cancer. However, impaired methylation of DNA and polyamines has often been proposed to be involved in carcinogenesis[30], so the combination of increased tHcy levels and impaired methylation capacity in patients has been proposed as being carcinogenic.[31] In lymphocytes, positive correlation between cellular tHcy levels and increased chromosome damage was shown.[31]

Patient with malignancies often have an increased risk of venous thromboembolic (VTE) disease[5] and as such being the second most common cause of death in cancer patients, second to the primary disease itself. The pathophysiology of this association has not been precisely defined. However, it has been reported that in cancer patients several pro-coagulant factors are increased.[32] Other established contributors to the VTE increased risk is oncological therapy as chemotherapy, hormonal adjuvant therapy, surgery, central venous catheters, immobility and inherited thrombophilia.[5,33,34] However, this oncological therapy can also influence the immunological response of treatment patients.[35] In women with advanced breast cancer hyperhomocysteinemia is common.[36] This observation could explain the high rate of venous thrombosis in women with metastatic breast malignancy.[6] Furthermore, the association between MTHFR C677T polymorfism and breast cancer has been reported. However a positive correlation has not been confirmed by all studies.[37] MTHFR C677T polymorphism is associated with changes in intracellular folate cofactors, affecting DNA methylation and synthesis via altered one-carbon transfer reactions. Of further notice on this association are potential ethnic differences.[38]

Al-Awadi *et al*.[39] demonstrated with nude mice implanting human breast, prostate and pancreas tumour cells leads to decreased plasma cysteine, homocysteine and methionine levels over a two-month period, which was a direct result from the progressing implanted tumour cells. In the case of methionine, the decrease was significant only due to progression of the breast tumours over a long time period. The results suggest that the sulphur amino acids cysteine, homocysteine and methionine can be potentially used as plasma or serum biomarkers for cancer progression.

Many other studies showed that the raised tHcy is related to the cancer itself and to the extent of the disease.[21,40] After remission of the cancer in children with acute lymphoblastic leukemia the tHcy levels returned to normal.[2]

Both plasma concentration of homocysteine and neopterin, a catabolic product of guanosine triphosphate-GTP and as such an immune system activation marker, are closely associated and elevated in patients with various types of disease.[3] From *in vitro* studies it has been shown that tumour cells and other proliferating cells release homocysteine.[41] This *in vitro* notion might be extrapolated to the *in vivo* situation and could explain why hyperhomocysteinemia is observed in patients with various kinds of cancers. Within cellular immune activation, T cells release large amounts of the cytokine IFN-γ, which stimulates human monocyte-derived macrophages and dendritic cells to produce neopterin.[42] For example, in cancer patients, increased urine and plasma neopterin concentrations have been reported, suggesting enhanced cellular immune activation.[43,44] Therefore, immune activation cascades might also be an important triggers for the accumulation of plasma homocysteine in various diseases, including malignancies.

When different tumours are compared, the frequency of increased neopterin concentration is much lower in patients with breast cancer that it is in patients with other types of cancers.[3] Although no association between neopterin and tumour size or lymph node status has been shown in women with breast cancer, follow-up examinations reveal that at diagnosis high urine neopterin concentrations are associated with shorter survival.[4] However, these authors conclude that plasma homocysteine and neopterin concentrations are only rarely elevated in breast cancer patients and they note that the activation and proliferation of immunocompetent cells rather than tumour cells proliferation is responsible for hyperhomocysteinemia in these breast cancer patients.

B vitamins for cancer prevention

Folate, vitamin B12, and vitamin B6, have a number of biologic roles that make them potentially important in cancer. Within DNA synthesis they function as coenzymes in the synthesis of purines and thymidylate. Diminished levels of these vitamins may result in misincorporation of uracil into DNA, leading to chromosome breaks and disruption of DNA repair.[45] As explained earlier both folate and vitamin B12 are involved in DNA methylation. Deficient folate and vitamin B12 levels can reduce the availability of S-adenosylmethionine, the universal methyl donor, for DNA methylation and may thereby influence gene expression.

Inadequate body levels of biologically active folate, vitamin B6, and vitamin B12 are primary determinants of high blood homocysteine levels.[46] Folic acid is a component of food which has been associated with lower cancer risk in epidemiologic studies.[9,10,47,48] Wide geographical variation and migrant studies in cancer incidence and mortality suggest that diet and other lifestyle factors as physical activity influence cancer risk.[49,50] Data on cancer incidence and mortality are available from 37 countries and analysis showed that incidence of colorectal cancer was inversely correlated with more cereals (grains) in the diet.[51] Folic acid present in a wide variety of plant foods, such the legumes, vegetables, fruits and whole grains is thought to be protective against colorectal cancer.[52,53] The lack of folic acid in animal cells studies resulted in DNA defects that resemble effects found in cancer cells.

It has been hypothesized in many epidemiologic studies[38,54,55] that cancer can be initiated by DNA damage (increasing DNA methylation, and by repairing and reducing formation of DNA strand breaks of p53 and Apc genes) caused by folic acid deficiency.[56,57] A deficiency of folic acid leads to a low level of thymidilic acid and alterations in the pool of nucleotides available for DNA and RNA synthesis. It is even suggested that adequate folate intake may be important in the prevention of breast cancer, particularly among women who consume alcohol.[58-60] Alcohol is a known folate antagonist and thus could increase an individual's requirement for folate intake. For vitamin B12, unlike folate, variation in amount absorbed rather than intake is the main determinant of plasma levels in Western populations.[61] In a prospective study analysis of collected blood from 195 case–control pairs, low plasma levels of vitamin B12 were associated with increased risk of breast cancer among postmenopausal women; however, low plasma levels of folate, and homocysteine were not associated with breast cancer risk.[62] Hypofolatemia and metabolic alteration in homocysteine, vitamin B12 could be associated with laryngeal cancer.[63] Therefore, a great effort was made to proof this association as it was made to find association between cysteine cathepsins as well as stefins and promoting and invasion of head and neck tumours.[63,64] Increased plasma vitamin B12 concentration may reduce the risk of rectal cancer.[65]

A recent animal study demonstrated that a B12-deficient diet, which was of insufficient severity to cause anemia or illness, disturbed normal homeostasis of one-carbon metabolism in the colonic mucosa and resulted in diminished genomic DNA methylation and increased uracil misincorporation in DNA, both of which are purported mechanisms for one-carbon metabolism-related colonic carcinogenesis.[66]

In a large prospective study on health care professionals, high intake of folic acid was found to be significantly correlated with low incidence of colorectal adenomas (polyps).[67] Therefore the diet regime and life style factors should be consider as primary prevention beside also important secondary prevention.[49,50,68] Case control studies have as well as found high folic acid intake to be correlated with low risk for either pancreatic cancer or breast cancer.[69,70] In Greece and in Argentina studies correlating breast cancer and diet found risk reductions from six to ten fold in subjects eating mostly vegetables rich of B vitamins.[71] The high risk of developing cancer in a lifetime in the North American and Western European societies might be related to the low intake of vegetables and particularly folic acid might be lacking in diets. Folic acid deficiency could be the permissive condition that enables DNA damage to occur and accumulate. This can lead to DNA damage and cancer.

Is synthetic folate fortification always good for us?

Many countries have implemented mandatory folic acid fortification of flour and grain products to reduce the risk of various diseases, especially neural-tube birth effects. Experimental evidence suggests that high doses of folic acid may enhance growth of cancer cells.[7,53,72,73] These effects have resulted in substantial increase in circulating folate and unmetabolized folic acid concentration.[72,74] Described

the beneficial effects of folate in preventing cancer, it is also well known that high intake of synthetic folic acid might mask vitamin B12 deficiency.[74] Experimental studies suggest that excessing folic acid may promote the progression of already existing preneoplasms.[7] Responsible mechanisms of high folates concentration causing cancer promoting effects include folates providing nucleotide precursors for the preneoplastic cells improving their replication and proliferation. Folates, methyl donors might lead to a de novo methylation and subsequent inactivation of tumour-suppressor genes, resulting in accelerated tumour progression.[7] The safe upper limit for folate intake as well as the safe upper folate concentration in blood are not known.[6] The mandatory fortification of food with folic acid, it dose and the time of intervention depends by the country's decision.[72]

Because the safety of folate might depend on its chemical structure (natural folate or synthetic folic acid), there is the question of potential adverse effects of circulating unmetabolized folic acid.[75]

Conclusions

Many data support a relationship between hyperhomocysteinemia, low B vitamins concentration and risk for various types of cancer. Defective metabolism of tHcy in carcinogenesis is well documented, but the pathophysiology of this association is not fully understood. Many authors suggest that factors contributing to folate status are not protective against certain type of cancer, so further studies are needed to explore folate studies in human.

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research article

Hemostatic efficacy of chitosan-based bandage for closure of percutaneous arterial access sites: An experimental study in heparinized sheep model

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Background. Most of the presently used percutaneous arterial closure devices (PACD) for hemostasis after interventional vascular procedures are effective, but carry risk of complications by deposition of a foreign body. A new promising externally applied PACD – chitosan-based HemCon Bandage (HCB) was explored in sheep. The HCB hemostatic efficacy and complications occurring with its use were compared to those with the standard manual compression (SMC).

Material and methods. Both superficial femoral arteries (SFA) of 9 heparinized sheep were catheterized with an 8F sheath for 5 minutes. After the sheath withdrawal, hemostasis with the HCB was compared with hemostasis achieved with SMC in the contralateral SFA. Iliac angiograms performed by carotid artery approach determined the hemostasis time.

Results. The HCB use shortened time to hemostasis with a mean time of 6.9 ± 3.9 minutes versus 10.8 ± 2.8 minutes for the SMC (P-value 0.019). Seven SFAs in the HCB group and only 1 SFA in the SMC group exhibited hemostasis in 5 minutes. All nine SFAs using the HCB showed femoral artery patency and demonstrated less hematoma (2/9) than in the SMC group (8/9). No complications developed in the HCB group, one SFA occlusion was seen in the SMC group.

Conclusions. The externally applied HCB in heparinized sheep was safe and effective. It significantly shortened time to hemostasis at the SFA access sites following 8F sheath removal. Proper application of the HCB was necessary to shorten hemostasis and prevent hematoma formation. The HCB should be tested in a clinically controlled study to evaluate its efficacy in humans.

Key words: arterial catheterization; hemostasis; closure devices; hemostatic pads; chitosan-based pad

Introduction

Since the introduction of percutaneous catheterization in 1953[1], manual compression over the puncture site has been the standard technique for achieving hemostasis in interventional radiology.[2] With diagnostic angiography using 5F to 6F catheters, manual compression followed by bed rest has been very efficient for achieving hemostasis and has led to less than 1% puncture site complications.[3] Introduction of therapeutic vascular procedures with the need for 8F and larger introductory sheaths, adjuvant anticoagulation and antiplatelets or thrombolytic therapy has led to an increase in complications. Arterial access site complications as high as 17% have been reported with interventional procedures, some of them requiring corrective surgical treatment.[4,5]

Since the early nineties, several types of percutaneous arterial closure devices (PACD) have been introduced to enhance hemostasis after interventional procedures and to decrease the rate of complications. These devices either replace or shorten the time of manual compression at the puncture

site.[5,6] Externally applied hemostatic patches and pads that accelerate the hemocoagulation process at the puncture site are one of the newest PACD types. Acceleration of hemostasis is caused by active ingredients of the patches and pads that contain procoagulants that potentiate clot formation.[7-9] All procoagulants require compression for hemostatis, but substantially reduce compression times. Most procoagulants require contact with blood for activation. There have been several clinical studies on the hemostatic efficacy of these patches and pads.[7-15] However, we have found only two experimental reports documenting the hemostatic efficacy of procoagulants in animals. One paper reported exploration of the efficacy of microfibrillar collagen and thrombin applied into the arterial puncture tract in dogs with the help of a balloon catheter.[16] The other report described a procoagulant (chitosan) installed into the arterial puncture tract in dogs.[17] Chitosan is a linear polysaccharide derived from chitin commercially extracted from marine arthropod shells. It is composed of positively charged molecules that attract red blood cells and platelets, thereby, promoting hemostasis. We report an exploration of the hemostatic efficacy of chitosan-based HemCon Bandage (HCB) (HemCon Medical Technologies, Portland, OR) and a comparison of these bandages with standard manual compression in a heparinized sheep model. We used sheep for testing since their arteries are similar in size to humans.[18,19] In addition, their coagulation and fibrinolytic systems are closer to those of humans when compared to canine and swine.[20]

Material and methods

The study protocol was approved by the Institutional Animal Care and Use Committee. Nine female sheep weighing from 56 to 70 kg were used in this study. A cardiac mobile system (GE/OEC 9800; GE Medical Systems, OEC, Salt Lake City, UT, USA) with digital imaging was used for fluoroscopy and angiography. Digital subtraction angiographies were performed with an injector (Medrad mark Plus, MEDRAD, Inc., Warrendale, PA, USA).

Preparation of animals and their anesthesia were described in previous paper.[18] After induction of general endotracheal anesthesia, the sheep were placed and secured with their backs on the radiographic table and their hind limbs in moderate abduction. The neck and both groins were shaved and prepped. The right common carotid artery (CCA) was exposed and a 9F, 50 cm long introducer sheath (Cook Medical, Bloomington, IN, USA) was retrogradely inserted into abdominal aorta. A standard dose of heparin (100 IU/kg) was then administered intra-arterially. Activated clotting times (ACT) were recorded at baseline prior to heparin administration, prior to arterial sheath removal and at the end of the procedures. A 5F multiside-hole catheter (Cook Medical) was then introduced through the 9F sheath for selective angiography of the external iliac arteries using an injection of 16 ml of Omnipaque (IOHEXOL 300 mg 1/mil, GE Healthcare, Princeton, NJ, USA) in 2 seconds.

The access sites in each animal were the superficial femoral arteries (SFA). One SFA received treatment with the HCB applied with manual compression and the contralateral SFA served as a control with the use of SMC. The sequences of the puncture sites and treatment modes were randomized. With selective iliac angiography, a road map image was created and SFA diameter was measured. Single wall access of the SFA was done under road map guidance with the 21 gauge needle of the micropuncture set (Cook Medical). An 8F sheath was then placed into the artery and left there for 5 minutes. During the sheath removal and prior to the use of the HCB 2″ X 2″ in size, mild nonocclusive pressure was first applied above the skin puncture site. After the sheath was completely removed, a small amount of blood was first allowed to seep on the skin access site to contact the bandage and initiate hemostasis. The bandage was then applied with digital nonocclusive pressure. In the control SFA, significant digital pressure was applied during the sheath removal to prevent blood penetration through the puncture tract, as used in clinical practice. Manual pressure was held in both the treatment and control groups for 5 minutes. Angiography was done immediately after pressure relief to confirm hemostasis. If angiography showed extravasation, compression was continued for a further 2.5 minutes. Angiography was then repeated every 2-1/2 minutes until no evidence of bleeding was seen. Compression was reapplied in the interval between repeat angiographies. Lack of extravasation was the endpoint. The study then proceeded on the contralateral SFA. Finally, angiography of each side was performed at about 30 minutes after cessation of bleeding to check the patency and status of the SFA. The access sites were then checked for hematomas defined as loss of definition of the fossa subinguinalis and raised appearance of the skin. The degree of hematoma at

TABLE 1. Comparison of angiographic findings, ACT values, hemostasis times and post procedure hematomas in 18 punctured superficial femoral arteries, 9 in the HCB and 9 in the control group

	HCB group	Control group	p value
	n = 9	n = 9	
SFA diameter (mm)	5.4 - 6.1	5.4 – 6.1	
Baseline ACT	106 – 161	(136 +/- 19.8)	
ACT prior sheath removal (sec)	205-1061 (404.4 +/- 262)	267- 480 (371.8+/-73.1)	0.737
Hemostasis time (min)	5-15 (6.9 +/- 3.9)	5-12.5 (10.8 +/- 2.8)	0.019
Artery patency at 30 min	9	8	
Subcutaneous Hematoma	2	8 (2 significant)	

TABLE 2. ACT values prior to sheath removal, hemostasis times and hematoma presence at puncture site in individual animals

HCB group				Control group		
AnimalNo.	**ACT**	**Hemostasis**	**Hematoma**	**ACT**	**Hemostasis**	**Hematoma**
1	348	5	0	344	12.5	+
2	268	5	0	412	7.5	+
3	447	5	0	257	12.5	+
4	800	12.5	+	1061	12.5	++
5	361	15	+	244	12.5	++
6	480	5	0	205	10	+
7	262	5	0	260	12.5	+
8	272	5	0	469	5	0
9	433	5	0	388	12.5	+
Mean+/-	**372±73.1**	**6.94±3.9**		**404±262**	**10.8±2.8**	

ACT values in seconds, hemostasis time in minutes; Hematoma grades 0 = none, + = minor, ++ = significant

the groin was graded: 0 = no hematoma, 1 = slight and 2 = significant. Groin area fullness with prominence <1 cm was considered slight hematoma. At the end of the study, the animals were euthanized.

Statistical analysis

Data were recorded into a worksheet (Excel 2007, Microsoft, Redmonds, WA, USA) and summary statistics (mean and standard deviation) were calculated. Student's t-test was used to determine if there was a statistically significant difference between the control and treatment with regard to achieving hemostasis. A value of $P<0.05$ was considered significant.

Results

SFA diameters ranged from 5.4 to 6.1 mm with all pairs being matched in size. The mean ACT prior to the sheath removal in the HCB group was 404.4 ± 262 seconds and in the control group 371.87 ± 73.1 ($p=0.737$). The mean time to achieve hemostasis in the HCB group was 6.9 ± 3.9 minutes, while the mean time of the control group was 10.8 ± 2.8 minutes ($p=0.019$). The results are summarized in Table 1 and 2. In the HCB group, hemostasis at 5 minutes post sheath removal was achieved in 7 of 9 SFAs (77.8%) (Figure 1). The other 2 SFAs exhibited hemostasis at 12.5 and 15 minutes, respectively. One of those two delayed times to hemostasis was equivalent to the control side and the other needed

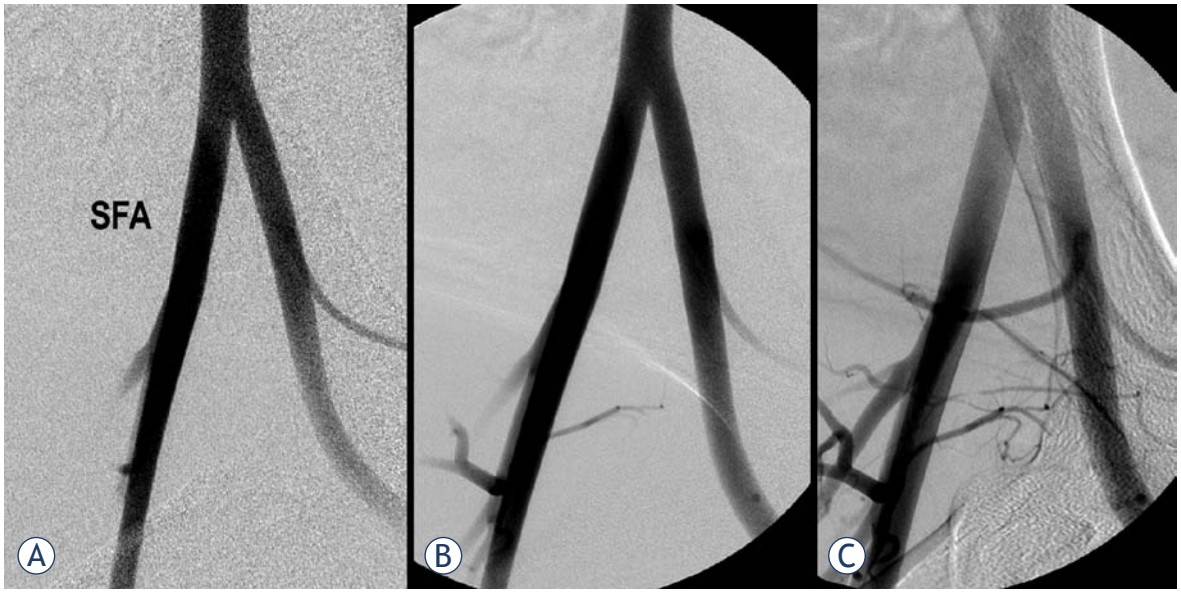

FIGURE 1. Hemostatic control with the chitosan-based bandage. A - Baseline angiogram prior to the superficial femoral artery (SFA) puncture. B - Angiogram obtained after 8F sheath withdrawal and 5 minutes chitosan-based bandage compression shows complete hemostasis. C - Angiogram obtained 30 minutes after sheath withdrawal shows patent SFA.

longer time to achieve hemostasis than the control SFA. Hemostasis was obtained at 5 minutes after sheath removal in one of 9 (11.1%) of the control group (Figure 1). Angiography at about 30 minutes after intervention showed no extravasation in either HCB or control group. All SFAs in the HCB group were patent without demonstrable arterial spasm. Eight of 9 (88.9%) SFAs in the control group were patent and spasm was found in 3 (33%) arteries. Two of these SFAs exhibited small defects, presumably thrombi at the access sites. One SFA was occluded. In the HCB group, there were two grade 1 hematomas, while in the SMC group there were eight, two of which were grade 2.

Discussion

Numerous PACDs are now available for achieving rapid hemostasis at percutaneous arterial access sites after diagnostic or interventional procedures. Madigan *et al*. in 2007 reviewed 14 PACDs.[6] Other new PACDs are being developed and/or tested.[7] Based on their principle mechanism of hemostasis, PACDs are categorized into four groups. The first three groups include biodegradable sealing plugs, suture-mediated devices and staple-mediated devices. They are very effective and have been the most frequently used PACDs. However, they have not been used without complications. Their use has been associated with infections due to deposition of a foreign body, bleeding, pseudo aneurysm, arteriovenous fistula and arterial occlusion.[5,6,18,21] The fourth group of PACDs – patches and pads have recently received close attention. They are topically applied and their procoagulant ingredients accelerate hemocoagulation at the access site without leaving any foreign material behind. Because of their action, they are called "noninvasive" PACDs.[7] The procoagulant components of the noninvasive PACDs include, among others, bovine thrombin (D-Stat-Dry-Vascular Solutions), poly-N-acetyl glucosamine derived from marine diatoms (Syvek Patch-Marine Polymer Technologies), polyprolate acetate (Clo-Sur Pad – Scion Cardiovascular) and chitosan obtained from exoskeleton of crustaceans (Chito-Seal, Abbot Vascular, HemCon®Bandage). Clinical studies of D-Stat-Dry, Syvek Patch, Clor-Sur Pad and Chito-Seal showed that these PACDs applied with compression reduce time to hemostasis after femoral artery catheterization compared with SMC and do not increase the complication rate when using 4-6 F sheaths.[8,10,12-14]

Literature is available on the hemostatic efficacy of the HCB in traumatic animal models, and on HCB use in trauma patients in the military, in emergency departments and during surgery.[22] However, its efficacy for hemostasis after femoral artery catheterization has not been documented by either experimental or clinical studies. Our experimental study demonstrates that the HCB can be effective in this setting. In our experimental heparinized sheep

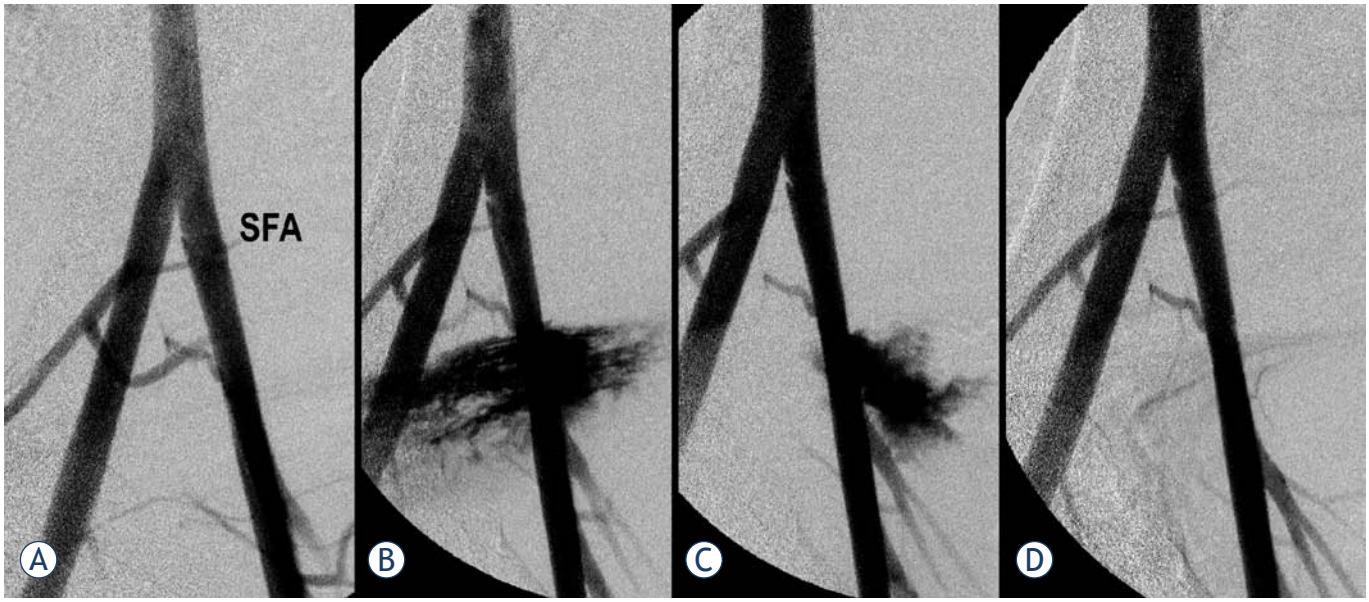

FIGURE 2. Hemostatic control with the standard manual compression. A - Baseline arteriogram prior SFA puncture. B - Angiogram obtained after 8F sheath withdrawal and 5 minutes standard manual compression shows extensive extravasation from the puncture site. C - After additional 2.5 minutes manual compression (total 7.5 minutes), there is decreased extravasation. D - After additional 2.5 minutes of manual compression (10 minutes total), complete hemostasis is achieved.

model using an 8F sheath, the HCB shortened the time to hemostasis to almost half of SMC alone. Hemostasis was achieved with HCB in 7 of 9 puncture sites within 5 minutes, in comparison to only one site in the control group that was within 5 minutes. The two instances where hemostasis with the HCB took the same amount of time or slightly longer time than in the control SFA was due to application of the bandage in a manner similar to that of standard compression where the needed blood to activate the HCB was not allowed to seep through the access tract. This finding strongly reinforces the need for the presence of blood at the access site to initiate the hemocoagulative action of chitosan. The HCB group also demonstrated less hematoma formation than the control group. The higher incidence of hematomas, however, was undoubtedly also related to release of pressure at the access site for performance of angiography before hemostasis could be established. No bleeding was found in both groups 30 minutes after compressions, but in two SFAs small defects suspicious of thrombi were found at the access sites after SMC.

The study limitations include the small sample size and the impossibility of performing a blind study due to the distinctive appearance of the HCB. Another limitation is the absence of data on time to ambulation and long-term efficacy of the closure. Important information about time to ambulation, thus, could not be evaluated. Another study should address these limitations and should also include histopathologic evaluation of the SFAs access site.

Conclusions

The chitosan-based HCB was effective and shortened the time to hemostasis at SFA access sites following removal of an 8F sheath in heparinized sheep. Proper application of the bandage, however, was necessary to shorten hemostasis and decrease hematoma formation. A controlled clinical study needs to be done for evaluation of hemostatic efficacy of HCB following endovascular interventions in humans.

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research article

3T MRI in evaluation of asbestos-related thoracic diseases – preliminary results

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Background. 3T high-field magnetic resonance imaging (MRI) scanners have recently become available for the clinical use and are being increasingly applied in the field of whole-body imaging and chest imaging as well. The aim of this study was to evaluate the diagnostic potential of 3 T MRI as a complementary imaging modality to CT in detecting the pathological changes of asbestos-related thoracic diseases.

Patients and methods. Fifteen patients with the asbestos-related thoracic disease were scheduled for 3T MRI. Five had a benign form of the disease and 10 had malignant pleural mesothelioma (MPM). From the patients with a benign form of the disease their last CT examination in digital form was acquired and patients with MPM were scheduled for CT examination with contrast media. The protocol of MR imaging consists of T2-weighted cardiac-gated breath-hold turbo spin echo (TSE) sequences in coronal, sagittal and axial plane and T1-weighted cardiac-gated breath-hold TSE black blood in axial plane. In T2-weighted sequences in axial plane, fat saturation was also used. CT examinations were obtained with the administration of the contrast medium from lung apices to the lower end of the liver. Images of 5 mm (mediastinum window) and 3 mm (lung window) in axial plan were reconstructed. MRI signal intensity of lesions and adjacent muscles on Syngo MultiModality Work Place were measured.

Results. Compared to muscles pleural plaques appeared hypo-intense to iso-intense on *T1* weighted images (in 100%) and also hypo-intense on *T2* fs-weighted images (in 100%). MPM appeared inhomogeneous hypo-intense to iso-intense on *T1*-weighted and hyperintense on *T2* fs-weighted images in all patients (100%).

Conclusions. These preliminary results pointed out that MRI was equal or even better compared with CT examination for detecting possible malignant potential of pleural changes in the asbestos-related pleural disease, using signal intensity measurements of *T2* fs-weighted images. The 3T MRI enabled the accurate determination of chest pathology and it could be used for imaging of patients with the asbestos-related thoracic disease. MRI is particularly valuable because a patient is not exposed to the harmful radiation which is important if imaging methods are used repeatedly, like in screening programs or in monitoring of treatment results. This finding turned us to propose 3T MRI imaging technique as a non-ionizing imaging method for the follow-up of patients with the isolated pleural form of the asbestos-related disease.

Key words: 3T, magnetic resonance imaging; asbestos-related thoracic disease; malignant pleural mesothelioma

Introduction

Asbestos is a generic term applied to a variety of naturally formed hydrated silicates, which were used because of their heat resistance properties. Asbestos-related thoracic diseases are benign pleural effusions, pleural plaques, diffuse pleural thickening, rounded atelectasis, asbestosis, mesothelioma, and lung cancer. Asbestos is a mainly cause for malignant mesothelioma, much more important than the potential other causes as Simian virus.[1] The incidence of malignant mesothelioma is expected to peak between 2010 and 2030 in industrialized countries despite the regulatory restriction during the 1980s and 1990s.[2]

CT is a gold standard tool for the detection of the asbestos-related thoracic disease although recent studies revealed that MRI was superior in

the detection of the invasive growth of malignant pleural mesothelioma (MPM) in diaphragm and endothoracic fascia or the detection of single chest wall focus.[3] A high radiation dose in repeated CT examination and the use of iodine contrast media in patients with renal disease, diabetes and known allergy must be considered.

3T high-field MRI scanners have recently become available for the clinical use and are now increasingly being applied in the field of the whole body imaging and the thoracic imaging as well. Due to the fact that the signal-to-noise ratio is directly related to the static magnetic field strength the spatial resolution can be increased and the examination time can be shortened.[4]

MRI of the lungs is limited because of physical and physiological factors such as low proton density, susceptibility effect, and respiratory movements as well as cardiac and vascular pulsation. Susceptibility artefacts, magnetic field distortion and motion artefacts are increased in high-field MRI. However, the signal loss from normal lung parenchyma due to the susceptibility effect and the theoretically increased signal from solid changes may result in a higher contrast between the normal lungs and pathological changes in high-field MRI.[5,6]

To reduce susceptibility artefacts the turbo spin-echo (TSE) with a short echo spacing sequence has been recommended.[7] To avoid respiratory movements the breath-hold technique in inspiration with the examination time under 20 seconds should be used[6] and to prevent cardiac and vascular pulsation artefacts ECG triggering must be applied. To avoid additional movements phase array coil should be properly attached with belt.[8]

The aim of our study was to evaluate the diagnostic performance of 3T MRI for detection and characterization of asbestos-related thoracic diseases in comparison to CT.

Patients and methods

Fifteen patients with the asbestos-related thoracic disease (ARTD) were scheduled for 3T MRI. Five had a benign form of the disease and 10 had MPM. From the patients with a benign form of ARTD their last CT examination in digital form, which was not older than one month, was acquired and patients with MPM were scheduled for CT examination with contrast media.

All patients with the benign form of ARTD were males and occupationally exposed to asbestos. The median age was 66 years in range from 53 to 76 years. In patients with MPM were 6 males and 4 females. The median age of this group was 62 years in range from 50 to 75 years. Only 3 males were occupationally exposed to asbestos, in others the history of the environmental exposure was described.

The study was approved by the national medical ethic committee of the Republic of Slovenia. A written consent was obtained from all patients.

MRI studies

MR studies were performed with the Trio team system (Siemens, Erlangen, Germany) equipped with the gradient system with a maximum gradient amplitude 40 mT/m and slew rate of 200 mT/m/ms. A matrix body coil with 6 elements in combination with a spine coil was used.

The protocol of MR imaging consists of *T2*-weighted cardiac-gated breath-hold TSE sequences in coronal, sagittal and axial plane and *T1*-weighted cardiac-gated breath-hold TSE black blood in axial plane. In *T2*-weighted sequences in axial plane Spectral pulse for saturation the signal from fat (SPIR – fs) was also used.

T2-weighted TSE with following parameters were performed:

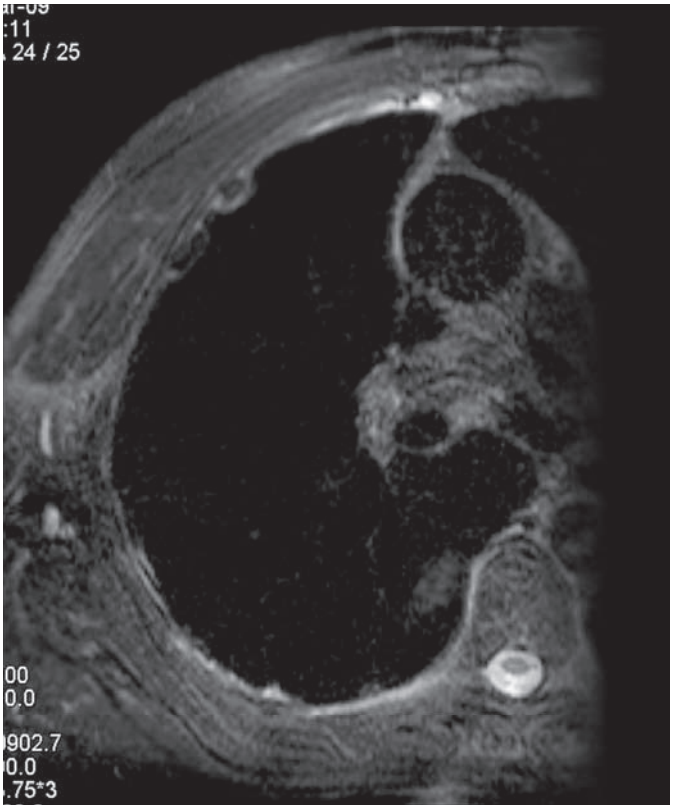
Long TR (repetition time) – depends on heart rate (two cardiac cycles were used)

- TE (echo time) 100 ms
- Slice thickness 5 mm with 1 mm gap
- Turbo factor 29 (Echo Train Length 5)
- Field of view between 350 to 400 mm (depends on the patient size)
- Matrix size 208 x 320 with interpolation
- Parallel imaging factor (iPAT) 2
- Acquisition time <20 seconds.

T1-weighted TSE with following parameters were performed:

- Short TR – depends on heart rate (one cardiac cycles were used)
- TE (echo time) 28 ms
- Slice thickness 5 mm with 2.5 mm gap
- Turbo factor 9 (Echo Train Length 11)
- Field of view between 350 to 400 mm (depends on the patient size)
- Matrix size 106 x 256 with interpolation
- Parallel imaging factor (iPAT) 2
- Acquisition time <14 seconds.

The examination time takes from 25 to 30 minutes depending on the patient's cooperation.



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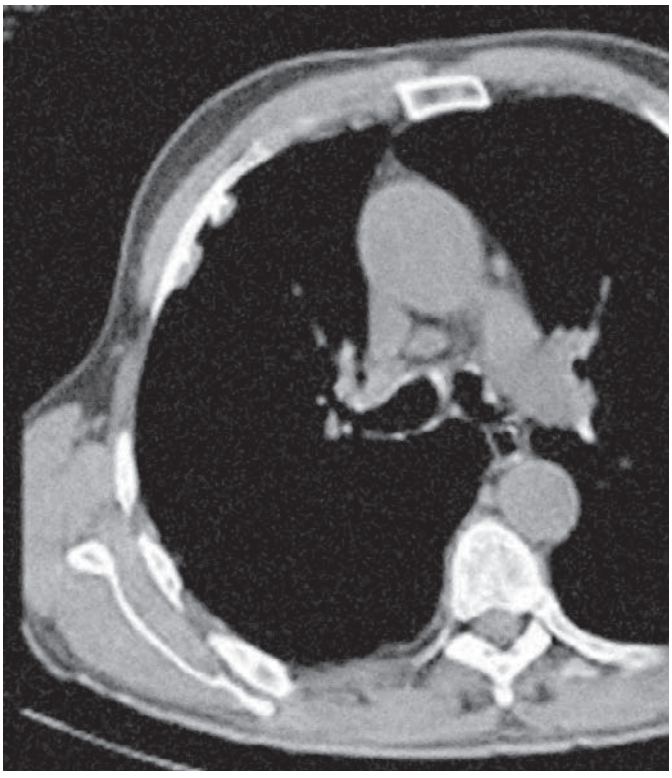

B

FIGURE 1 A, B. MRI and CT images of the same patient. (A) *T2* fs-weighted MRI image in axial plane shows benign hypo-intense pleural plaques with hyper-intense rim. (B) On CT image calcification on the lateral side of the plaques are more delineated than on MRI images.

CT examinations

CT examinations were obtained with Somatom 16 or Definition scanners (Siemens, Erlangen, Germany) with the administration of the contrast medium using a power injector with 2 ml/s flow. CT scans with 120 kV and 100 mAs were performed from lung apices to the end of the liver. Kernel B31f medium smooth for mediastinal window (W: 350, C: 35) and B80f ultra sharp for the lung window (W: 1600, C: -600) were used. Images of 5 mm (mediastinal window) and 3 mm (lung window) in axial plan were reconstructed.

Measurement of MRI signal intensity

MRI signal intensity of lesions on Syngo MultiModality Work Place were measured to establish their hyper intensity or hypo intensity compared to the signal intensity of muscles.

On *T1* and *T2* fs-weighted images in axial plane the circular region of interests (ROI) with area expanse from 0.3 to 0.4 cm^2 were drawn. The region with the pronounced artefact was avoided. Data were collected and arranged regarding to the characteristics of the lesion.

MRI and CT examinations were assessed by two radiologists experienced in chest imaging.

Results

Compared to muscles benign pleural plaques appeared hypo-intense on *T2* fs-weighted images and on *T1* weighted images. In some plaques a hyperintense rim between the hypo-intense plaque centre and lung parenchyma on *T2* fs- weighted images was found (Figure 1). A diffuse pleural thickening was more distinctive on MRI than on CT images.

MPM appeared inhomogeneous hypo-intense to iso-intense on *T1*-weighted and hyperintense on *T2* fs-weighted images. SPIR was used to saturate the signal from the fat and consequently a high signal compared to the muscle was only found in malignant lesions. In our study 3T MRI shows the extent of the tumour and accompanying pleural solid and fluid components with the greater accuracy compared to CT examination (Figure 2).

Results of signal intensity measurements on *T1*-weighted images (Figure 3) have shown that the MR signal from benign pleural plaques compared to muscles was hypo-intense to iso-intense in all patients (100%). In eight patients with MPM

(80%) the measured MR signal was hypo-intense, iso-intense in one patient (10%) and hyper-intense in one patient (10%).

Results of measurements on *T2* fs-weighted images (Figure 4) have shown that the MR signal from benign plaques compared to muscles was hypo-intense in all patients (100%) and hyper-intense in patients with MPM (100%).

Discussion

The benefit of 3T high field MR scanners which have recently become available for the clinical use is higher signal-to-noise ratio. For this reason the examination time can be shortened and the spatial resolution can be improved.[4] These facts are the reason that MRI is expanding on the field of chest imaging and becoming comparable imaging modality to CT and proposed as a valuable alternative to certain patient groups.[9]

The most significant advantage of MRI of MPM is its excellent contrast resolution of soft tissues. Recent studies have demonstrated that MRI is superior to CT in evaluating of MPM invasive growth in diaphragm and abdominal cavity, invasion of endothoracic fascia and mediastinal structures.[3,10] Falaschi *et al.*[11] analyzed the potential usefulness of MR signal intensity in differentiating the malignant from the benign pleural disease and concluded that the hypo-intense signal in pulse sequences with long TR is a reliable predictive sign of the benign disease. In our study the intensity of the signal measured in benign pleural plaques compared to muscles on *T1*-weighted images were hypo-intense to iso-intense. On *T2* fs-weighted images the signal intensity from MPM compared to muscles was hyper-intense in all patients.

CT using high resolution protocols is superior to MRI in imaging of parenchymal and early interstitial involvement in ARTD, but MRI achieved a comparable interobserver agreement in detecting pleural plaques compared to CT and a higher interobserver agreement in revealing other pleural pathologies.[2,7] In the same article it is also stated that a hyper-intense rim of benign asbestos plaques next to lung parenchyma on *T2* weighted images possibly correlate to reduced plaque collagen fibrils and that diffuse pleural thickenings are more pronounced on MRI than on CT. In our small group of patients with asbestosis (altogether 5 patients) we found that imaging of the asbestos pleural disease with 3T MR protocols was comparable with CT imaging showing almost the same extent and nature (calcified and non-calcified) of pleural plaques, but statistically significant conclusions on this statement needs to be confirmed on the larger number of patients.

The incidence of ARTD is rising but the pattern of ARTD has been changing due to the intensity of exposures. The incidence of MPM will probably increase at least twenty years after the interdiction of asbestos.[13] This fact requires the evaluation of screening programs for the detection of early stage malignant changes in a high risk group. MRI is a very suitable imaging method in repeatedly screening programs because the patient is not exposed to a harmful radiation. MRI can be also used in monitoring patients treated with chemotherapy.

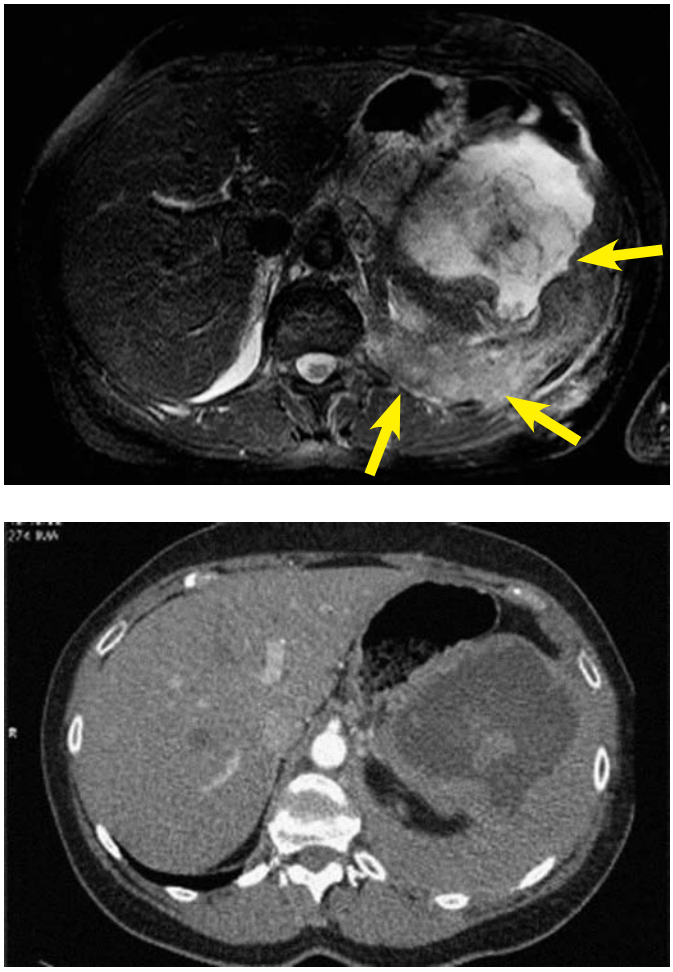

FIGURE 2 A, B. MRI and CT images of the same patient. (A) *T2* fs-weighted MRI image in axial plane shows chest wall and diaphragmatic invasion and the extent of the tumor in abdominal cavity (arrows). (B) Contrast enhanced CT image on the same position shows worse contrast resolution compared to MRI.

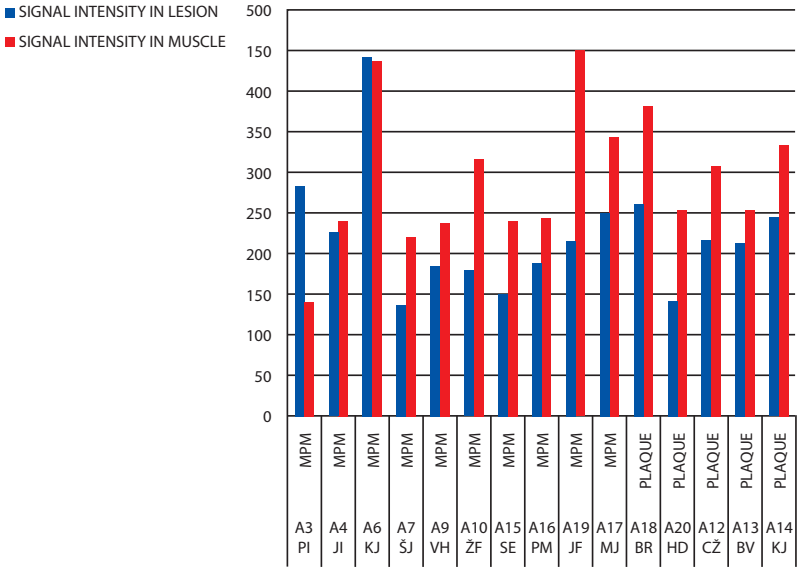

FIGURE 3. Signal intensity measurement on T1 fs-weighted images.
MPM = malignant pleural mesothelioma

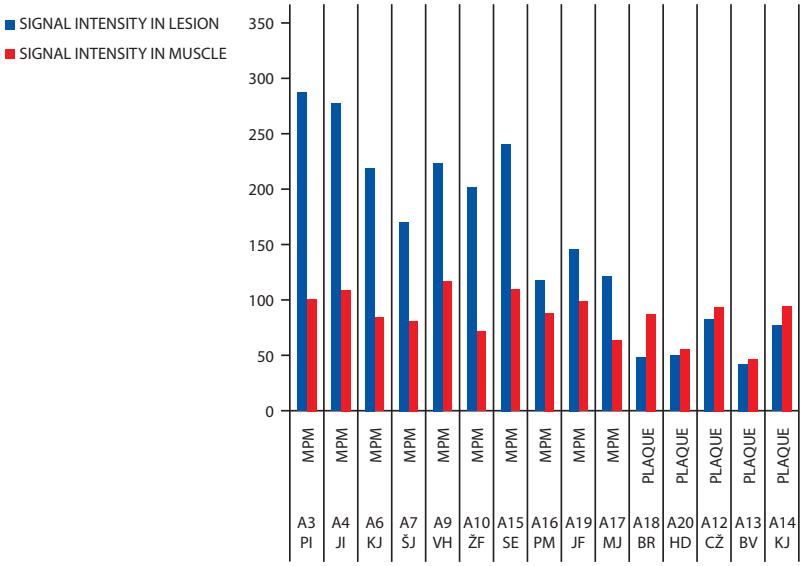

FIGURE 4. Signal intensity measurement on T2 fs-weighted images.
MPM = malignant pleural mesothelioma

The results of our preliminary study show that CT is still a gold standard in imaging patients with thoracic diseases, also because the large number of CT scanners is available. 3T MRI is a promising method which can be nowadays used as a complementary method.

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research article

Reliability of diffusion weighted MR imaging in differentiating degenerative and infectious end plate changes

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Part of this study was presented as an poster presentation in *17th Symposium Neuroradiologicum of European Society of Neuroradiology (ESNR)* Paris, France, 2002.

Background. The aim of the study was to investigate the value of diffusion weighted MR imaging in the diagnosis of Modic type 1 change, which may be confused with the acute infectious spondylodiscitis on conventional MR imaging.
Patients and methods. Twenty-seven patients with erosive intervertebral osteochondrosis, Modic type 1 and 18 patients with spondylodiscitis were included in this retrospective study. All images were acquired using on 1.5 Tesla MR units. Lumbar spinal MR imaging of 45 patients were retrieved from a digital database of a radiology record system and evaluated by one experienced radiologist. Patients with Modic type 1 change had CT slices obtained from the diseased disc space and the affected vertebrae.
Results. Bone marrow adjacent to the vertebral end plate in both Modic type 1 change and acute spondylodiscitis were hypointense on T1-weighted images. On T2-weighted images corresponding levels of vertebral end-plates showed hyperintense signal intensity in both group. All the patients with spondylodiscitis and Modic type 1 change were hyperintense and hypointense on diffusion-weighted MR images, respectively.
Conclusions. Our findings suggest that diffusion weighted MR imaging is an useful method in differentiating Modic type 1 changes from acute spondylodiscitis, both of which may mimic each other, either on clinical or conventional MRI findings.

Key words: Modic type 1 change; spondylodiscitis; magnetic resonance imaging; diffusion-weighted imaging; vertebral end-plate.

Introduction

Vertebral end-plate abnormalities of the lumbar spine are commonly seen on MR images.[1] Of these abnormalities most of them are frequently associated with degeneration.

Degenerative vertebral end-plate changes were first described independently by Roos *et al.*[2] and Modic *et al.*[3] as being a feature associated with the degenerative disk disease. These changes, also called as erosive intervertebral osteochondrosis (EIVO), were classified into three groups by Modic *et al.*[3] Type 1 change is vascular granulation tissue, demonstrated as low signal intensity on T1-weighted images (T1WI) and high signal intensity on T2-weighted images (T2WI). They are associated with fissuring of cartilaginous end-plate and increased vascularity within the subchondral bone marrow on the histological examination.[4] Type 2 change is fatty infiltration of the end-plates, dem-

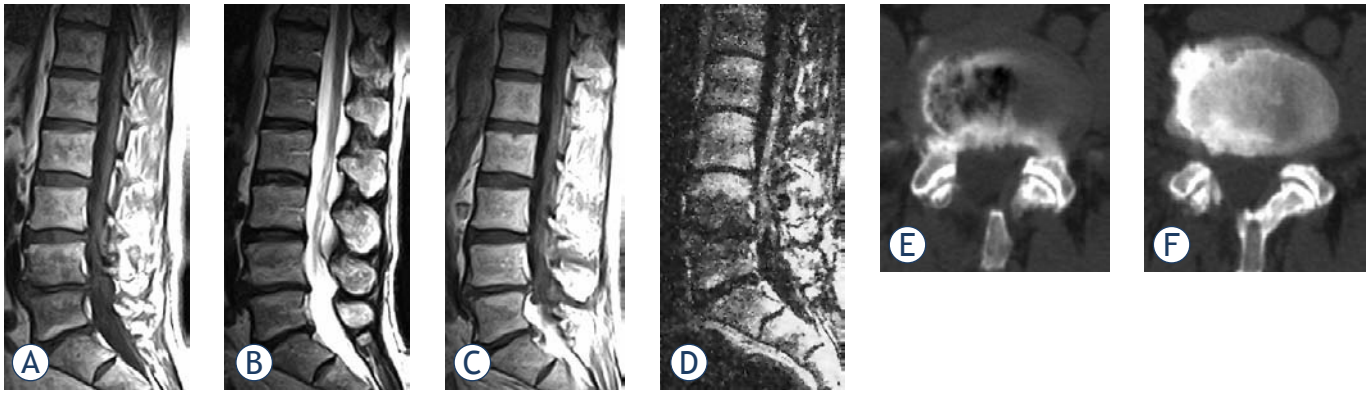

FIGURE 1. Sagittal MR images from 53 years old male patient with low back pain. A) T1-weighted MR image demonstrating low-signal intensity changes adjacent to the L4-5 disk, and B) T2-weighted MR image of the same level demonstrating high-signal intensity changes. C) On post-contrast T1-weighted images end-plates disclose signal intensity increase, which can also be seen in spondylodiscitis. D) On DWI end-plates showing low signal intensity changes consistent with Modic type 1 change at L4 through L5. E) and F) Axial CT slices obtained from the L4-5 disk level showing discal vacuum phenomenon and sclerosis, supporting the diagnosis of degenerative disc disease.

onstrated as hyper intense signal intensity on T1WI and hyper intense or isointense signal intensity on T2WI. In such cases biopsy reveals the fatty replacement of the marrow, which is thought to be the result of marrow ischemia.[4] Type III changes consist of reduced signal intensity on both T1- and T2-weighted images representing bone sclerosis.[3]

Intervertebral disk space infections typically give rise to vertebral marrow oedema, manifesting as areas of low signal intensity on T1WI and high signal intensity on T2WI.[5]

Thereby, type 1 Modic changes may cause a diagnostic dilemma in patients with low back pain since sometimes it resembles the MRI features of spondylodiscitis.[6]

The diffusion weighted imaging (DWI) has recently been used in the spine by many authors, mainly for the differentiation of benign and malign oedema of the vertebral body.[7-9] DWH might be also useful in the differential diagnosis of benign from malignant lesions in other organs.[10] It has been reported that benign fracture oedema depicts hypoisointensity on DWI whereas malign infiltration of the vertebrae discloses hyperintensity. To distinguish the benign from the malignant differences is crucial to choose the right treatment.[11] DWI has also been used in spondylodiscitis. It has been shown that DWI reveals hyperintensity in the affected vertebrae and the paravertebral infectious soft tissue in acute spondylodiscitis.[12] The purpose of this investigation was to evaluate the usefulness of diffusion weighted MR imaging for the differentiation of Modic type 1 changes from acute spondylodiscitis, both of which may mimick each other, either on the clinical evaluation or conventional MRI findings.

Patients and methods

Patients

Forty-five patients (18 patients with acute spondylodiscitis and 27 patients with Modic type 1 change) who underwent lumbar MRI examinations between January 2001 and December 2008 were included after a review of a digital database of a radiology record system. They were identified from a total of 1400 MR imaging examinations of the lumbar spine with a low back pain performed during this time at our institution. Patients with signal abnormalities limited to having previous surgery, recent vertebral fracture, metastatic disease, and pregnancy were excluded from the study.

Spondylodiscitis were proven with CT-guided biopsy in 12 patients. In 6 patients the diagnosis of spondylodiscitis were based on laboratory findings.

The diagnosis of Modic type 1 change were proven either by clinical or laboratory findings. In order to confirm the diagnosis radiologically two year-follow-up MRI was assessed. On the follow-up Modic type 1 in 8 of 27 patients partially converted Modic type 2 and 14 of 27 patients fully converted Modic type 2. Five of 27 patients were stable, and showed no change. CT slices were obtained from the diseased disc space and the affected vertebrae in all the patients to support the diagnosis.

Spondylodiscitis was diagnosed in the presence of paravertebral or epidural signal abnormalities with or without abscess formation. If such findings were absent, three of the following four criteria had to be fulfilled for the disk-space infection: signal abnormality of the bone marrow adjacent to the in-

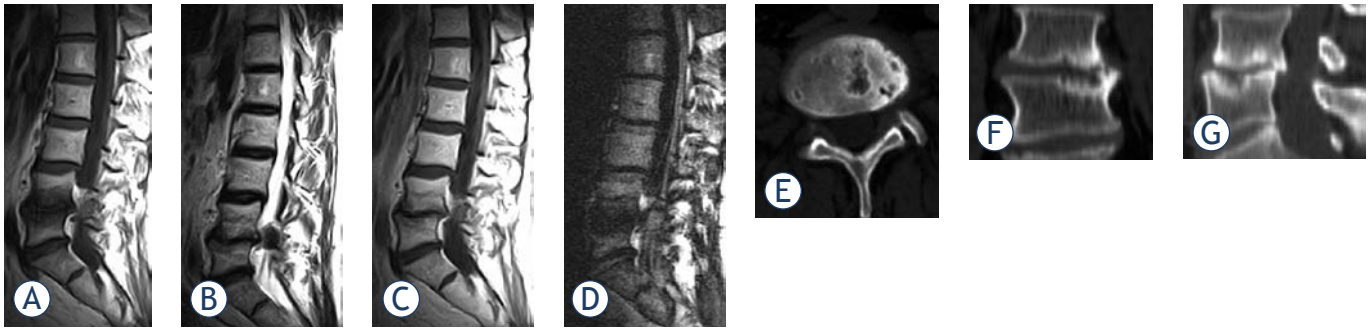

FIGURE 2. Sagittal MR images from 48 years old male patient with low back pain. A) Sagittal T1-weighted and B) Sagittal T2-weighted MR images showing low and high signal respectively at both end-plates of L4-5. C) On post-contrast T1-weighted images same level demonstrating heterogenic contrast enhancement. D) On DWI end-plates demonstrating low signal intensity changes consistent with Modic type 1 change at L4-5. E) Axial CT slices obtained from the L4-5 disk level and F) Coronal and G) Sagittal reconstructed CT image showing sclerosis on both end-plates of L4-5.

tervertebral disk (hypointense on T1-weighted images and hyperintense on T2-weighted images, signal not well demarcated); loss of the low-intensity vertebral endplate on T1-weighted images; hyperintensity of the disk on T2-weighted images; and disk enhancement after the injection of gadopentetate.

All patients gave a written informed consent to use their clinical data for the study purposes. The study protocol was approved at the research ethics review committee of the hospital.

Imaging technique

A standard lumbar MRI protocol was employed to all patients. All images were acquired using on 1.5 Tesla MR units. All MR studies were performed on a 1.5 T unit (Magnetom Vision, Siemens, Erlangen, Germany) with gradient echo-planar capabilities and a standard phased array surface receiver coil for imaging the spine. The imaging protocol included axial and sagittal T1-weighted spin-echo sequences (552/ 12[TR/TE]), axial and sagittal T2-weighted turbo spin-echo (4000/120[TR/TE]) sequences, sagittal STIR(3600/60[TR/TE]) sequences, and sagittal diffusion-weighted sequences. Sagittal spinal DW images (b = 150 s/mm^2) were acquired in the same plane and orientation as used in the routine sequences by using a reversed fast imaging with steady-state precession (PSIF) sequence (TR/TE 1.400 ms/100 ms; field of view 320×80 mm; section thickness 5 mm; intersection gap 0.5 mm; sections 6; matrix 128×256; echo train length 69 and one excitation) with spectral presaturation and inversion recovery (SPIR). In addition, axial and sagittal fat-suppressed T1-weighted images were obtained after IV infusion of 0.1 mmol/kg of gadopentate dimeglumine.

Image assessment

All MR images were reviewed and evaluated by one radiologist specializing in MR imaging of the spinal system. The abnormal levels were classified as either infected or degenerative.

Signal intensity changes of the related disc and vertebral body marrow adjacent to the end plates of the degenerative spine on the conventional spin-echo sequence MR and the diffusion weighted MR were compared with those of spondylodiscitis.

We categorized the signal intensity of the abnormal vertebra on T1-Weighted images as hypointense relative to the presumed normal marrow. The signal of the abnormal vertebra on T2- weighted images was categorized as hypointense, isointense or hyperintense relative to the areas of the presumed normal marrow. On the diffusion-weighted images, the areas of the abnormal signal intensity were categorized as hypointense, isointense and hyperintense with respect to the normal marrow.

Statistical analyses

The statistical analysis was carried out by using Statistical Package of Social Science (SPSS), version 13.0.

Results

The mean ages of patients with Modic type 1 change (Figures 1, 2) and spondylodiscitis (Figure 3) were 52.2 years (range, 24-77 years) and 55.8 years (range, 18-85 years), respectively.

These 27 patients had a total of 62 Modic changes and 18 patients with acute spondylodiscitis had

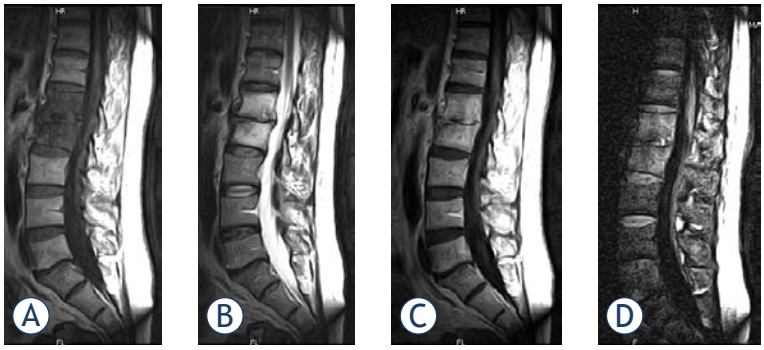

FIGURE 3. Sagittal MR images from 38 years old male patient with acute spondylodiscitis. A) T1-weighted image showing complete homogenous hypointensity at the L1 and L2 vertebrae corpus and adjacent intervertebral disc. There are also osteophytic changes on end-plate and loss of L1-2 intervertebral disc space. B) T2-weighted images showing hyperintense signal intensity corresponding to the same level. C) Postcontrast image showing homogenous enhancement of disc and adjacent vertebrae corpus. D) DWI revealing high signal intensity relative to neighbouring normal vertebrae.

46 vertebral involvement. Four patients with type 1 Modic changes and 5 patients with spondylodiscitis had more than two vertebral involvements.

On CT slices of affected vertebrae in patients with Modic type 1 change, 5 patients (18.5%) had discal vacuum phenomenon, 22 patients (81%) had well-defined sclerosis, and 4 patients (14.8%) had erosions of vertebral endplates without bone destruction.

Bone marrow adjacent to the vertebral end plate in both Modic type 1 change and and acute spondylodiscitis were hypointense on T1-weighted images. The hypointense areas in the vertebral end plates on T1-weighted images in patients with Modic type 1 change enhanced either moderately or strongly on postcontrast images, a finding which could not be differentiated from a disc space infection. On T2-weighted images corresponding levels of vertebral end-plates showed hyperintense signal intensity in both groups. On diffusion-weighted MR images with relatively low b values, all vertebral body marrow and end-plates with Modic type 1 change showed hypointense to normal signal intensities. Conversely, all vertebral body marrow and end-plates with acute infectious spondylodiscitis showed increased signal intensities when compared to presumed normal vertebrae.

Discussion

MR imaging (MRI) is commonly used in the diagnosis of patients with low back pain (LBP) and sciatica.[1] In the search for causes of LBP, vertebral end-plate signal changes have come into focus. Vertebral end-plate changes are bone marrow and end-plate lesions visible in MRI.

Different pathological processes can involve vertebral bone marrow adjacent to the end-plates, including degenerative disc disease, infection and tumours, and these may present with a variety of signal intensity as shown by MRI.

After the initial description of Modic changes some studies have attempted to identify the cause of such changes. Modic type 1 changes were found to be associated with fissuring of the cartilaginous end-plate and increased vascularity within the subchondral bone marrow on the histological examination.[4] Vertebral end-plate changes consistent with bone marrow oedema may also be seen in infective discitis, following intraosseous disc herniation (Schmorl's nodules) and within 3 months of chemonucleolysis.[13-15]

Spondylodiscitis is an infection of the intervertebral disk and the adjacent vertebrae, with or without associated epidural or psoas abscesses. It is a serious disease both due to its long-term course and the possible outcomes.[16]

Type 1 Modic change and spondylodiscitis may both reveal similar symptoms, mainly LBP. Clinical and laboratory findings such as white blood cell count, erythrocyte sedimentation rate and elevated body temperature are, supportive but not confirmatory evidence in infectious spondylodiscitis.[17]

Stirling *et al*.[18] suggested a theory that bacteria might play a causative role in LBP in Modic type 1 changes and that patients might benefit from the antibiotic treatment. Stirling *et al*.[18] found bacteria in disc material from 19 of 36 patients with severe sciatica and Albert *et al*.[19] showed that 17 out of 32 patients with Modic type 1 changes and persistent LBP achieved long-lasting pain relief after the long-term antibiotic treatment. But Wedderkopp *et al*.[20] showed no evidence of bacteria in vertebrae in Modic type 1 changes in their recent study, although in this study possible presence of bacteria in the disc adjacent to the Modic type 1 changes in the vertebrae cannot be ruled out.

Fayad *et al*.[21] found that patients with chronic LBP and predominantly type 1 Modic changes had a better short-term relief of symptoms following intradiskal steroid injection than those with predominantly type 2 changes, which further supports the inflammatory nature of Modic type 1 changes and the role of inflammation in the generation of LBP.

Although the aetiology of the inflammatory process in the vertebrae is still unknown in Modic type 1 change, there are two accepted possible

theories.[20] The most well known theory is that the process is a part of the ''normal'' degenerative process of the spine, where dehydration of the nucleus and loss of disc height biomechanically leads to unphysiological load and shear forces causing microfractures followed by inflammation in the vertebral end-plate and adjacent bone marrow.[20] A second theory is that anaerobic bacteria with low virulence enter the vertebra from the bloodstream, resulting in infection, which is represented on MRI as Modic type 1 changes.[20]

On MRI, type 1 change reveals hypointensity on T1-weighted and hyperintensity on T2-weighted images. Affected bone marrow may show mild or strong contrast enhancement after the intravenous contrast administration. Differentiation of these findings from those of spondylodiscitis may not be always possible based on the conventional MRI findings alone.[6,21,22] CT may be helpful in the differentiation showing discal vacuum phenomenon or well-defined sclerosis and erosions of vertebral endplates without bone destruction, findings that support the diagnosis of EIVO. In our patient group, CT revealed these changes compatible with EIVO at the end-plates adjacent to the degenerated disc.

DWI imaging has been successful to some extent in the differentiation of benign versus pathologic compression fractures of the vertebral body.[7-9,12] It has been shown that benign bone marrow oedema has revealed hypointensity on DWI and malign vertebral compressions have disclosed hyperintensity in the vertebral bone marrow.[7,9] Recently it has been reported that infective discitis has also shown hyperintensity on DWI as well as malignancies of the affected vertebral bone marrow.[12] However, in this study we have found hypointensity on DWI at the Modic type 1 disorders.

In general, histopathologic findings of the type 1 bone marrow are fibrovascular tissue totally replacing normal marrow elements.[5] A possible explanation for our results on diffusion-weighted MR imaging is that in the fibrovascular degenerative change, the increased free water of bone marrow caused by depletion of normal marrow elements leads to an increase in the extracellular volume fraction which produces low signal intensity in diffusion-weighted MR imaging. In contrast, in spondylodiscitis the reduction of the extracellular volume due to densly infiltrated inflammatory cells might lead to the increase in the signal intensity on diffusion-weighted MR imaging.

Although there are some clues for the diagnosis of type 1 changes on routine MRI, it sometimes may be very difficult to differentiate it from early onset spondylodiscitis. Intervertebral disk space infections typically give rise to vertebral marrow edema, manifesting as areas of low signal intensity on T1WI and high signal intensity on T2WI, thereby mimicking type 1 Modic changes. The contrast enhancement in the disk and endplates may occur in both conditions. Moreover, the enhancement of the intervertebral disc itself is not a definitive rule for spondylodiscitis, since sometimes this cannot be detected. Because of desiccation and dehydration, the disk often appears normal or hypointense on T2WI in degenerative disc disease, whereas its T2WI signal intensity is typically increased in spondylodiskitis.[21] If there is enhancing paravertebral soft tissue mass adjacent to the intervertebral disc space, the MRI findings should orient the diagnosis toward an infectious process.[6] The vertebral endplates are usually preserved in degenerative disc disease rather than destroyed or eroded with the bone destruction and the presence of these findings should also remind the possibility of disk space infection.[23] However, in the absence of these findings, the decision should be made between EIVO type I and spondylodiscitis. We believe that DWI can make this differentiation easily and eliminate the necessity of CT in these groups of patients.

There are some limitations to the study presented here. The interpretations of the MR images were performed by only one experienced radiologist. Thus, the interobsever variability and accuracy associated with a less experienced radiologist was not assessed. Another limitation was the small number of patients who participated in the study. The diagnosis of Modic type 1 was relied on the clinical and radiological follow-up and we had no histopathologic correlation in our study. Further studies comparing histopathologic results and DWI findings are needed.

In clinical practice, most radiologic and clinical findings are sufficiently supportive for the differential diagnosis of pathological processes involving vertebral body marrow adjacent to the end-plates. But, sometimes Modic type 1 change causes a diagnostic dilemma in patients with low back pain since it has almost the same conventional MR imaging and clinical findings with the acute spondylodiscitis. Moreover postcontrast MR imaging will not lead to the differential diagnosis since the Modic type 1 change will also enhance as well as spondylodiscitis. However, DWI appears to be useful in the differentiation of EIVO from the acute spondylodiscitis with distinct documented features in this study.

For this reason, when results of clinical and conventional MR findings are equivocal, diffusion-weighted MR imaging may provide an excellent differential diagnosis between degenerative fibrovascular change of the spine and pyogenic spondylitis.

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case report

Post-traumatic high-flow priapism treated by endovascular embolization using N-butyl-cyanoacrylate

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Background. Priapism, persistent erection without arousal, can be classified into low-flow (venous or ischemic) and high-flow (arterial or non-ischemic). The diagnosis of high-flow priapism can be confirmed by colour Doppler and arteriography and it is usually treated by the endovascular embolization.

Case report. We present a case of a 20-year-old man with a post-traumatic high-flow priapism as a result of the previous perineal trauma. After a period of watchful waiting and an unsuccessful attempt at endovascular embolization using the resorptive gelatinous foam he was successfully treated by the endovascular embolization using N-butyl-cyanoacrylate.

Conclusions. High-flow priapism can be successfully treated by the endovascular embolization, but the optimal choice of the embolization agent and a careful technique is essential.

Key words: priapism; endovascular embolization; angiography; Doppler duplex ultrasonography; MRI angiography

Introduction

Priapism is a relatively rare condition characterized by the persistent erection in the absence of sexual arousal. There are two main subtypes: the more common ischemic, or low-flow, characterized by the impaired outflow from the corpora cavernosa, and non-ischemic, or high-flow, most often caused by trauma, characterized by the formation of arteriocavernous fistulas and increased inflow of blood to the corpora cavernosa. While the painful low-flow priapism and the associated decreased oxygenation of cavernous tissue can quickly lead to a cavernous fibrosis and permanent damage to penile tissues and is, therefore, an urological emergency, high-flow priapism is often painless and can persist for months or years, in most cases without a permanent damage of penile tissues, but sometimes with the reduced potency.[1]

The diagnosis of high-flow priapism can be confirmed by colour Doppler[2], which can also be used to characterize the number and location of arteriocavernous fistulas and concomitant arterial pathology such as pseudoaneurysms. Colour Doppler is also useful in the follow-up, avoiding the repeated angiography with its risks and the ionizing radiation dose, although the MR angiography is usually necessarily to evaluate the effect of the radiological invasive interventional procedures.[3]

There are many treatment options in high-flow priapism: those mentioned most often are watchful waiting[4], Doppler-guided compression[5], endovascular highly selective embolization and surgery. Because the more aggressive treatment methods are associated with a small but significant rate of the permanent erectile dysfunction, an initial watchful waiting period is commonly indicated. The surgery in high-flow priapism usually consists of the ligation of a cavernous artery or its branch and is reported to have the highest permanent erectile dysfunction rate, thus it is usually the last treatment option.

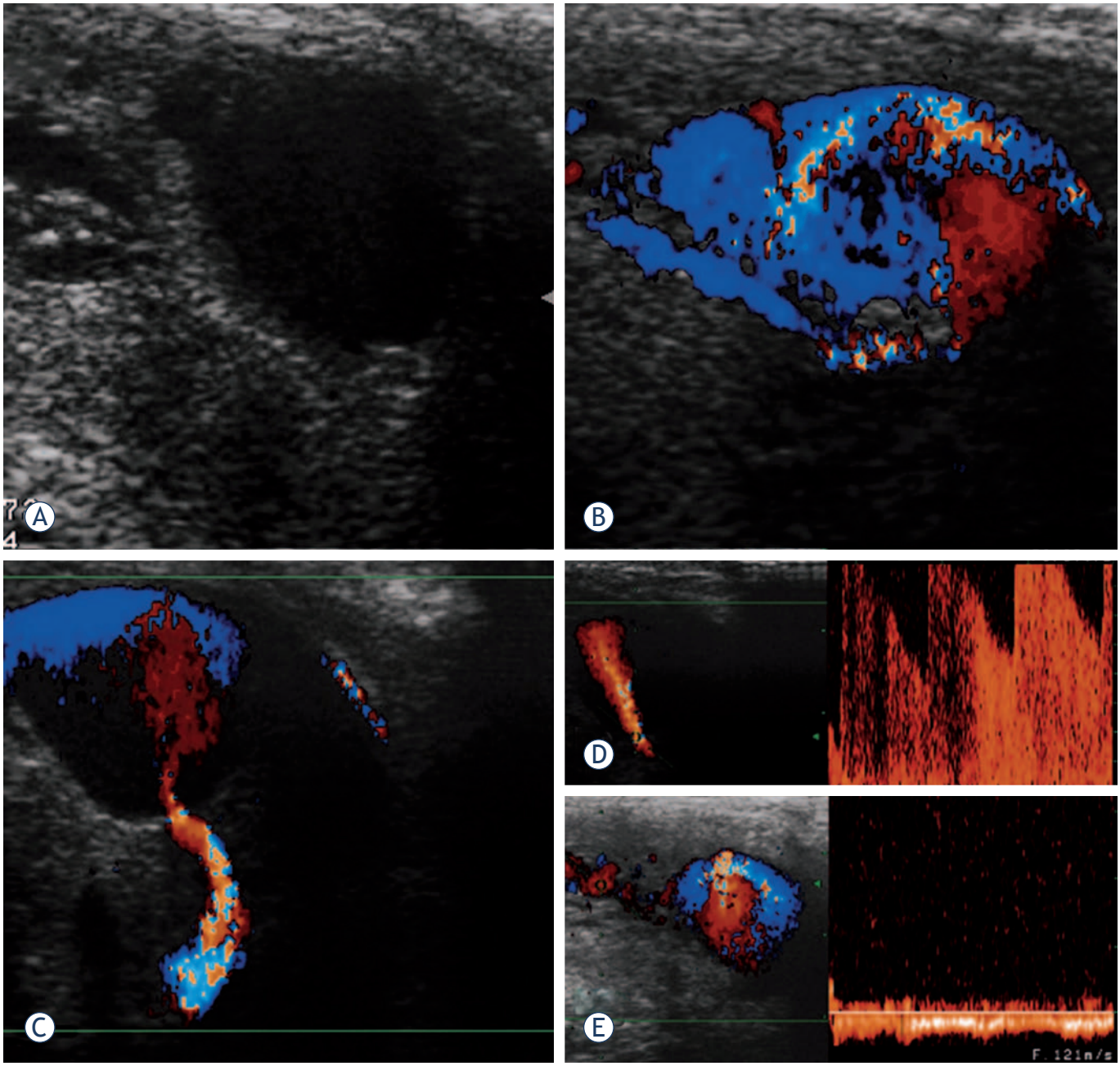

FIGURE 1 A. Gray-scale ultrasound depicts anehoic region, within corpus cavernosum. **B.** Colour Doppler ultrasound with multiple colour signals. **C.** Doppler sonogram of cavernous artery which fills the pseudoaneurysm. **D.** Pulsed Doppler analysis with aliasing phenomena due to turbulent high-velocity flow in the cavernous artery. **E.** Venous drainage in the *corpora cavenosa* on Doppler sonogram.

Case report

A 20-year old patient presented with priapism caused by previous perineal trauma. Gray-scale ultrasound depicted anehoic region, 14.7 x 12.7 mm in size, within corpus cavernosum (Figure 1A) Colour Doppler ultrasound showed multiple colour signals due to the extravasation of blood (Figure 1B). The pulsed Doppler analysis confirmed typical to-fro signals into suspected cavernoma and a high velocity flow in the cavernous artery which fills the pseudoaneurysm (Figure 1C and 1D). Venous drainage in corpora cavenosa was also found on Doppler examination (Figure 1E)

The initial arteriography confirmed a pseudoaneurysm of the right cavernous artery with an arteriocavernous fistula (Figure 2). A smaller arteriocavernous fistula was also present on the left cavern-

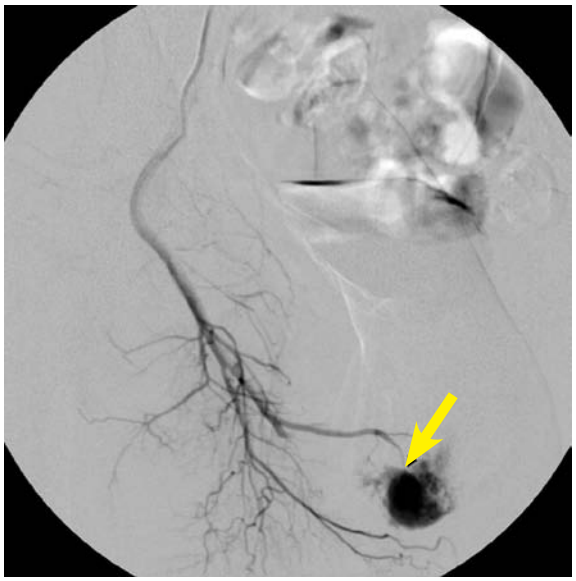

FIGURE 2. Selective angiography before embolization shows the arteriocavernous fistula (marked with an arrow).

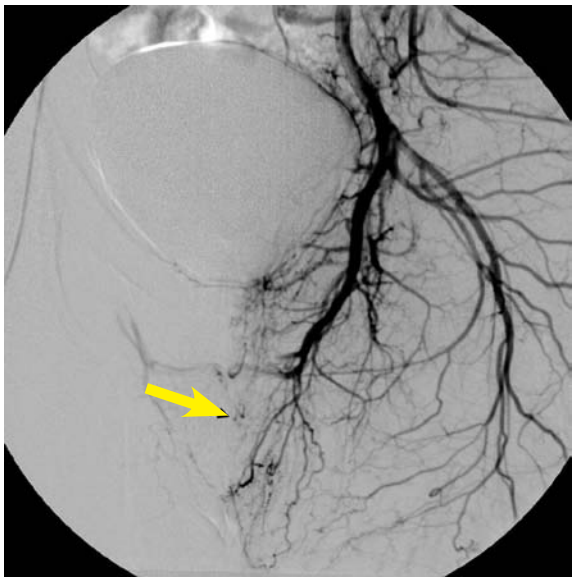

FIGURE 3. Selective angiography after the second embolization shows the occlusion of arteriocavernous fistula (filling artery marked with an arrow).

ous artery. The communication of the left and right internal pudendal artery was noted, with the blood from the left pudendal artery flowing to the right and contributing to the filling of the pseudoaneurysm on the right.

After a six-month period of watchful waiting priapism did not resolve spontaneously and a more aggressive approach was decided upon with an attempt at highly selective embolization of the fistulas of both cavernous arteries using the resorptive gelatinous foam. One-month follow-up showed a recurrence of the right-sided fistula necessitating another embolization procedure during which the superselective catheterization was performed and a microcatheter was inserted into the pseudoaneurysm on the right cavernous artery. The embolization agent used was N-butyl-cyanoacrylate (Glubran II, GEM S.r.l., Viareggio, Italy). Two-month follow-up showed the closure of arteriocavernous fistulas with the persistence of pseudoaneurym on the right that had morphed into a small cavernoma, which was embolized using additional N-butyl-cyanoacrylate. The end-result was a complete occlusion of the fistula (Figure 3). Priapism was successfully resolved and the patient remained symptom-free and regained the erectile function. Contrast-enhanced MR angiography follow-up at 6 months showed no recurrence of the fistula (Figure 4).

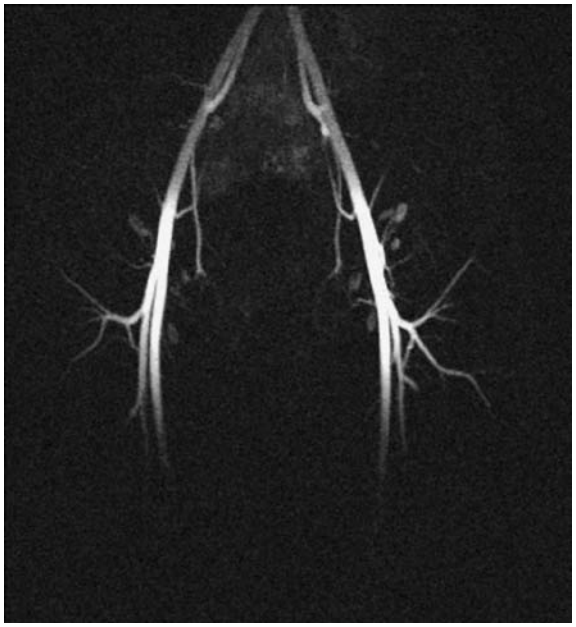

FIGURE 4. Contrast-enhanced MR angiography, late follow-up: a maximum intensity projection of the pelvic vessels in the late arterial phase shows no abnormalities.

Discussion

The endovascular selective embolization of the pathological arteriocavernous communication is firmly established as the invasive treatment of choice in high-flow priapism.[6,7] It is commonly performed using microcatheters and a range of embolization materials: autologous clots, gelatinous foam, endovascular coils[8] or N-butyl-cyanoacrylate.[9,10] Autologous clots and gelatinous foam are often preferred because of their spontaneous degradation and a reportedly lower risk of the permanent erectile dysfunction, but could have a greater recurrence rate.

After our first unsuccessful attempt at embolization using resorptible embolization materials we switched to N-butyl-cyanoacrylate (Glubran II) which could provide faster and more efficient occlusion of the fistula. The cyanoacrylate embolization is permanent and carries a higher risk of ischemia of the vessel in question, and it consequently requires a better embolization technique and more experienced interventionists capable of introducing the catheter and the embolization material directly into the site of the fistula. In our case the treatment was facilitated by the fistula being positioned on a pseudoaneurysm of the cavernous artery. Even though the fistula was occluded after the first embolization session with N-butyl-cyanoacrylate we elected to perform an additional session to obliterate the residual cavernoma in order to prevent a possible recanalization of the fistula and recurrence.

Our case showed that embolization using N-butyl-cyanoacrylate (Glubran II) could be used as a second-line treatment in patients with recurrence after the first embolization attempt with resorptible materials.

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research article

Antigen expression on recurrent meningioma cells

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Introduction. Meningiomas are intracranial brain tumours that frequently recur. Recurrence rates up to 20% in 20 years for benign meningiomas, up to 80% for atypical meningiomas and up to 100% for malignant meningiomas, have been reported. The most important prognostic factors for meningioma recurrence are meningioma grade, meningioma invasiveness and radicality of neurosurgical resection. The aim of our study was to evaluate the differences in antigenic expression on the surface of meningioma cells between recurrent and non-recurrent meningiomas.

Methods. 19 recurrent meningiomas and 35 non-recurrent meningiomas were compared regarding the expression of MIB-1 antigen, progesterone receptors, cathepsin B and cathepsin L, using immunohistochemistry.

Results. MIB-1 antigen expression was higher in the recurrent meningioma group (p=0.001). No difference in progesterone receptor status between recurrent and non-recurrent meningiomas was confirmed. Immunohistochemical intensity scores for cathepsin B (p= 0.007) and cathepsin L (p<0.001) were both higher in the recurrent than in the non-recurrent meningioma group.

Conslusions. MIB-1 antigen expression is higher in recurrent compared to non-recurrent meningiomas. There is no difference in expression of progesterone receptors between recurrent and non-recurrent meningiomas. Cathepsins B and L are expressed more in recurrent meningiomas.

Key words: meningioma; recurrence; tumour markers; proliferation index, MIB-1 antigen; cathepsin B; cathepsin L

Introduction

Meningiomas represent 10-20% of primary intracranial tumours and show a wide range of histomorphological subtypes. According to signs of malignancy in their histological picture, they are classified by the WHO classification as benign meningiomas (BM - grade I), atypical meningiomas (AM - grade II) and malignant meningiomas (MM - grade III). Although in general meningiomas are considered as slow-growing benign tumours, recurrence rates are quite high. Over a 20 year period, the recurrence rate for BM is reported to be 10-26%, for AM 50-80% and for MM 78-100%.[1-4] The average recurrence-free interval ranges between 4 and 6.5 years. The most important risk factors for the meningioma recurrence are the meningioma grade, meningioma invasiveness and thoroughness of neurosurgical resection, which is a principal approach for any surgical treatment in the brain tumour patient.[5,6] Meningioma invasiveness has been observed in all meningioma grades.[1]

Biological markers with the prognostic value for the meningioma recurrence have been sought in the last decades. The proliferation index Ki67 (Ki67 index), assessed as the percentage of MIB-1 positive cells in the area of greatest proliferation, is the most frequently used proliferation index for meningiomas. The Ki67 index was shown to correlate with higher malignancy grades of meningiomas and was observed to be significantly more expressed in recurrent meningiomas compared to non-recurrent ones.[7] However, the Ki67 index has not been confirmed as a statistically significant predictor of recurrence in gross-totally removed benign meningiomas.[8,9]

The loss of progesterone receptors (PR), which are normally expressed in two thirds of BM, seems

to correlate with a higher meningioma grade and the decreased recurrence-free survival time of patients with meningiomas.[9-12] Other biochemical factors, like the epithelial membrane antigen, S100, activated caspase 3 and its inhibitor survivin, HER2, metalloproteases MMP-2 and MMP-9 and others have all been proposed as tumour markers significant for the meningioma recurrence.[13-19]

Cathepsins are intracellular cysteine proteases normally present in most tissues. A high expression of cathepsins B and L was found in several malignant tumours.[20-24] A high expression of cathepsin D was observed in benign meningiomas.[25] A higher expression of cathepsins B in L was found in AM and MM2 [3,27], compared to BM. Cathepsins B and L were found to be expressed more in recurrent meningiomas.[26,28] However, only three recurrent meningiomas were studied and the need for the evaluation of cathepsins B and L expression on a larger series of recurrent meningiomas, was emphasized.[28]

In the present study, we focused on expression of the MIB-1 antigen, PR, cathepsin B and cathepsin L on meningioma cells. The aim of the study was to compare recurrent and non-recurrent meningiomas. Differences between three meningioma grades were also sought.

Patients and methods

In our retrospective study, 54 patients (32 female and 22 male), aged from 19 to 78 (mean 55.5 years), operated for meningioma at the University Medical Centre Ljubljana in the years 1996 - 2001, were selected randomly for the study. Thirty-three meningiomas were diagnosed as BM, 11 as AM and 10 as MM. Meningiomas were operated on by different surgeons and they were described by surgeons as gross-totally removed, graded as Simpson I or Simpson II. All meningiomas were intracranial: 24 cranial base, 16 hemispheric and 14 parasagittal meningiomas were studied. Nineteen patients were in the recurrent meningioma group, and 35 patients were in the non-recurrent meningioma group. In the recurrent meningioma group, the mean time to the recurrence from the first operation was 4.5 years (1-14 years). In the non-recurrent meningioma group, tumours have not recurred for at least 5-10 years (mean 6.5 years).

Tissue samples were fixed in formaldehyde and embedded in paraffin wax. 5μm thick sections stained with haematoxylin and eosin (H&E) were assessed by the pathologist with regard to grade of malignancy according to the WHO classification. In the recurrent meningioma group, 6 tumours (34%) were benign, 5 (23%) were atypical and 8 (43%) were malignant. In the non-recurrent group, 27 (75%) were benign, 6 (18%) were atypical and 2 (7%) were malignant.

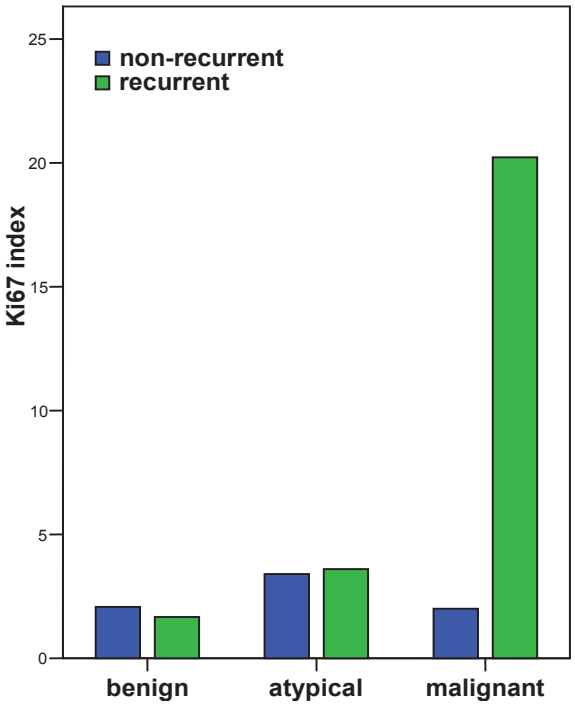

FIGURE 1. Expression of MIB-1 antigen in different meningioma grades comparing non-recurrent and recurrent meningiomas.

Immunohistochemistry (IH) was performed on tissue sections cut from the most representative paraffin block of each tumour using the standard procedures. IH was performed according to the routine protocols used in everyday practice at the Institute of Pathology, Ljubljana. Primary mouse monoclonal antibodies against human cathepsin B and human cathepsin L (Krka, Novo mesto), primary mouse anti-human Ki67 monoclonal antibodies (clone MIB-1, No 7240, DAKO, Denmark) and primary mouse anti-human progesterone receptor antibodies (clone PgR 636, No M3569 diluted, DAKO, Denmark) were used. Antibodies were incubated with slides for 26 min at 40°C. The Ki67 index was calculated with the help of the Leica Q Prodit computer program (Leica, Germany), counting the percentage of MIB-1 labelled nuclei in the most affected region. Positive or negative progesterone receptor status was determined. Tumours were considered positive even if there were only a few cells with a positive IH reaction. The intensity of the IH reaction between cathepsin B and L anti-

bodies and the tumour cells was scored from 0 to 5 by two independent observers. Cases with a different score at the beginning were discussed and the agreement was reached. Intensity and frequency of immunostaining for cathepsins B and L was scored with: 0 no staining; 1 very mild; 2 mild, 3 moderate, 4 strong and 5 very strong staining observed.

Statistical analysis was performed, using SPSS 16.0 for Windows (SPSS Inc., USA). The recurrent meningioma group was compared with the non-recurrent one. Variables used in the analysis included meningioma grade, absence or presence of recurrence, proliferation index, PR status, and IH intensity scores for cathepsins B and L. Differences in expression of biological markers were analyzed using independent-samples T-test. Significance of differences between recurrent and non-recurrent meningiomas, as well as between different histological subgroups, was given as the p value; $p<0.05$ was considered significant.

Results

Differences between recurrent and non-recurrent meningiomas

Proliferation index

The proliferation index was higher in the recurrent meningioma group ($p=0.001$), regardless of the tumour grade.

Progesterone receptor status

No differences in PR status between recurrent and non-recurrent meningiomas were confirmed. Ten out of 19 recurrent and 18 out of 35 non-recurrent meningiomas had a positive PR status. PR status did not correlate with other biological markers.

Protein expression of cathepsins B and L

IH intensity scores for cathepsin B ($p=0.007$) and cathepsin L ($p<0.001$) were both higher in the recurrent than in the non-recurrent meningioma group.

Differences between meningioma grades

The proliferation index increased with the meningioma grade (Figure 1). A higher proliferation index ($p=0.006$) and a significant loss of PR ($p=0.002$) were observed in MM compared to BM (Figure 2). Higher IH intensity scores for cathepsin B ($p=0.007$), and for cathepsin L ($p=0.006$), were observed in MM compared to BM (Figures 3 and 4).

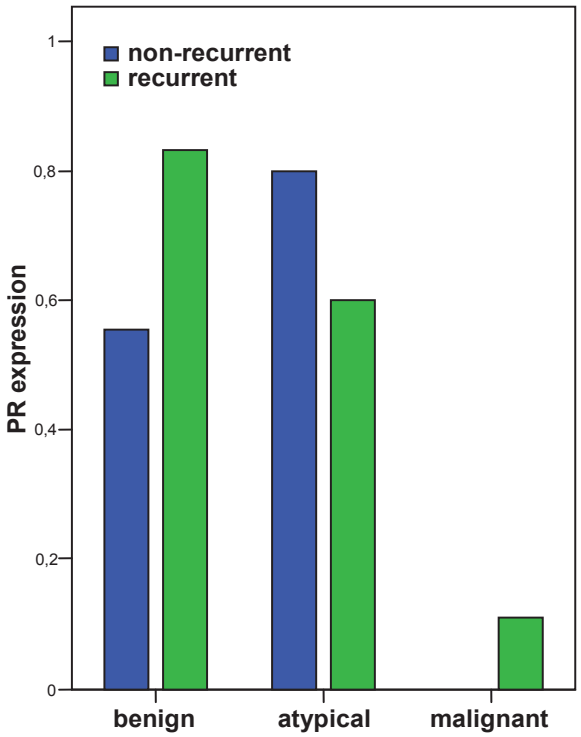

FIGURE 2. Expression of PR in different meningioma grades comparing non-recurrent and recurrent meningiomas.

Differences between recurrent and non-recurrent meningiomas in the BM subgroup

In the subgroup of 33 BM, recurrent BM expressed more cathepsin L ($p=0.035$) than non-recurrent BM.

Discussion

In our series of 54 meningiomas, treated in a single institution, we showed that MIB-1 antigen, cathepsin B and cathepsin L were expressed more in recurrent compared to non-recurrent meningiomas. No difference in PR expression between recurrent and non-recurrent meningiomas was noticed.

Comparing different meningioma grades, we showed that higher meningioma grades express more MIB-1 antigen, less PR and more cathepsins B and L. Differences between BM and MM were statistically important in all four parameters. Differences between BM and AM as well as differences between AM and MM were statistically insignificant.

Although often studied and even used in everyday diagnostics of meningiomas, the prognostic significance of the Ki67 index remains poorly de-

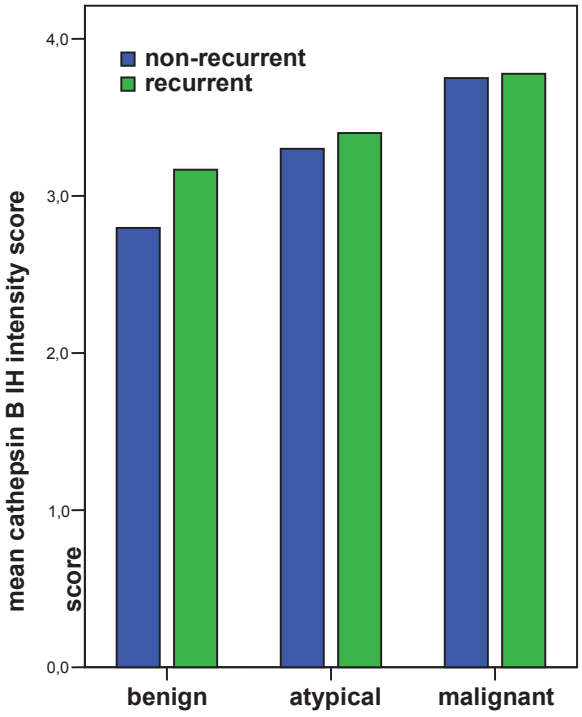

FIGURE 3. IH intensity score of cathepsin B expression in different meningioma grades comparing non-recurrent and recurrent meningiomas.

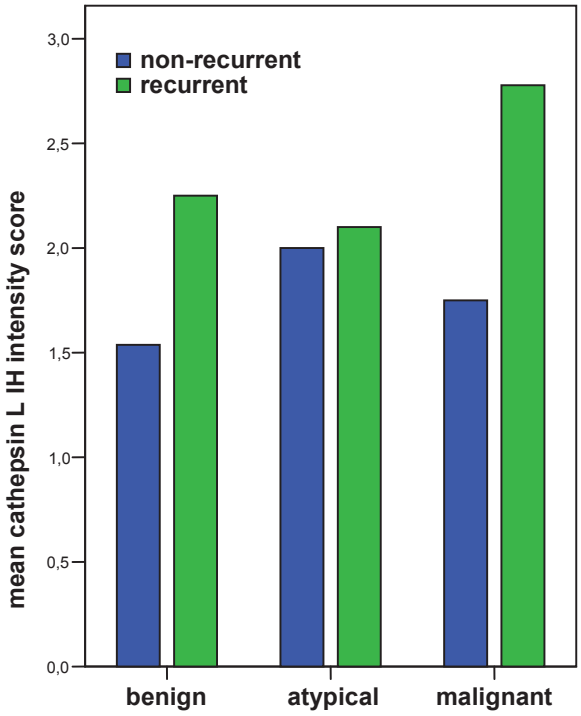

FIGURE 4. IH intensity score of cathepsin L expression in different meningioma grades comparing non-recurrent and recurrent meningiomas.

fined.[9,29,30] The Ki67 index significantly increases from BM through AM to MM, but there is a considerable overlap between different grades.[9,29,31] In a series of primary meningiomas of all three grades, Perry *et al.* reported a Ki67 index ≥ 4.2% associated with the decreased recurrence-free survival in univariate but not in multivariate analysis.[29]

No correlation between the outcome and the Ki-67 index was found in 600 cases of benign meningiomas.[9] The principal limitation of the Ki67 index seems to be the lack of standardization of the technique and difficulties in defining cut-off values.[9,29,30] Our study confirms an important role of the Ki67 index in meningioma grading, suggesting different cell proliferation rates in different meningioma grades (Figure 1).

No differences in PR expression between recurrent and non-recurrent meningiomas were found in our study. PR were expressed more frequently in female patients` meningiomas. A significant loss of PR was observed in MM (Figure 2). The loss of PR could be responsible for malignant progression of meningiomas with PR acting protectively. According to our observations, meningioma recurrences are most frequent for female patients at the onset of menopause. More research work is required to confirm this observation.

Suggesting the invasive nature of recurrent meningiomas, most research work has been focused on proteases. Lysosomal proteases cathepsin B and L have been associated with tumour invasiveness.[20-22] They were considered as factors contributing to invasiveness of meningiomas.[26-28] Higher expressions of cathepsin B, metalloprotease-2 and metalloprotease-9 were also detected in meningiomas, histologically described as invasive.[18,32]

In the present study, we found that cathepsins B and L were expressed more in recurrent meningiomas compared to non-recurrent ones. This finding is in accordance with previous reports about cathepsin expression in recurrent meningiomas which so far have not been confirmed on a larger series.[28] Our study shows obvious differences between the two groups, suggesting recurrent meningiomas were biologically different – *i.e.* more invasive than non-recurrent meningiomas. Our findings support the idea of cathepsins as indicators of invasiveness of meningiomas. They suggest that meningiomas recur not only due to a higher cell proliferation marked by the Ki67 index but also due to a more powerful invasiveness of meningioma cells, marked by higher cathepsin B and L expression.

Our study also confirms that higher expressions of cathepsins B and L correlate with a higher men-

ingioma grade, as already shown in previous studies.[26-28] However, since invasiveness is also found in BM, the meningioma grade is probably not directly correlated to expression of cathepsins B and L. Several cellular mechanisms of cellular proliferation, invasiveness and others are responsible for the malignant transformation of meningiomas.

The expression of antigenic markers,the Ki67 index and cathepsins B and L seem to correlate with a tendency of meningioma to recur. Measuring the expression of these three antigens on meningioma cells could have prognostic value already at the first appearance of a meningioma. These antigenic markers could be proposed as prognostic indicators of the recurrence-free survival of patients with meningiomas. A larger multivariate study on a larger population is needed to confirm the prognostic value of MIB-1 antigen, PR, cathepsin B and cathepsin L on meningioma cells.

A higher probability of an individual meningioma to recur would mean an alarm to start a more aggressive therapeutic approach for the patient. This would include more frequent control check-ups, more frequent control MRI scans and possibly immediate postoperative irradiation with the proper radiation delivery.[33] A more frequent follow-up is particularly important since the median time to recurrence in meningiomas is rather long (4.5 years), which gives us enough time for therapeutic intervention.

Conclusions

The recurrence rate of meningiomas, especially of AM and MM is quite high. So far, the most important known prognostic factors for the meningioma recurrence have been meningioma grade, meningioma invasiveness and completeness of neurosurgical resection. Since the WHO grading system alone does not always correctly predict the biological behaviour of meningiomas, antigenic markers with prognostic significance are being sought. The MIB-1 antigen, cathepsin B and cathepsin L are shown to be expressed more on cells of recurrent meningiomas compared to non-recurrent ones. Expression of these antigens could possibly help us to assess the risk of meningioma recurrence with each individual meningioma patient.

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research article

Frequent MGMT (0^6-methylguanine-DNA methyltransferase) hypermethylation in long-term survivors of glioblastoma: a single institution experience

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Background. The aim of this retrospective study was to analyse the MGMT (0^6-methylguanine-DNA methyltransferase) promoter methylation status in long-term surviving (≥ 3 years) patients with glioblastoma multiforme (GBM).
Methods. The methylation status of the MGMT promoter was determined by bisulfite modification of the DNA and subsequent methylation-specific polymerase-chain-reaction (MSP). DNA was extracted from routinely formalin-fixed and paraffin-embedded tumour tissue samples.
Results. MSP yielded interpretable results in only 14 of 33 (42%) long-term surviving patients with GBM. A methylated band was seen in 3 of 14, methylated as well as unmethylated bands in 8 of 14 and an only unmethylated band in 3 of 14 patients, thus, yielding MGMT promoter methylation in 11 of 14 patients. The two groups of patients with methylated and unmethylated MGMT promoter status were too small to draw any firm statistical conclusions.
Conclusions. Long-term surviving patients with GBM have very frequently intratumoural MGMT promoter methylation. This phenomenon discriminates long-term survivors from a non-selected group of patients with GBM. The standardization of the MSP for the determination of the MGMT promoter methylation status seems to be necessary in order to make this methodology a more reliable one.

Key words: glioblastoma multiforme (GBM); high grade glioma; MGMT promoter methylation; hypermethylation; long-term survival

Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumour in adults. It represents the most frequently encountered type of glial tumours and can also occur in children.[1,2] Median survival is generally only slightly longer than one year based on multimodal approaches consisting of maximal feasible resection, radiotherapy and chemotherapy. A substantial step forward in the treatment of GBM was reached by the randomized phase III trial by Stupp *et al.*, demonstrating a significantly longer survival in patients treated with temozolomide in addition to radiotherapy followed by adjuvant temozolomide with a median survival of 15 months and a five-year survival rate of 9.8%.[3]

Distinct from unselected GBM patients, who survive about one year, there is a small subgroup

of 1% - 5% of patients with GBM that survive at least 3 years after the diagnosis of GBM.[4-12] They are designated as long-term glioblastoma survivors. This period of 36 months survival was also adopted in our study as the lower limit for long-term surviving GBM patients; yet, there is no generally accepted definition. All histologic diagnoses of the putative long-term surviving GBM patients have to be reviewed because in about one half of the cases the histologic diagnosis of GBM is reclassified to represent a less malignant tumour, namely oligodendroglioma, malignant mixed oligodendroglioma-astrocytoma or anaplastic astrocytoma.[6-10,13] Although the histologic aspect of the tumours from long-term survivors does not differ from that of classical survivors, it is postulated that the long-term surviving patients are a subgroup of GBM patients with a different biological behaviour, a different therapeutic responsiveness and a distinct genetic characterization.

Clinical parameters such as young age, high Karnofsky performance status and the extent of radicality of surgery are associated with a better prognosis despite the histology of a GBM.[4,6,8,10,14,15] Scott *et al.* found additional factors as the neurologic function and the dose of radiotherapy applied in their recursive partitioning analysis to be important prognostic variables.[16] The period of symptoms before the diagnosis in long-term GBM survivors in contrast to average GBM patients is significantly longer.[10] A significantly lower Ki-67-labeling index compared to controls has been described in tumours from long-term survivors.[10] Such patients exhibit fewer genetic aberrations than typical GBM patients.[7] Like in oligodenroglioma patients the loss of 19q is exclusive to the long-term survivors.[1] Usually 6q loss, 10q loss and 19 q gain are associated with the short-term survival[7] whereas mdm2 overexpression is less likely exhibited by the long-term GBM survivors.[5] Molecular parameters, which can determinate the step of tumour malignancy[17], are also important in GBM patients.[5] The overexpression of the protein p53 and the nuclear p53 expression are significantly more frequently found in long-term surviving patients.[5] A better molecular characterization of long-term GBM patients is achieved by examining of multiple markers suggesting that differing patterns of genetic lesions may discriminate between the long and the short-term survival of GBM patients.[7]

It has become clear that cancers in general arise from both genetic and epigenetic changes. Epigenetic changes, such as hypermethylation, may inactivate genes without changing the base sequence. Analysing a different promoter methylation status of key regulator genes implicated in apoptosis and inflammation hypermethylation of TMS1/ASC was significantly more frequent in long-term surviving GBM patients and DAPK promoter hypermethylation was only found in the long-term subset compared to unselected GBM patients.[4] Martinez *et al.*[18] found a significantly higher methylation rate of MGMT in long-term GBM patients compared to unselected GBM patients. The MGMT gene is located on chromosome 10q26. Methylation of the gene promoter is associated with the loss of MGMT expression which results in diminished DNA-repair activity. Tumour cells lacking MGMT are prone to cell death induced by alkylating substances such as temozolomide. In this process the alkyl-group is transferred to the active site of the MGMT protein that thereby becomes irreversibly inactivated and subsequently degraded, requiring resynthesis. Although O^6-methylguanine accounts for less than 10% of the lesions induced by alkylating agents, it plays a major role as a trigger for cytotoxicity and apoptosis. If left unrepaired, e.g. due to epigenetic silencing of the MGMT gene or depletion the MGMT protein by saturation of the process, O^6-methyl guanine persists in the DNA.[19] Recently, Hegi *et al.*[20], Glas *et al.*[21] and Sonoda *et al.*[22] described promoter methylation of MGMT as an independent favourable prognostic factor. Patients with GBM containing a methylated MGMT promoter benefited from temozolomide, whereas those who did not have a methylated MGMT promoter did not have such a benefit.[20]

To further characterize long-term glioblastoma patients genetically we investigate retrospectively the MGMT promoter methylation status by the bisulfite modification of the DNA and subsequent methylation-specific polymerase-chain-reaction (MSP) in formalin-fixed and paraffin-embedded tumour tissue samples of 33 long-term survivors with GBM from a single centre.

Patients and methods

Patient recruitment

Primary and secondary GBM patients surviving longer than 36 months after the diagnosis were retrospectively identified in a single centre, the Department of Internal Medicine I, University of Vienna, Vienna, Austria starting from the year 1995

up to 2003. The histologic diagnosis of GBM according to the World Health Organization (WHO) classification of the brain tumours was confirmed by the pathology review by M.P. All patients have been treated with alkylating agents. The clinical data were evaluated by checking patients` records, the presence and the extent of oedema by reviewing the radiologic films. A cognitive impairment was assessed by analysing the dialogues between the treating physicians and the patients; additionally, the functional capacities regarding ADL (activities of daily living) and IADL (instrumental activities of daily living) documented as reported by the relatives were scored. This study has been approved by the local ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The informed consent for samples and the data analysis from each patient had been obtained.

MGMT promoter methylation analysis

A MGMT promoter methylation status was analysed using methylation-specific PCR (MSP) as described by Hegi *et al.*[20] In brief, genomic DNA was isolated from paraffin sections of GBM tissue using Ex-Wax DNA Extraction (Chemicon, Temecula, California, USA). The DNA was subjected to bisulfite treatment at 56°C for 16-20h. Then the DNA was purified using Wizard DNA Clean-Up System A7280 (Promega, Madison, Wisconsin, USA). MSP was performed in a two-step "nested" approach using previously defined primer sets.[20] The PCR products were separated on two percent agarose gels. A glioblastoma case with a known methylated MGMT promoter was used as the positive control and water was used as the negative control for MSP analysis.

MIB-1 proliferation index immunohistochemistry

Tumour sections (3-5 micrometers thick) were immunostained with a monoclonal mouse anti-Ki-67 antibody (clone MIB-1, Dako, Glostrup, Denmark) at a dilution of 1:50 for 25 minutes. For the determination of the MIB-1 proliferation index, the fraction of labelled nuclei per 500 tumour cell nuclei was manually counted using an eye grid and was expressed as percentage.

Statistical analysis

Time to progression reached from the date of the first neurosurgical procedure or diagnosis of glioblastoma to the time of the first objective evidence of tumour progression or the time of censoring. Survival time was defined as the time lapse from the initial surgery or diagnosis to the patient's death or the time of censoring. Time to progression and survival were estimated using the Kaplan-Meier method. The influence on time to progression and overall survival by sex, age, presence of primary or secondary glioblastoma, side and region of the brain of the primary tumour, presence of cognitive impairment, presence of oedema and Karnofsky performance status was calculated by the log-rank test. For the analysis of the influence of the age of the formalin-fixed or paraffin embedded tumour tissue on the feasibility of determination of the MGMT promoter methylation status a χ^2-test was used. All statistical analyses were performed using the SPSS software 15.0.

Results

In this retrospective analysis 35 long-term surviving patients with GBM were identified from one centre. 33 of them were confirmed GBM patients after the histologic review indicating a percentage of 6% of revised histologies. The two diagnoses of the reclassified histologies were anaplastic oligodendroglioma and anaplastic astrocytoma. As 40 patients per year with primary GBM are treated in the institution this results in about four patients per year becoming long-term surviving GBM patients. This corresponds to an estimated percentage of 10% of long-term survivors in the institution. The median follow-up was 54.2 ±SD 26.1 months. The patient's characteristics are shown in Table 1.

Karnofsky performance score

The postoperative Karnofsky performance score of the long-term surviving patients was at least 80%. At the time of writing two women and three men were professionally still active, the two women as computer clerks with full time employment, one of the men as a teacher for mathematics in a vocational school, one as a farmer and the third as a pizza cook.

TABLE 1. Patient characteristics

	N (%)
Number of patients	33
Age (years) median (range)	38 (22-66)
Sex	
Male	22 (66.7)
Female	11 (33.3)
Performance status acc. to Karnofsky (%)	
60	1 (3.1)
70	3 (9.1)
80	11 (33.3)
90	14 (42.4)
100	4 (12.1)
Oedema	
≤ 1 cm	11 (33.3)
> 1 cm	19 (57.6)
n.e.	3 (9.1)
History of glioblastoma	
Primary glioblastoma	31 (93.9)
Secondary glioblastoma	2 (6.1)
Localisation of the tumour	
Frontal	12 (36.4)
Parietal	4 (12.1)
Trigonal	2 (6.1)
Temporal	8 (24.2)
Occipital	1 (3.0)
Frontoparietal	1 (3.0)
Insula	1 (3.0)
Parietooccipital	2 (6.1)
Thalamus	2 (6.1)
Side of tumour localisation	
Right	10 (30.3)
Left	22 (66.7)
Bilateral	1 (3)
Extent of resection	
Biopsy	4 (12.1)
Subtotal	10 (30.3)
Total	19 (57.6)
Cognitive impairment	
Yes	11 (33.3)
No	22 (66.7)
Stroke	
Yes	2 (6.1)
No	31 (93.9)
Initial chemotherapy	
Temozolomide	6/33 (18.2%)
CCNU	15/33 (45.5%)
Fotemustine/Dacarbacine	12/33 (36.4%)

N = numbers, n.e. = not evaluable

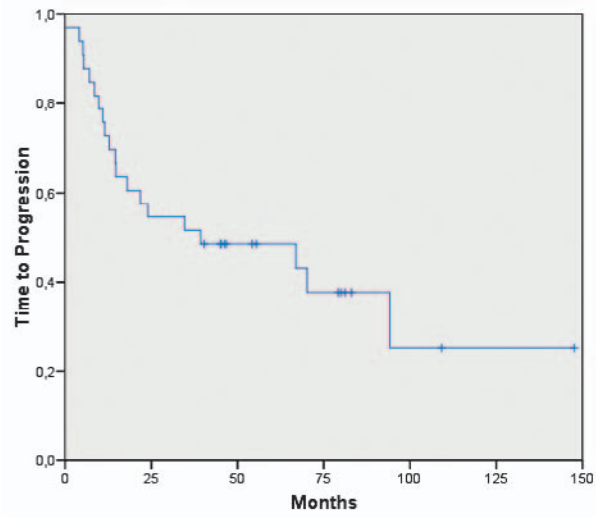

FIGURE 1. Time to progression of all long-term surviving patients with glioblastoma multiforme (n=33).

Local relapses

20 of 33(61%) patients suffered a local relapse, eleven of them after gross total resection of the primary tumour.

Median time to progression

The median time to progression (TTP) was 39 months [95% CI: 0; 105.6] (Figure 1). Patients with a biopsy at initial diagnosis had a median TTP of 5.3 months [95% CI: 0; 17.8 months] (n=4), patients with a subtotal resection had a TTP of 39.3 months [95% CI: 0-92 months] (n=10) and patients with a total resection one of 66.9 months [95% CI: 0; 145.3 months] (n=19), respectively. The clinical parameters age, sex, oedema, side and region of the brain of the primary tumour and Karnofsky performance status did not impact on the TTP. Patients with the unmethylated MGMT promoter had a time to progression of 7, 39 and 79+ months whereas patients with methylated MGMT promoter had a TTP of 5 to 56+ months.

Survival

At the time of evaluation, 15 patients were alive, seven of them without tumour recurrence for up to 151+ months. The median survival was 83 months [95% CI; 43.8-122.3] (Figure 2). Patients with a subtotal resection survived 47.2 months [95% CI: 28.8-65.6 months] and patients with a total resection 83.0

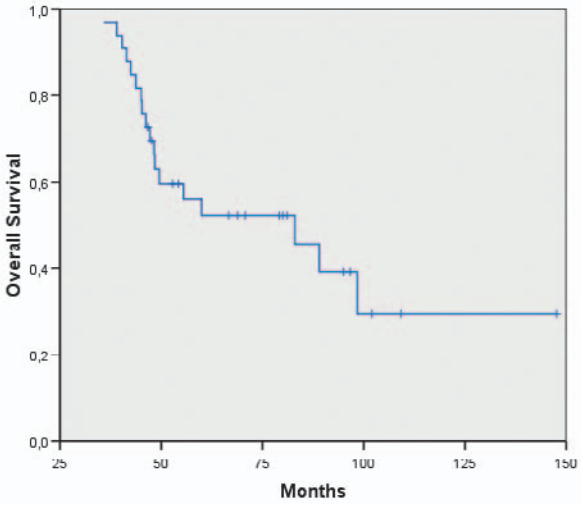

FIGURE 2. Survival of all long-term surviving patients with glioblastoma multiforme (n=33).

months [95% CI: 43.2; 122.9 months], respectively. The clinical parameters age, sex, oedema, side and region of the brain of the primary tumour and Karnofsky performance status did not impact on the survival. Of note, nine of the patients with a local relapse survived longer than five years. Patients with the unmethylated MGMT promoter had a survival of 43, 79+ and 97+ months, respectively. Patients with the methylated MGMT promoter had a median survival of 48 ± SD 0.97 months.

MIB-1 scoring

In seven patients the immunohistochemical staining of MIB-1 was determined. The mean MIB-1 score was 29.1%, the median 30.3% (range 12.1 - 49%).

MGMT promoter methylation status (Table 2)

Only in 14 of 33 (42%) patients the determination of the MGMT promoter methylation status by MSP yielded interpretable results. There was no linear correlation of the success rate to the age of the paraffin block (p=0.5). Of 14 patients with interpretable MSP results, three of 14 patients had a methylated MGMT promoter, three of 14 patients an unmethylated MGMT promoter and 8 of 14 patients partly a methylated and partly an unmethylated MGMT promoter. Thus, the MGMT promoter methylation was found in 11 of the 14 patients. These two groups of patients with the methylated and the unmethylated MGMT promoter status, respectively were too small to draw reliable conclusions based on statistical testing.

TABLE 2. MGMT promoter determination

	N (%)
Feasible	14 (42)
Methylated	3 (21.4)
Unmethylated	3 (21.4)
Methylated and unmethylated	8 (57.2)
Not feasible	19 (58)

Discussion

Reports on the MGMT promoter methylation status in long-term surviving patients with glioblastoma multiforme are scarce. 78.5% of long-term survivors presented with MGMT promoter hypermethylation. This is in the same range as reported by Martinez *et al.*, Sonoda *et al.* and Krex *et al.*[18,22,23] This high proportion of patients with the MGMT promoter methylation is in clear contrast the 44% (range 25- 68%) determined from 13 different studies of unselected patients with GBM.[18,20,24,34] However, the high rate of methylated tumours in the long-term surviving patients let suggest that MGMT promoter methylation is of paramount importance for response to the actual standard therapy with alkylating agents in GBM.[3] The proof of this principle is eagerly awaited in form of the results of the prospective Intergroup trial RTOG0525/EORTC26052 which will not yet be presented for the first time at ASCO 2010 testing dose-intense temozolomide in comparison to standard-dose temozolomide dependent on the MGMT promoter methylation status in GBM patients.

Although 33 patients were initially included in our analysis of long-term surviving patients with GBM, the paraffin embedded tissue blocks of only 14 out of 33 (42%) patients were suitable for the MGMT hypermethylation test by MSP. Because of the small number of patients it was impossible to determine whether the MGMT methylation status was of prognostic impact in our patient cohort. The MGMT promoter methylation status as a prognostic factor in long-term surviving GBM patients should be further evaluated in prospective studies.

The statistical analysis did not show a significant difference between older paraffin embedded tissue in contrast to younger patient samples

(p=0.5); however, only higher patient numbers in the subgroups could provide reliable significant results. The success rate of the methylation specific PCR determination on paraffin-embedded tumour samples is highly variable and centre dependent.[20] Hegi *et al.* reported on a median success rate of 75% (range 0-100%)[20], Brandes *et al.* of 66%.[29] In contrast to these results Aldape *et al.* found prospectively a success rate of 91% in 995 patients with GBM.[35]

In the literature several reasons for the low success rate of MSP testing in paraffin-embedded tumour tissue of patients with GBM compared to fresh frozen tissue are discussed. Frequently only a very small amount of partially degraded DNA is recovered due to extensive necrosis and scarcity of malignant cells. Herrlinger *et al.* observed that 17% of the tumour specimens did not contain enough DNA.[33] Especially in tumour biopsies tumour cells are not easily found.[24] Hau *et al.* recommended a good quality paraffin embedded tissue that is not overfixed.[19] The accumulation of normal cells in the tumour, including infiltrating lymphocytes, may complicate accurate assessment of MGMT.[28] The best results with methylation-specific PCR are obtained with cryopreserved tumour specimens, thus avoiding the fixation-related deterioration of the quality of DNA.[36] 8 of 14 our patients exhibited both the methylated and the unmethylated MGMT promoter. Our observation is in the same range as reports by Martinez *et al.*, Blanc *et al.*, Criniere *et al.*, Cankovic *et al.* and Gonzalez-Gomez *et al.* who found that the majority of the methylated tumours also exhibited an unmethylated band, which may arise from either normal cells within the tumour sample or from a tumour cell side population.[18,26,31,34,37] Our experience shows that MGMT promoter methylation testing may be technically challenging. Several methods including multiplex ligation probe amplification MLPA, real time quantitative polymerase chain reaction (quantitative rt-PCR), have been proposed as potential alternatives to conventional MSP. These methods need to be critically evaluated in future studies and reliable cut-off values for the prognostication and the prediction have to be prospectively validated.[38,39]

Two of 33 (6%) patients of our study suffered from secondary GBM. This percentage was markedly lower than the incidence of 20% reported by Steinbach *et al.*.[6] In these two patients with secondary GBM in our study the MGMT promoter methylation determination was not feasible due to technical reasons.

The determination of the proliferative activity in form of MIB-1 evolved a low median score of 30.3 (range 12.1-49). This observation correlated well with the results reported by Ho *et al.*, demonstrating a cut-off value of ≥ 35 being related to worse outcome in unselected GBM patients.[40] However, due to sampling differences, there has no clear prognostic impact of Ki-67 on the survival of GBM patients been detected.[10,22,41]

In addition to the MGMT methylation status, we compared clinical parameters of our long-term GBM patients like age, Karnofsky-performance score, ratio male/female, localisation of the primary tumour, extent of surgery, laterality of the primary tumour, incidence of cognitive impairment and of ischemic events, incidence of relapses, median survival, to those of other reports of long-term survivors with GBM. Most authors included patients surviving ≥ 3 years, Vertosick *et al.* those > 4 years, McLendon *et al.*, Steinbach *et al.* and Salvati *et al.* patients surviving ≥ 5 years and Morita *et al.* those ≥ 7 years.[6,8,11-13]

The median age at diagnosis of the patients of this series was 38 years, which is only slightly younger than the median of 41 years (range 37-51 years) observed in 10 different studies[5,6,8,10-13,18,23,42] and 12 years younger than the average age at diagnosis of unselected GBM patients (median 53 years ± 0.55 years).[10]

One of the most important prognostic factors in cancer patients[43], the Karnofsky performance score at the beginning of radiochemotherapy in our 33 patients reached median 90%. This equals the median of 90% (range 80-90%) observed in nine other studies of long-term surviving GBM patients[5-7,10-12,18,23,42] and is clearly higher than the median of 76.1% of unselected GBM.[10] The sex ratio male:female in our patient cohort was 67:33, quite similar to other studies. There was no predilection in laterality or in localization in a given cerebral lobe, as in the other series of long-term surviving GBM patients.[6,13,23]

The radiological parameter "extent of oedema > 1 cm" at diagnosis of GBM was present in 58% of our patients and did not impact on time to progression or survival in this series. In other studies of long-term surviving patients with GBM oedema was not investigated as a prognostic factor. However, in average patients with GBM, oedema larger than 1 cm has been reported to influence the survival, negatively.[44,45]

Of note, a gross total resection was achieved in 58% of the patients of this series. This has been reported accordingly in the series by Scott et al. with 40%, Salvati et al. with 46% and Hottinger et al. with 48% but not by Mc Lendon et al. with 27%.[8,10,12,42]

Obtaining a total gross resection appears to be of paramount importance for achieving a long-term survival in GBM patients. A considerably lower percentage of gross total resections of about 40% were recorded in studies of unselected patients.[3] However, in this series four patients (12%) underwent only a biopsy of the primary tumour. In three of these four patients the MGMT status was not evaluable and in the remaining patient the MGMT promoter was unmethylated. This raises hope that even patients without tumour debulking and with unmethylated MGMT promoter status can eventually achieve a long-term survival.

61% of our patients relapsed locally. This was in line with three other reports of long-term surviving GBM patients specifying a percentage ranging from 45-73%.[10,12,42] Of note, we did not observe distant relapses in the cohort of long-term surviving patients.

In 33% of our patients a cognitive impairment was recorded. A similar rate of 28% has been reported by Hottinger *et al.*[42]

Two of 33 (6%) of our patients suffered from an ischemic event, this was nearly identical to the 10% observed by Steinbach *et al*. but clearly lower than the 23% found by Hottinger *et al*..[6,42] Further trials will have to evaluate the incidence of ischemic events in long-term surviving patients with GBM, to identify risk factors and establish preventive strategies.

In summary, this series of patients achieving a long-term survival after the diagnosis of GBM illustrates the validity of the prognostic factors developed in the nomogram by Gorlia *et al*.[15] on the patients of EORTC and NCIC trials as well as of other series with long-term surviving patients with GBM: young age, extensive tumour resection, favourable performance status and treatment according to the standard of care, as well as a high percentage of glioblastomas with MGMT promoter methylation. The definitive role of MGMT promoter methylation in directing tailored chemotherapy in GBM patients will be elucidated in the large randomized international intergroup trial RTOG0525/EORTC26052 stratifying GBM patients by MGMT methylation status and randomizing for standard temozolomide in contrast to dose-dense temozolomide therapy. MGMT promoter methylation testing represents a substantial step forward in the treatment of patients with glioblastoma multiforme and enables us to better understand the mode of action of alkylating therapies and the course of the disease. Further, new treatment options exploiting the MGMT promoter methylation mechanism may add to the improvements achieved in this disease.

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case report

A clinical case of the penile metastasis from the rectal carcinoma

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Background. Penile metastases are rare and usually secondary to genitourinary and colorectal cancer.
Case report. We present a case of a 77-year-old man with penile metastasis who was operated for rectal carcinoma. He was referred to our clinic for penile ulcerous lesion, semierectile penis and voiding dysfunction. Imaging studies showed nodular lesion at glans penis and multiple bone metastases. He did not respond to chemoradiotherapy and he had bad prognosis.
Conclusions. Imaging methods and biopsy may help to clarify the diagnosis but the treatment modalities are insufficient in these patients.

Key words: penis; metastasis; rectum; carcinoma

Introduction

The metastatic involvement of the penis is extremely rare, despite rich vascularisation between the penis and the neighbouring organs. The first report of secondary penile malignancy from an adenocarcinoma of the rectum was defined by Eberth.[1] The commonest sites of primary malignancy are genito-urinary organs, rectum and recto-sigmoid areas.[2] In literature less than 300 penile metastases were reported; 50 of them are originated from colorectal carcinoma.[3] Penile metastases lead to semi-erectile penis/priapism and skin lesions.[2] Like in colorectal lesions MRI is the most useful method for the accurate diagnosis in suspected penile metastases.[4,5] An open biopsy is definitive for a the accurate diagnosis. A high rate of suspicion is required to detect them.

In this article, we report a case of a penile metastasis secondary to a rectal carcinoma three years after the surgery.

Case report

A 78-year-old male presented in March 2002 with a 2 months history of change in bowel habit and rectal bleeding. The examination revealed a tumour of the lower rectum. An abdomino-perineal excision was performed and the pathological examination revealed a Dukes C undifferentiated adenocarcinoma with the lymph nodes involvement (15/22). He had received adjuvant chemo-radiotherapy postoperatively. He remained well until 2 years later, when he presented with urogenital complaints: penile ulcerous lesion, semierectile penis and voiding dysfunction.

FIGURE 1. Physical examination showed indurations, oedema and ulcerative lesions of glans penis.

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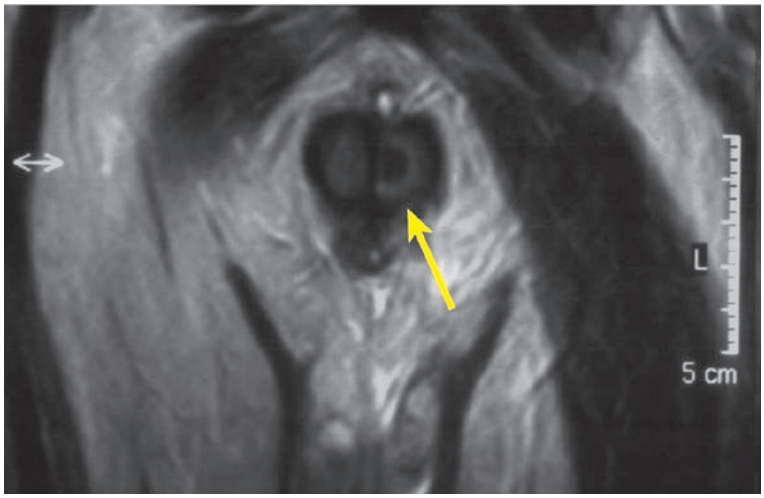

FIGURE 2. Axial T2-weighted MR image was showed nodular metastatic involvement of the penis shaft (arrow).

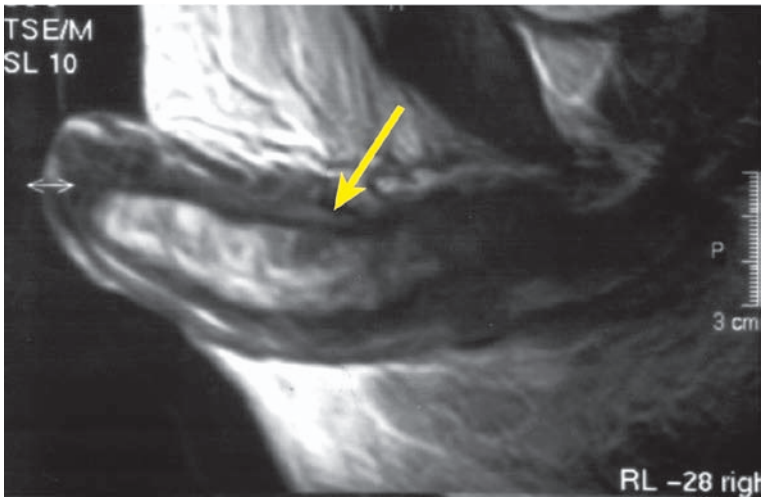

FIGURE 3. Sagittal T2-weighted MR image of the penis, demonstrating multiple metastatic nodules (arrow).

A follow-up examination was performed in April 2005 following the development of indurations, hyperaemia, and oedema of the penis and demonstrated 0.5 cm and 0.3 cm ulcerative lesions on glans penis (Figure 1). The examination of the superficial inguinal lymph nodes and the abdominal examination showed no remarkable signs. There was also no history of trauma or infectious disease.

A complete blood cell count showed an increased white blood cell count of 16 x 10^3/ μL (normal range: 5-10.0 x 10^3/μL), anaemia (with a haemoglobin of 10.2 g/dL), normal level of PSA but increased levels of CEA (66.6 ng/ml, normal value <3 ng/ml) and CA19.9 (67.5 U/ml, normal value <37 U/ml). Penile US was normal for *corpus spongiosum* and *cavernosus*. CT demonstrated metastases of several thoracic vertebras and sacroiliac joint. Furthermore, MRI of the penis showed multiple pathological focuses in T2 signal and a nodular lesion that measured 15 mm in size, occupying the glans penis (Figures 2,3). The patient underwent excisional biopsy of the glans penis and inguinal lymph node, which revealed metastatic adenocarcinoma consistent with his rectal carcinoma. The patient received chemotherapy for metastatic disease until disease progression and unacceptable toxicity. The order of drug administration each week was irinotecan (80 mg/m^2 i.v. day 1) followed by leucovorin (500 mg/m^2 i.v day 1) and 5-fluorouracil (2300 mg/m^2 continuous infusion day 1). Two days after chemotherapy administration of the third cycle the patient was admitted to our department with fatigue, weakness, dysuria and hypotension. Increased level of BUN, creatinin and decreased level of blood count were registered. Despite the whole blood transfusion, the values of blood count decreased (down to 5.4 g/L) during hospitalization. He was admitted for further medical therapy but during this admission his general condition deteriorated and he died with progressive disease.

Overall it appeared that this patient had a disseminated metastatic rectal carcinoma, with an unusual settled for the penis.

Discussion

The penile metastasis in the course of rectal cancer is an unexpected complication, despite its proximity to the rectum and its rich vascularity. Recently published studies showed that the penile metastases are associated with prostate, urinary bladder cancer, and infrequently with rectal carcinoma.[6] There have also been some reports regarding disseminated metastases from the oeasophagus, pancreas and stomach carcinoma. There are a few pathways of metastasis to penis; frequently, retrograde venous route, embolism to arterial system, retrograde lymphatic spread into the penile lymphatic channels, direct extension, and operative manuplations. The retrograde venous transportation suggested the main pathways to penis metastasis.[1]

The most common symptoms and signs are dysuria, voiding dysfunction, perineal pain, priapism, penile nodules and mass.[6] In our case we found voiding dysfunction, semi-erectile penis and ul-

cerative lesions of the glans penis. Perineal pain, which it was a feature in one-third of patients in the series[5], was not found in our case. We found a three-year interval between the primary tumour and penile metastasis. However, there are reports of the metastatic involvement years after the rectum carcinoma, but, penile metastases were usually reported on the average of 13 months after the primary carcinoma.[1] The lung, vertebra and liver metastasis can be associated with the penile metastasis in patients with rectal carcinoma[2] which, in our case, we found vertebral metastases at presentation. This might reflect the fact that penile metastases reflect the disseminated disease.

We believe that it would be beneficial in the differential diagnosis to take into consideration the findings related to voiding dysfunction, priapism and ulcerous lesions of the penis. Peyronie's disease, traumatic and infectious skin and syphilitic lesions or primary carcinoma of penis, must also be in the differential diagnosis of the penile metastasis.[2,7]

Tissue tumour markers: AFP, CEA, HCG can be used for differential diagnosis. Penile US and CT is the first choice for imaging but its sensitivity is limited. Cavernosogram was reported a preferable application in some patients.[8] MRI reported as a very useful method even at the beginning of the symptoms.[5] MRI demonstrates multiple metastatic nodules, with a low signal intensity and is intense with the surrounding *corpus cavernosum* on T1-weighted images, and low signal intensity against the high background intensity of the cavernous bodies on T2-weighted imaging.[9] Biopsy of the penile lesions is a favoured diagnostic modality to confirm the diagnosis as outlined in our case report. Regardless of the length to metastasis and difference in the treatment of the metastatic focus, the metastasis of the penis reflects a widely disseminated disease and poor prognosis.

Treatment options are palliative with local surgery, systemic chemotherapy and local radiotherapy.[4,7] Surgery (panectomy) remains the only treatment with a long survival but mostly a patient dies in a year. Radiotherapy has an average survival of 8 months, whereas chemotherapy has not been studied for metastases from the rectum carcinoma. In our case, metastases did not respond to palliative therapy and showed progression of the local disease. Despite the studies showed that rectal primaries had longer surviving comparing to genitor-urinary primary, we did not find any forms of result.[7,8,10]

In conclusion, penile metastases are rarely seen as a form of metastasis among rectal cancer patients. Imaging methods may help to clarify the diagnosis, but penile lesions should be biopsied to confirm the diagnosis. On the other hand, the treatment modalities are insufficient for a long survey and quality of life in these patients.

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research article

A fully electronic intensity-modulated radiation therapy quality assurance (IMRT QA) process implemented in a network comprised of independent treatment planning, record and verify, and delivery systems

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Background. The purpose of this study is to implement an electronic method to perform and analyze intensity-modulated radiation therapy quality assurance (IMRT QA) using an aSi megavoltage electronic portal imaging device in a network comprised of independent treatment planning, record and verify (R&V), and delivery systems.

Methods. A verification plan was generated in the treatment planning system using the actual treatment plan of a patient. After exporting the treatment fields to the R&V system, the fields were delivered in QA mode with the aSi imager deployed. The resulting dosimetric images are automatically stored in a DICOM-RT format in the delivery system treatment console computer. The relative dose density images are subsequently pushed to the R&V system. The absolute dose images are then transferred electronically from the treatment console computer to the treatment planning system and imported into the verification plan in the dosimetry work space for further analysis. Screen shots of the gamma evaluation and isodose comparison are imported into the R&V system as an electronic file (e.g. PDF) to be reviewed prior to initiation of patient treatment. A relative dose image predicted by the treatment planning system can also be sent to the R&V system to be compared with the relative dose density image measured with the aSi imager.

Results. Our department does not have integrated planning, R&V, and delivery systems. In spite of this, we are able to fully implement a paperless and filmless IMRT QA process, allowing subsequent analysis and approval to be more efficient, while the QA document is directly attached to its specific patient chart in the R&V system in electronic form. The calculated and measured relative dose images can be compared electronically within the R&V system to analyze the density differences and ensure proper dose delivery to patients.

Conclusions. In the absence of an integrated planning, verifying, and delivery system, we have shown that it is nevertheless possible to develop a completely electronic IMRT QA process.

Key words: EPID; IMRT; QA; paperless; portal dosimetry; PACS: 87.53.Bn, 87.53.Kn

Introduction

Intensity-modulated radiation therapy (IMRT) involves complex treatment plans that are completely patient specific in order to highly conform delivered dose to the treatment volume, thus improving normal tissue sparing as compared to more traditional radiotherapy techniques.[1,2] As a consequence, the complexity and uniqueness of these treatment plans demand patient-specific pretreatment quality assurance (QA) of all IMRT treatments. Standard methods of IMRT QA involve ionization chambers, diode arrays and radiographic films, often used in some combination to provide verification of absolute dose, field geometry, number of monitor units, etc. However, these tra-

ditional QA methods carry some distinct disadvantages, especially in the clinic that delivers a large number of IMRT treatments. These methods can be exceedingly time and resource demanding, requiring calibration and constancy checks of ionization chambers, set-up and calibration of diode arrays, calibration of films and expensive processing (unless self-developing dosimetry film is chosen as a more convenient yet still expensive alternative).

Furthermore, for all of the QA methods listed above, the QA analysis report may not be readily available in electronic form, demanding direct attachment to the patient paper chart (or manual scanning into the patient electronic chart, a process that is still not paperless). The disadvantages of paper charts are well known and well documented[3] - including illegible signatures, notes and prescriptions; inaccessibility to multiple reviewers at one time; difficulties in locating charts and inability to access them remotely; etc. Meanwhile, the benefits of implementing an entirely paperless electronic medical record process have also been expounded in the literature.[3-7] A study published by the National Institute of Health and the Journal of the American Medical Association concluded that "EMRs will eventually become the standard of care," citing that electronic patient charts provide complete, legible, and organized patient information in a format that is accessible at any time, even to multiple viewers in multiple (even remote) locations.[3] Given the current shift toward adopting electronic medical records over paper charts, it is all the more important that the pretreatment IMRT QA process be fully electronic: no films, no printing and no scanning of QA reports, treatment plans and other documents.

In recent years, it has been demonstrated that an electronic portal imaging device (EPID), previously employed to replace radiographic portal images for patient alignment, can effectively be used for absolute dose measurement and pretreatment IMRT verification.[8-12] In EPID IMRT QA, portal dosimetric images are compared to respective portal dose predictions created by a treatment planning system (TPS) using geometric and dosimetric tools (such as dose profiles and gamma evaluation).[13-15] Thus, with a properly calibrated and commissioned EPID, all qualitative and quantitative data necessary for verification of an IMRT fluence is acquired in a single exposure, and all information is readily available in electronic form allowing for the possibility of an entirely paperless IMRT QA process.

A significant roadblock to the paperless EPID IMRT QA process is the common situation in which the treatment planning, record and verify (R&V), and radiotherapy delivery systems are not manufactured by the same vendor and thus communication between these systems is not entirely integrated. The purpose of this study is to implement a fully electronic method to perform and analyze patient-specific IMRT QA using an EPID in a network comprised of independent treatment planning, R&V, and delivery systems. The advantages of such a QA process over standard methods of IMRT QA include:

1. Excellent efficiency, acquiring complete qualitative and quantitative information in a single exposure for each field, with no processing and no other calibration than the absolute and relative dose calibrations of the EPID (at intervals suggested by the vendor).
2. Excellent resolution compared to ionization chambers and diode arrays, with arrays as high as 1024x768 pixels with 0.392 mm pixel pitch (Varian PortalVision aS1000, Varian Medical Systems, Palo Alto CA).
3. Possibility of weekly QA by quick acquisition of EPID relative dose density images and comparison within the R&V system to TPS predictions of those dose densities.
4. IMRT QA report electronically attached to the patient chart within the R&V system in a paperless process with no manual attachment or tracking of QA reports, thereby decreasing the probability of errors (e.g. misplacement of QA document, etc.).

Methods

We have commissioned an electronic portal dosimetry system consisting of an amorphous silicon (aSi) EPID (Varian PortalVision aS1000), coupled to a Varian Trilogy linear accelerator with the Varian Millinium Multi-Leaf Collimator (MLC, 120 leaves). The PortalVision aS1000 is a 40x30 cm^2 flat-panel, indirect detection EPID with a matrix of 1024x768 pixels with 0.392 mm pixel pitch. For this study, all EPID images were acquired at the minimum SSD of 105 cm with gantry and collimator at zero degrees (unless the collimator needed alternate positioning to avoid regions of high backscatter in the EPID).[16] The EPID was fully calibrated using the procedures supplied by the vendor[17], using the following intervals: the dark field background correction and flood field relative dose calibration were both performed weekly; while the absolute dose calibration was performed each day that the EPID was

in use for IMRT QA (also employing the diagonal dose profile correction suggested by Bailey *et al*.[16]). The beam symmetry, energy and output were verified each week. The TPS employed for this study is Varian Eclipse (Version 8.6, including Portal Dosimetry Version 8.2.24), and the R&V system is the vendor-independent Impac Mosaiq (Version 1.6, Elekta Oncology Systems, Norcross GA).

From TPS to the R&V system

Our electronic QA process begins with a patient-specific radiotherapy treatment plan created in the TPS using inverse-planning IMRT techniques based upon the patient's 3-D computed-tomography (CT) data and the dose criteria predefined by the radiation oncologist. Each specific treatment field within this plan contains 320 control points that dictate the dynamic motion of the MLC leaves. Firstly in this process, the TPS uses the input geometric and dosimetric criteria to calculate an ideal fluence matrix referred to as the optimal fluence. Secondly, the optimal fluence is sent to the Leaf Motion Calculator which incorporates various mechanical and geometric aspects of the delivery system (*e.g.* MLC beam transmission, minimum leaf gap, maximum leaf speed, MLC position deviation tolerance, etc.) to calculate leaf trajectories for the fluence that the system can capably deliver, known as the actual fluence.[18] Routine IMRT QA is partly designed to check the accuracy of these beam models and parameters. If the necessary LINAC collimator jaw settings are beyond a certain separation (approximately 15 cm), the TPS splits the treatment field into multiple overlapping carriages, maintaining maximum degrees of freedom in MLC position and motion. After the treatment plan is completed and approved, the plan is electronically exported to the R&V system as a DICOM-RT file which includes all necessary patient information and delivery information, such as number of monitor units (MU), dose rate, collimator settings, and dynamic MLC positions.

From R&V system to LINAC delivery

The R&V system communicates the delivery parameters from the TPS to the delivery system (and allows for automatic field setup), and further provides an electronic medical record (EMR) which tracks the fractions and doses that have been delivered to the patient, the delivery system settings for each field and fraction delivered, portal images and IMRT QA dosimetric images acquired with the EPID (or scanned films), among other information. When the treatment plan is delivered, whether for pretreatment QA or actual treatment delivery, the R&V system communicates the field setup and delivery information to the LINAC delivery system as an RTP file and stands by to record the subsequent delivered parameters and capture the acquired images. The IMRT QA process also checks the accuracy of communication and file transfer between the R&V and delivery systems for each delivered field.

From image acquisition to the electronic medical record

In order to acquire IMRT dosimetric images with the Varian delivery system and portal imager, the EPID is positioned with the center of the detecting surface aligned to the LINAC cross-hairs and at the desired SSD (minimum of 105 cm, maximum of 140 cm). Since the Varian TPS is programmed to predict non-transit EPID response, no phantom or other buildup is placed between the source and the EPID detecting surface. The delivery system is prompted by the user to acquire a portal dose image for each field, and the image must be acquired in "Integrated Acquisition" mode, meaning that the EPID continuously collects data throughout the duration of beam-on time (with maximum readout of 20-30 frames per second[19]) with no dependence on the timing of LINAC beam pulses, and sums all the collected data from one acquisition to form one image. The patient plan is delivered from the R&V system in QA Mode such that the delivery does not contribute to the tracking of patient dose delivery, but the chart reviewer can see whether or not the fields have been delivered for QA. When the delivery of a single field is complete, the delivery system calculates two images simultaneously from one acquisition: (1) an integrated relative dose image of the fluence (Figure 1, right panel), and (2) an absolute dose image computed from the EPID response and the most recent calibration data for the appropriate energy and dose rate (Figure 2). The absolute dose image can be collected from the delivery system treatment console computer via portable drive or network (we have used both methods), but cannot be automatically exported to the R&V system since this system has no information about the dosimetric calibration of the imager. However, a filter can be set up within the R&V system to automatically collect the relative dose

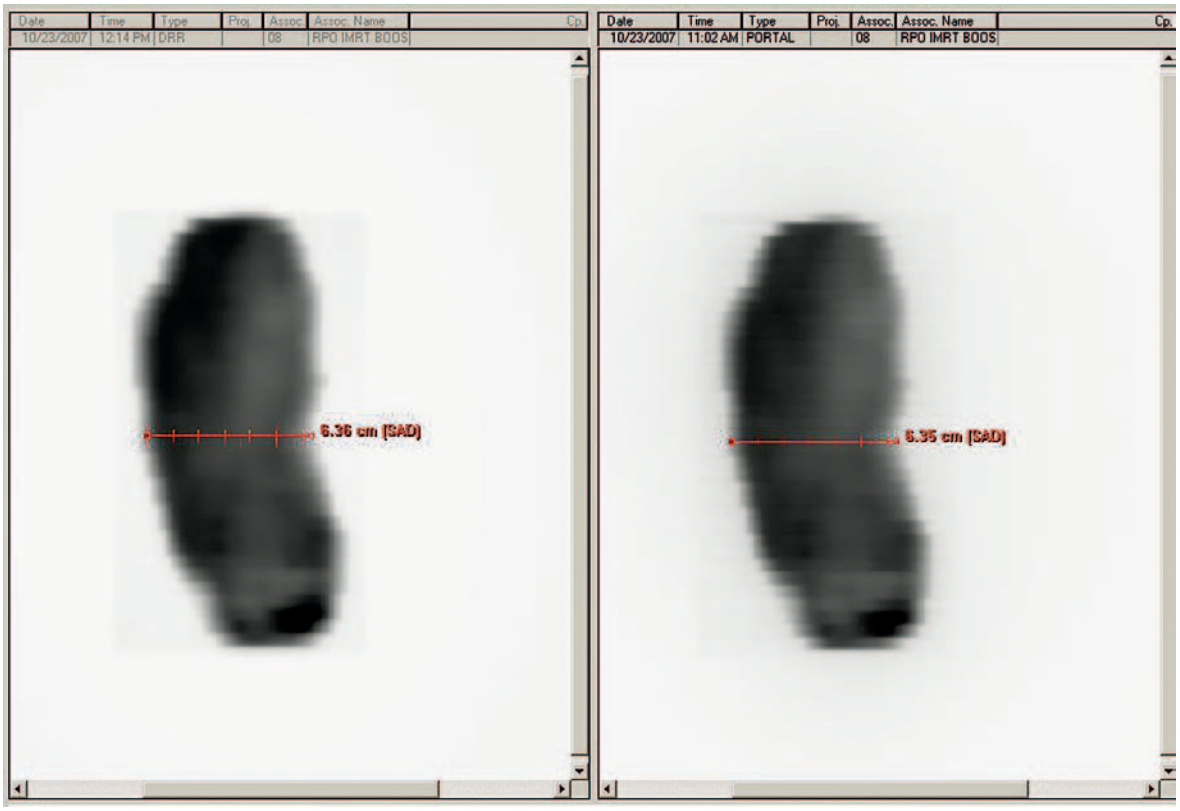

FIGURE 1. 2D integrated relative dose images displayed in the R&V software: 1) acquired using the EPID in integrated acquisition mode (right); and 2) predicted by and exported from the TPS (left). These images are saved within the patient's EMR, attached directly to the appropriate treatment field, and can be compared with various measuring tools within the R&V software (for example, the measuring tool illustrated in the figure).

density image and attach it to the respective field within the patient's EMR (in DICOM-RT format).

Results and discussion

Analysis of the acquired EPID images takes two paths, one for the relative dose density image and one for the absolute dose image.

Qualitative analysis

As mentioned in the previous section, the relative dose density image is collected by the R&V system and attached to the specific patient field. To check the field geometry and relative dose distribution, a respective planned relative dose density map must be exported from the TPS (for the same SSD at which the EPID image was acquired) and similarly attached to the specific field. In this manner, the planned fluence and the acquired fluence can be placed side by side in the R&V software for qualitative comparison (Figure 1). This process is analogous to comparing a TPS printout of the fluence at a certain SSD to a radiograph exposed to the same IMRT field at the same SSD as the printout. The R&V software contains a number of measuring tools which can be used to compare the field size, leaf position, qualitative dose distribution, etc.

Though this type of QA does not contribute substantial amounts of information to the absolute dose QA (discussed below) when performed only once, it does have one distinct advantage. Currently, daily QA for IMRT treatments is virtually non-existent (though some institutions are pursuing *in vivo* QA with EPIDs).[20-22] However, one of the main objectives of patient-specific QA is to ensure that the electronic files containing treatment and delivery system information accurately reflect what was planned and approved in the TPS. If the EPID were used to take a quick non-transit image of one or two fields in the radiotherapy plan (much the same way portal images are currently used for patient positioning), these EPID images can easily and quickly be compared to the fluences already exported from the TPS and stored in the patient's EMR. In this way, radiotherapy professionals can quickly verify

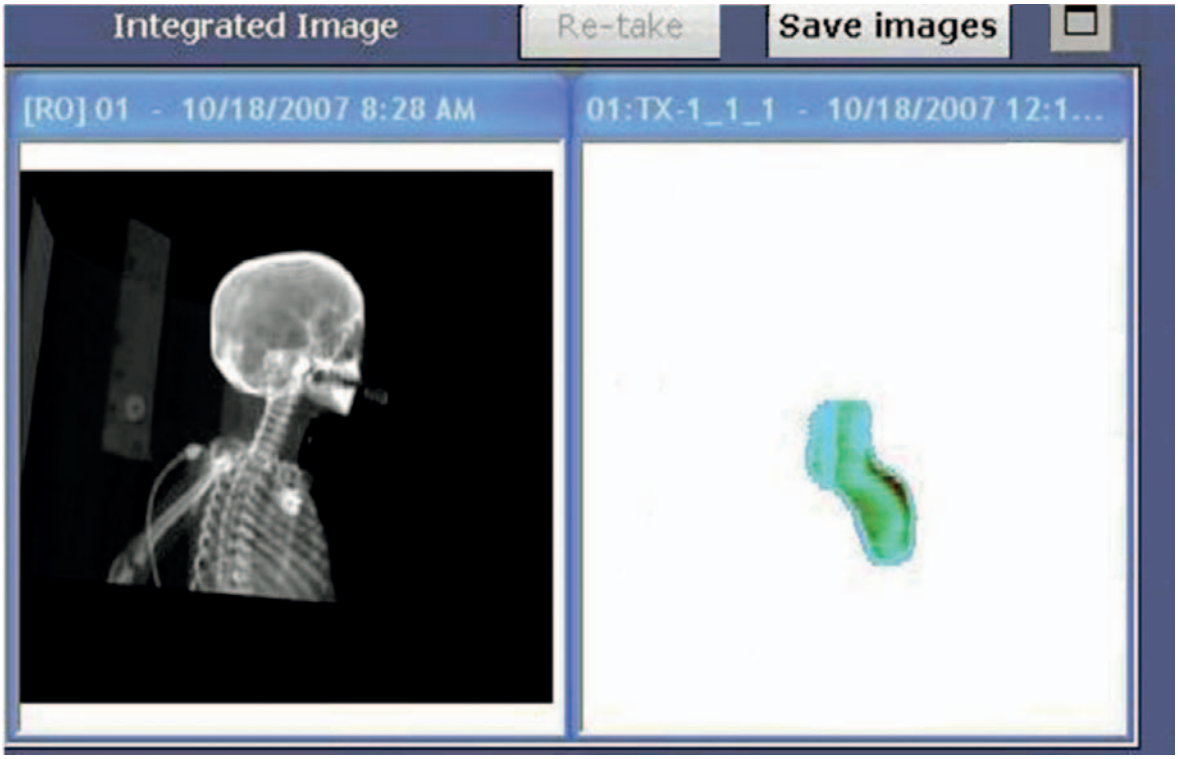

FIGURE 2. Absolute dose image computed from the EPID response and the most recent calibration data for the appropriate energy and dose rate, as displayed by the delivery system computer upon acquisition. This image is exported from the delivery system treatment console computer to the TPS for comparison to the calculated portal dose prediction for the appropriate field and SSD.

that, throughout the course of treatment and daily file transfer, the correct treatment fields and DMLC positions are being delivered accurately. Using this technique, it may also be possible to catch mechanical problems (such as errors in MLC leaf and collimator jaw positions) before the patient is treated, even between the extensive monthly LINAC QA intervals. Thus, this quick, qualitative analysis with the grayscale EPID image could be used on a weekly basis to provide fast and efficient system QA, much as weekly port films (with static MLC) are used to provide clinical treatment QA.

Quantitative analysis

To complete quantitative analysis on the absolute dose EPID image, this file must first be exported from the delivery system treatment console computer via portable drive or network connection, and imported to the computer with which the analysis will be completed. It is possible to perform this analysis via custom made software[10,12,18], commercially available software modalities alternative to the TPS in use[23] (see EPIDose, SunNuclear, Melbourne, FL), or the portal dose prediction and analysis capabilities of the TPS in use. We currently employ the Varian Portal Dosimetry algorithm (Dosimetric Portal Image Calculation, DPIC) within the Eclipse TPS to create portal dose predictions for the aS1000 PortalVision EPID at desired SSD. Commissioning of this algorithm requires capturing two vendor-specified EPID images (at two different SSDs), the diagonal beam profile measured during LINAC commissioning (*i.e.* along the major diagonal of a 40 x 40 cm^2 field of desired energy at d_{max} in water), and the EPID acquisition of field-size output factors for various field sizes specified by the vendor.[24,25] To perform IMRT QA with the PortalVision EPID, the Varian TPS has been programmed to predict the response of the EPID to an IMRT field delivered with no buildup or phantom between the MLC and the EPID, following the methods pioneered by Van Esch *et al*. in 2004.[11,20]

With the EPID dose image imported into the TPS and the respective portal dose prediction calculated, these two planar dose maps can be evaluated through dose difference analysis, gamma evaluation, dose profile line scans, isodose comparisons, various measuring tools, etc. (Figure 3). For gamma evaluation and dose evaluation, the region of interest can be selected to only include the area of

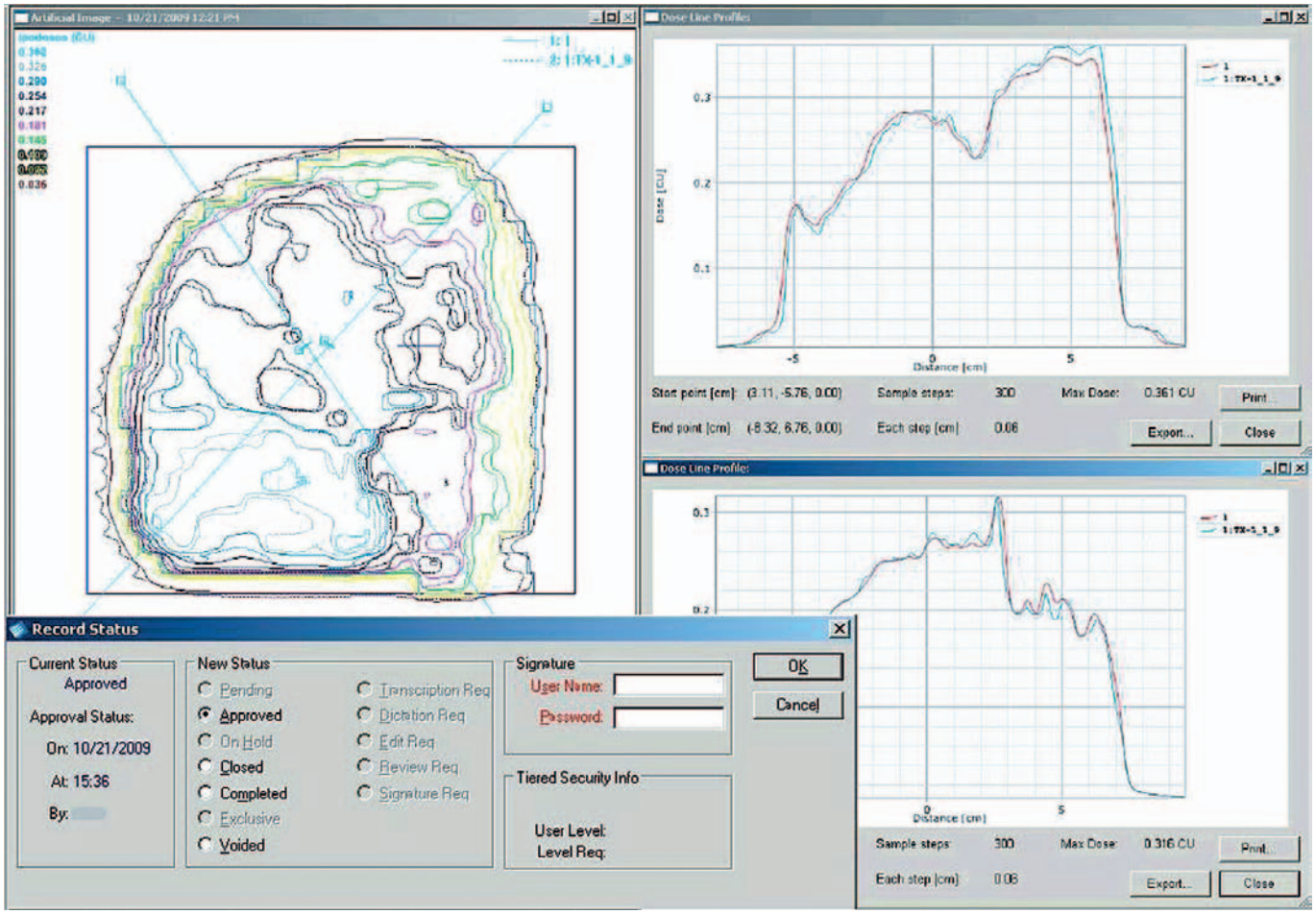

FIGURE 3. Portal dose prediction and acquired EPID absolute dose image as compared in the TPS via: 1) predicted vs. measured isodose lines (left panel); and 2) predicted vs. measured dose line profiles (right two panels). This analysis (in PDF or other desired format) is attached to patient's EMR in the R&V software for approval prior to treatment. The bottom left panel shows the record status dialogue window within the R&V system, including reviewer options such as "pending," "approved," "voided," etc.

the detector within the collimator jaws, or a low-dose threshold can be specified by the user which effectively limits the analysis to the image within the collimator jaws. The resolution of the EPID image and subsequent analysis is far superior to ionization chambers and 2D arrays, while the ease of calibration and image analysis is far more resource and time efficient than the use of films.

To complete the IMRT QA report, the QA analysis can be easily and electronically transferred from the TPS to the patient's EMR in the R&V system by copying the screen to any standard word processing or image editing software, or the screen can similarly be printed to PDF or postscript with the appropriate open-source software installed. A QA report can thus be created for each field within the radiotherapy plan and electronically attached to the patient's chart, requiring no paper, no films, no scanning documents, and no searching for misplaced QA reports. Furthermore, the R&V system can be set up such that this QA analysis must be approved before the fields are treated (see the status dialogue window in Figure 3).

Conclusions

Our radiotherapy department does not have integrated planning, R&V, and delivery systems - and yet we have shown that even in this hybrid environment it is nonetheless possible to develop a completely electronic IMRT QA process. Given the current demand for paperless patient charts, developing a paperless IMRT QA process is vital, even in systems that understandably include components made by diverse vendors. The process suggested in this study is paperless, filmless, time saving and reliable, enabling the pretreatment IMRT QA

process to be far more efficient. Furthermore, QA analysis documentation can be directly attached to its specific patient EMR within the R&V system, eliminating searching for documents and running around to obtain signatures, while greatly reducing the risk of misplacing or losing the QA report. The calculated and measured relative dose density images can be viewed electronically side by side within the patient's EMR to quickly and qualitatively analyze the density differences, field sizes and MLC trajectories, ensuring proper dose delivery to patients - even on a weekly basis. The absolute dose EPID images can be analyzed quickly and thoroughly with custom software or programs supplied by the TPS vendor or a secondary vendor, providing an absolute dose verification system that is of substantially higher resolution than arrays of diodes or ionization chambers, and substantially more efficient than exposing, processing, calibrating, scanning, analyzing and storing films.

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research article

Effect of 905 MHz microwave radiation on colony growth of the yeast *Saccharomyces cerevisiae* strains FF18733, FF1481 and D7

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Background. The aim of this study was to evaluate the effect of weak radiofrequency microwave (RF/MW) radiation emitted by mobile phones on colony growth of the yeast *Saccharomyces cerevisiae*.

Materials and methods. *S. cerevisiae* strains FF18733 (wild-type), FF1481 (*rad1* mutant) and D7 (commonly used to detect reciprocal and nonreciprocal mitotic recombinations) were exposed to a 905 MHz electromagnetic field that closely matched the Global System for Mobile Communication (GSM) pulse modulation signals for mobile phones at a specific absorption rate (SAR) of 0.12 W/kg.

Results. Following 15-, 30- and 60-minutes exposure to RF/MW radiation, strain FF18733 did not show statistically significant changes in colony growth compared to the control sample. The irradiated strains FF1481 and D7 demonstrated statistically significant reduction of colony growth compared to non-irradiated strains after all exposure times. Furthermore, strain FF1481 was more sensitive to RF/MW radiation than strain D7.

Conclusions. The findings indicate that pulsed RF/MW radiation at a low SAR level can affect the rate of colony growth of different *S. cerevisiae* strains.

Key words: microwave radiation; *Saccharomyces cerevisiae;* colony growth

Introduction

Microwave radiation is a type of non-ionizing electromagnetic radiation widely used in industry, commerce, medicine and for private purposes, especially in mobile communication. In recent years, the use of mobile phones has accelerated, resulting in increasing exposure of the environment to weak radiofrequency microwave (RF/MW) radiation generated by these devices. Although the average exposure levels are low compared to exposure limits, public concern about the potential hazard on human health is growing.[1] Numerous experimental studies evaluating the biological effects caused by RF/MW radiation are controversial and no unanimous conclusion has been reached.[2-4]

It is well-documented that yeasts are representative of eukaryotes, including human cells, in many aspects of fundamental cellular processes.[5] Many experiments, with the yeast *Saccharomyces cerevisiae* as a model organism, can be performed under biologically and technically well-controlled conditions after exposure to microwave radiation.[6]

The objective of this study was to evaluate the potential effect of 905 MHz RF/MW radiation similar to that emitted by mobile phones on colony growth of *S. cerevisiae* strains FF18733, FF1481 and D7.

Materials and methods

Yeast strains

This experiment was carried out using three *S. cerevisiae* strains. The FF18733 strain (*MATa leu2-3,112 trp1-289 ura3-52 his7-2 lys1-1*) is a wild-type, whereas the derived FF1481 strain (*MATa leu2-3,112 trp1-289 ura3-52 his7-2 lys1-1 rad1::LEU2*) is deficient in nucleotide excision repair.[7] The D7 strain (*MATa/α ade2-40/ade2-119 trp5-12/trp5-27 ilv1-92/ilv1-92*) is

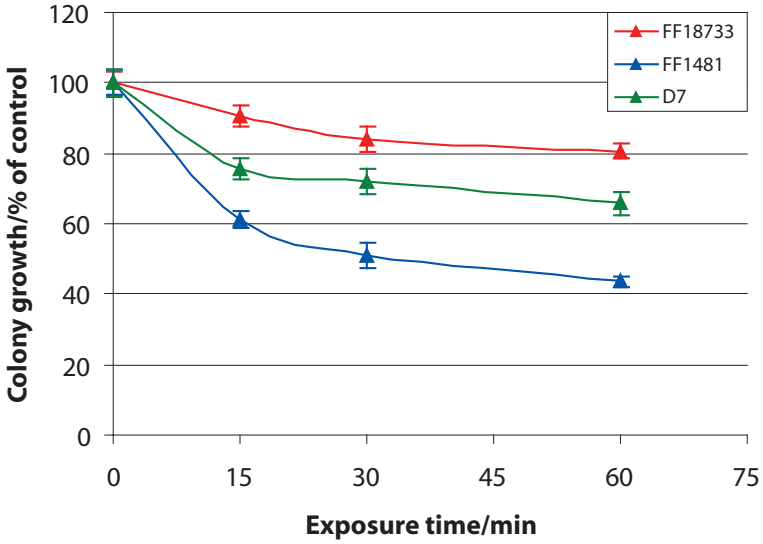

FIGURE 1. Colony growth of yeast *S. cerevisiae* strains FF18733, FF1481 and D7 after 15-, 30- and 60-minutes exposure to 905 MHz microwave radiation. Values represent means and standard deviations.

relatively genetically unstable. Therefore, changes in mitotic crossing-over, mitotic gene conversion and reverse mutations may occur spontaneously.[8]

Experimental procedures

A preculture of three strains of *S. cerevisiae* was suspended in yeast extract liquid (YEL) and grown for 48 h at 28°C. Precultured cells (2 x 10^6 cells/ml) were then suspended in YEL and grown for 18 h at 28°C. Half of each culture (2 x 10^8 cells/ml), prepared in triplicate, were exposed to 905 MHz microwave radiation for 15, 30 and 60 minutes, whereas the other half served as a control. After radiation treatment, yeast cells were inoculated on solid complete growth medium and grown overnight at 28ºC. Thereafter, the number of colonies of the three strains (irradiated and non-irradiated) was counted under a magnifier.

Exposure conditions

An electromagnetic field was generated using a Gigahertz Transversal Electromagnetic Mode Cell (GTEM-cell) model 5402 (ETS™ Lindgren, USA) equipped with a signal generator (Antrisu MS27211B, Japan), signal amplifier (RF 3146 Power Amp Module, RF Micro Devices, Greensboro, USA) and a signal modulator (RF 2722 Polaris Chip, RF Micro Devices, Greensboro, USA). The signal amplifier was used to amplify the RF/MW signal induced by the signal generator, whereas the signal modulator was used to modulate a continuous wave to pulse signal used in the Global System for Mobile Communication (GSM) mobile phones. Yeast suspensions were exposed to 905 MHz RF/MW with the GSM basic signal modulation for 15, 30 and 60 minutes. Inside the GTEM-cell, the electromagnetic field strength was 10 V/m, and the temperature was 28°C. The average specific absorption rate (SAR) for a single cell was 0.12 W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells.[9]

Statistical analysis

Statistical analyses were carried out with descriptive statistics. Significant differences in colony growth were determined using the Student's t-test. Values of *P* lower than 0.05 were considered statistically significant.

Results

Figure 1 shows the colony growth of three *S. cerevisiae* strains after 15-, 30- and 60-minutes exposure to 905 MHz RF/MW radiation similar to that emitted by mobile phones at SAR of 0.12 W/kg. The number of non-irradiated colonies of each strain was taken as 100% and the percent of irradiated colonies after different exposure times was calculated with respect to this control sample. Following a 15-, 30- and 60-minutes exposure to RF/MW radiation, the wild-type strain FF18733 did not show statistically significant changes in colony growth compared to the control sample. Irradiated strains FF1481 and D7 demonstrated statistically significant reduction of colony growth compared to non-irradiated strains after all exposure times. The data indicate that RF/MW radiation decreased colony growth of strains FF18733, FF1481 and D7 resulting in a 19.30±2.06%, 56.37±1.49% and 34.29±3.21% growth reduction after 60-minutes exposure, respectively.

Discussion

Users of mobile phones are exposed to weak microwave radiation. In this context, the possible effects of RF/MW radiation on genetic material are

very important. Many studies on mammalian cells failed to find microwave-induced DNA damage and cell proliferation[10-12]; in contrast with exposure to ionizing radiation where the DNA damage is well known.[13] Other studies have reported that modulated RF/MW radiation is capable of causing DNA lesions and inhibition of cell proliferation.[14,15]

In this study, we estimated the effect of mobile phones radiofrequency of 905 MHz on the yeast *S. cerevisiae* strains FF18733, FF1481 and D7. Strains FF1481 and D7 demonstrated a statistically significant difference in colony growth after 15-, 30- and 60-minutes exposure to pulsed RF/MW radiation at SAR 0.12 W/kg. Therefore, these strains showed increased sensitivity to RF/MW radiation and reduction of colony growth was time-dependent. An earlier experiment with the yeast *S. cerevisiae* demonstrated either an increased (up to 15%) or decreased (up to 38%) cell growth rate by certain frequencies of microwave radiation within a 41.6-41.8 GHz band.[16-18]

It is known that microwave radiation may occur directly by DNA lesion and/or indirectly by damage to DNA repair mechanisms. Strain FF1481 of *S. cerevisiae* is deficient in nucleotide excision repair due to an insertion of the functional *LEU2* gene at the *RAD1* locus and *rad1* becomes non-functional. Rad1, in complex with Rad10, exhibits single-stranded DNA endonuclease activity and cleaves 3'-ended single-stranded DNA at its junction with the duplex DNA.[19] Since we observed a significant decrease of *rad1* mutant cell proliferation, it seems that pulsed RF/MW radiation at a low SAR level during short exposure times could induce DNA damage in *S. cerevisiae* cells.

Mitotic recombination is necessary during mitosis for the repair of DNA single- and double-strand breaks, and mutagenic lesions generated by exposure to chemicals and radiation.[20] Strain D7 of *S. cerevisiae* is commonly used to detect reciprocal (crossing-over) and nonreciprocal (gene conversion) mitotic recombinations and reverse mutations. Besides evaluation of the RF/MW effect on cell growth rate of strain D7, we estimated the induction of mitotic gene conversion and reverse mutations in strain D7 after 15-, 30- and 60-minutes exposure to 905 MHz RF/MW at SAR 0.12 W/kg. The frequency of gene conversion at the *trp* locus and reverse mutation at the *ilv* locus showed only a slight tendency to increase compared to the control sample (data not shown). Preliminary results indicate that modulated RF/MW radiation with a low SAR value did not affect either the rate of gene conversion nor reverse mutations in strain D7. Gos *et al.*[21] reported that mobile phone fields at 900 MHz with SAR of 0.13 and 1.3 W/kg did not exhibit any effect on mutations or recombinations in *S. cerevisiae* cells either in the absence or presence of genotoxic stress.

In conclusion, our study showed that three *S. cerevisiae* strains exhibit different patterns of colony growth after 15-, 30- and 60-minutes exposure to a mobile phones radiofrequency of 905 MHz at SAR 0.12 W/kg. Strains FF1481 (DNA repair mutant) and D7 (relatively genetically unstable) demonstrate an increased sensitivity to RF/MW radiation in comparison to strain FF18733 (wild-type). The data indicate that pulsed RF/MW radiation at a low SAR level could induce DNA damage in *S. cerevisiae* cells. This points to the need for further studies of DNA repair mechanisms in yeast cells.

Acknowledgments

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doi:10.2478/v10019-010-0025-9

Radioterapija v kombinaciji z zdravili usmerjenimi proti tumorskemu žilju

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Izhodišča. Glede na odločilno vlogo tumorskega žilja pri razvoju malignih tumorjev je bilo veliko naporov vloženih v razvoj zdravil usmerjenih v tumorsko žilje. Razvitih je bilo veliko različnih zdravil, ki jih v splošnem uvrščamo v dve skupini. Antiangiogena zdravila se vpletajo v proces angiogeneze in v tumorjih zavirajo nastanek novih žil. Žilno razdiralna zdravila uničujejo obstoječe tumorsko žilje in v tumorju povzročajo ishemijo s posledično nekrozo tumorja. Kljub velikemu terapevtskemu potencialu obeh skupin zdravil postaja jasno, da bodo klinično najbolj uporabna v kombinacijah z drugimi vrstami protirakavega zdravljenja. Radioterapija je zelo široko uporabljan način zdravljenja, ki je povezan z drugačnimi terapevtskimi izzivi in je zato kombinacija obeh pristopov smiselna.

Zaključki. Za učinkovito uporabo zdravil usmerjenih proti tumorskemu žilju v kombinaciji z radioterapijo obstaja močna biološka podlaga. Izkazalo se je, da antiangiogena in žilno razdiralna zdravila vplivajo na tumorsko mikrookolje na takšen način, da povečajo odgovor tumorja na obsevanje. Rezultati predkliničnih in prvih kliničnih raziskav so potrdili terapevtski potencial te nove strategije zdravljenja, vendar so opozorili tudi na večjo možnost stranskih učinkov na zdrava tkiva.

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Hiperhomocisteinemija in vloga B vitaminov pri raku

Plazar N, Jurdana M

Izhodišča. Bolniki z rakom imajo pogoste srčno-žilne zaplete in predstavljajo tveganje za razvoj venske tromboze. Epidemiološke raziskave poročajo, da so bolezni srca in ožilja povezane z povišano koncentracijo homocisteina v krvi (hiperhomocisteinemija), ki se pojavlja tudi pri raku. Homocistein je aminokislina v krvi, ki nastaja pri presnovi metionina in ga v telo vnašamo s hrano. Zato na raven homocisteina v krvni plazmi močno vpliva prehrana pa tudi genetski dejavniki. Prehrambene učinkovine z najmočnejšim vplivom so B vitamini: folna kislina, vitamina B6 in B12. Vloga B vitaminov je pomembna za normalno vzdrževanje strukture DNK-ja. Raziskave opravljene na živalih in ljudeh so pokazale, da pomanjkanje folatov vpliva na proces metilacije in sintezo DNK-ja ter posledično na visoko raven homocisteina v krvi. Zaradi pomembne vloge folatov mnogo raziskav že vrsto let proučuje omenjeno povezavo.

Zaključki. Hiperhomocisteinemija je eden glavnih skupnih značilnosti povezanih z vensko in arterijsko trombozo pri mnogih rakavih obolenjih, patofiziologija slednje povezave pa še ni povsem pojasnjena. Ker ima metabolizem B vitaminov, predvsem folatov ključni pomen pri razvoju hiperhomocisteinemije pri raku, smo v preglednem članku navedli literaturo, ki opisuje tovrstno povezavo.

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doi:10.2478/v10019-010-0021-0

Učinkovito zaustavljanje krvavitve s prevezo iz hitina po perkutani punkciji arterije. Eksperimentalna raziskava na hepariniziranih živalih

Kranokpiraksa P, Pavcnik D, Kakizawa H, Uchida BT, Jeromel M, Keller FS, Rösch J

Izhodišča. V raziskavi smo testirati učinkovitost preveze iz hitina, da bi zaustavili krvavitev po perkutani punkciji povrhnje femoralne arterije in po uporabi žilnega uvajala velikosti 8 F. Učinkovitost preveze iz hitina smo primerjali z metodo standardne ročne kompresije.

Material in metode. Pri devetih hepariniziranih ovcah smo perkutano punktirali povrhnjo femoralno arterijo obojestransko in vanjo za 5 minut uvedli žilno uvajalo velikosti 8 F. Po odstranitvi žilnega uvajala iz obeh punkcijskih mest smo randomizirano zaustavljali krvavitev s kompresijo s prevezo iz hitina ali pa s standardno ročno kompresijo. Obe kompresiji smo izvajali 5 minut. Nato smo uspešnost zaustavitve krvavitev preverjali angiografsko. Kadar smo angiografsko prikazali krvavitev iz punkcijskega mesta, smo nadaljevali z ročno kompresijo in ponavljali angiografske prikaze v razmaku 2,5 minut, dokler nismo popolno zaustavili krvavitve.

Rezultati. Po 5-ih minutah kompresije s prevezo iz hitina smo uspešno zaustavili krvavitev pri sedmih živalih (77,8 %). Srednje trajanje kompresije, s katero smo s prevezo iz hitina zaustavili krvavitev (6,9 ± 3,9 minut), se je statisticno pomembno (p=0,019) razlikovalo od srednjega trajanja pri standardni ročni kompresiji (10,8 ± 2,8 minut). Po zaustavitvi krvavitve s kompresijo s prevezo iz hitina je bil pretok neoviran v vseh devetih arterijah (100%). V tej skupini smo odkrili, da je hematom manjši (2/9) v primerjavi s kontrolno skupino (8/9).

Zaključki. Uporaba kompresije s prevezo iz hitina po odstranitvi žilnega uvajala iz arterije v primerjavi s standardno ročno kompresijo statistično pomembno skrajša čas, v katerem smo dosegli hemostazo. Pri tem ni bilo več zapletov. Za skrajšanje časa za hemostazo in preprečitev hematoma je pomembna pravilna uporaba preveze iz hitina. Pričakujemo lahko, da bomo takšno, prevezo, ki jo sedaj uporabljamo za zaustavitev krvavitve pri poškodbah, v prihodnosti uporabljali tudi za zaustavitev krvavitve po diagnostičnih in terapevtskih endovaskularnih posegih.

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Uporaba 3T MR slikanja pri odkrivanju bolezni, povezanimi z azbestom

Podobnik J, Kocijančič I, Kovač V, Serša I

Izhodišča. Namen raziskave je bil preveriti možnosti uporabe 3T MR slikanja pri odkrivanju in spremljanju bolezenskih sprememb, ki so posledica izpostavljenosti azbestnemu prašenju.

Bolniki in metode. V raziskavo smo vključili 15 bolnikov, 5 z azbestno boleznijo in 10 z malignim plevralnim mezoteliomom (MPM). Bolniki z azbestno boleznijo so nam v digitalni obliki posredovali svoje prejšnje CT preiskave, ki niso bile starejše od enega meseca. Pri bolnikih z MPM pa smo zaradi primerjave sami opravili CT preiskavo, ki je bila potrebna pred pričetkom zdravljenja. Pri vseh smo naredili MR slikanje prsnega koša. Protokol je bil sestavljen iz *T2* obteženih pulznih zaporedij v koronarni, sagitalni in transverzalni ravnini ter *T1* obteženih pulznih zaporedij v transverzalni ravnini. Vse meritve smo naredili, ko je bolnik zadrževal dih in z uporabo EKG prožilca. Pri *T2* obteženih pulznih zaporedjih smo uporabili tudi SPIR tehniko za zasičenje signala iz maščevja. CT preiskave smo naredili z uporabo jodnega kontrastnega sredstva. Slikali smo področje od pljučnih apeksov do spodnjega roba jeter. Rekonstruirali smo slike v transverzalni ravnini debeline 5 mm v mediastinalnem oknu ter 3 mm v pljučnem oknu. Na delovni postaji *Singo MultiModality Work Place* smo izmerili intenzivnost MR signala v leziji in v mišici v isti rezini.

Rezultati. Plevralni plaki imajo na *T1* in *T2* obteženih slikah v primerjavi z mišico hipointenziven signal. Na *T1* obteženih MR slikah je MPM izrazito nehomogen in v primerjavi z mišico hipointenziven, na *T2* obteženih slikah pa hiperintenziven.

Zaključki. Raziskava je pokazala, da je MR slikanje prsnega koša primerna diagnostična slikovna metoda za prikaz sprememb, ki so posledica izpostavljenosti azbestu. T2 obtežene slike lahko celo nakazujejo maligno naravo bolezni. Pri MR slikanju bolniki niso izpostavljeni škodljivemu ionizirajočemu sevanju, zato lahko to slikovno metodo uporabljamo pri spremljanju rezultatov zdravljenja ali razvoja bolezni. Kontrastna sredstva, ki se uporabljajo pri MR slikanju so varnejša in manj nefrotoksična v primerjavi z jodnimi kontrastnimi sredstvi, kar je zlasti pomembno pri bolnikih, ki jih zdravimo s kemoterapijo.

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Zanesljivost difuzijskega magnetnoresonančnega slikanja pri razlikovanju med degenerativnimi in vnetnimi spremembami terminalne plošče

Oztekin O, Calli C, Kitis O, Adibelli ZH, Eren CS, Apaydin M

Izhodišča. Namen raziskave je bil preveriti uporabnost difuzijskega magnetnoresonančnega (MR) slikanja pri razlikovanju med degenerativno spremembo Modic tip 1 in akutnim infekcijskim spondilodiscitisom. S konvecionalnim MR slikanjem je razlikovanje med obema patološkima spremembama težavno in pogosto nezanesljivo.

Boniki in metode. V retrospektivno raziskavo smo vključili 27 bolnikov z erozivno intervertebralno osteohondrozo Modic tip 1 in 18 bolnikov s spondilodiscitisom. Magnetnoresonančne preiskave smo naredili z napravo 1,5 Tesla. Izkušen radiolog je pregledal MR slike lumbalne hrbtenice 45 bolnikov. Bolnikom s spremembo Modic tip 1 smo naredili tudi CT slikanje prizatedega vretenca in medvretenčne ploščice.

Rezultati. Slika kostnega mozga je bila v bližini terminalne plošče tako pri bolnikih z Modic tip 1 kakor tudi pri bolnikih s spondilodiscitisom hipointenzivna na T1 poudarjenih slikah. Na T2 poudarjenih slikah pa je bila pri obeh vrstah bolnikov hiperintenzivna. Slika Modic tip 1 je bila pri difuzijski MR hipointenzivna, pri spondilodiscitisu pa je bila hiperintenzivna.

Zaključki. Naša raziskava je pokazala, da z difuzijsko MR preiskavo lahko zanesljivo ločimo med degenerativno spremembo Modic tip 1 in akutnim spondilodiscitisom, s konvecionalnim MR slikanjem in tudi s kliničnim pregledom pa je ločevanje nezanesljivo.

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Embolizacija z N-butyl-cyanoakrilatom pri znotrajžilnem zdravljenju priapizma z visokim pretokom po poškodbi

Radoš M, Šunjara V, Sjekavica I, Štern Padovan R

Izhodišča. Priapizme (trajna erekcija brez vzburjenja) delimo v nizko pretočne (venske ali ishemične) in visoko pretočne (arterijske ali neishemične). Diagnozo visoko pretočnega priapizma ugotovimo z barvno Dopplersko ultrazvočno preiskavo in arteriografijo ter ga običajno zdravimo z znotrajžilnim zapiranjem fistule.

Prikaz primera. Opisujemo primer 20-letnega bolnika z visokopretočnim tipom priapizma, ki je nastal po poškodbi perineja. Bolnika smo najprej opazovali in nato neuspešno zdravili z embolizacijo fistule, pri kateri smo uporabili resorptivno gelatinozno peno. Ponoven endovaskularni poseg, ki smo naredili embolizacija z N-butyl-cyanoakrilatom, je bil uspešen.

Zaključki. Visokopretočni priapizem lahko uspešno zdravimo z znotrajžilnim posegom, pri čemer je pomembna optimalna izbira embolizacijskega sredstva in natančna izvedba posega.

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Izraženost antigenov pri ponavljajočih se meningiomih

Vranič A

Izhodišča. Meningiomi so intrakranialni tumorji, ki se pogosto ponovno pojavljajo. V literaturi opisujejo, da pride do ponovitve pri 20% benignih, do 80% atipičnih in do 100% malignih meningiomih. Najpogostejši napovedni dejavniki za ponovni vznik bolezni so gradus meningioma, invazivnost v možganovino in radikalnost nevrokirurške resekcije. Namen naše raziskave je bil oceniti razlike med ponavljajočimi se meningiomi in meningeomi, ki se ne ponavljajo, v izražanju nekaterih antigenov na površini njihovih celic.

Metode. Primerjali smo 19 ponavljajočih se in 35 ne ponavljajočih se meningiomov. S pomočjo imunohistokemije smo ocenjevali razlike v izražanju antigena MIB-1, progesteronskih receptorjev, katepsina B in katepsina L.

Rezultati. Izražanje MIB-1 antigena je večje pri ponavljajočih se meningiomih (p=0,001). V izražanju progesteronskih receptorjev nismo opazili statistično pomebnih razlik med skupinama. Imunohistokemično ocenjeno izražanje katepsina B (p= 0,007) in katepsina L (p<0,001) je bilo večje v skupini ponavljajočih se meningiomov.

Zaključki. V raziskavi smo ugotovili, da je pri ponavljajočih se meningiomih večkrat izražen antigen MIB. V prihodnje bi to ugotovitev lahko uporabili kot dodaten napovedni dejavnik, ki bi nam poleg standardnih dejavnikov pomagal pri ocenjevanju tveganja za ponovitev bolezni.

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doi:10.2478/v10019-010-0023-y

Pogostnost hipermetilacije MGMT (0^6-metilgvanin-DNK metiltransferaza) pri bolnikih z glioblastomom in dolgotrajnim preživetjem; izkušnje dunajske ustanove

Baur M, Preusser M, Piribauer M, Elandt K, Hassler M, Hudecm M, Dittrich C, Marosi C

Izhodišča. Namen retrospektivne raziskave je bila analiza metilacijskega statusa promotorja za MGMT (0^6-metilgvanin-DNK metiltransferazo) pri bolnikih z glioblastomom (GBM), pri katerih smo ugotovili dolgotrajno preživetje (≥ 3 leta).

Metode. Metilacijski status promotorja za MGMT smo ugotavljali z bisulfidno modifikacijo DNK in nato z verižno polimerazno reakcijo specifično za metilacijo. DNK smo izločili iz vzorcev tumorja, ki so bili fiksirani v formalinu in vključeni v parafinske bloke.

Rezultati. Vzorce, primerne za interpretacijo smo z verižno polimerazno reakcijo dobili le pri 14 od 33 (42%) bolnikih z GBM in z dolgotrajnim preživetjem. Metilirane proge smo videli pri 3 od 14 bolnikih, metilirane in nemetilirane pri 8 od 14, samo nemetilirane pa pri 3 bolnikih. Metiliacijo promotorja za MGMT smo tako dokazali pri 11 od 14 bolnikih. Skupini bolnikov z metiliranim in nemetiliranim statusom promotorja za MGMT sta bili premajhni za kakršnekoli zanesljive statistične zaključke.

Zaključki. Bolniki z GBM, pri katerih smo ugotovili dolgotrajno preživetje, imajo pogosto prisotno intratumorsko metilacijo promotorja za MGMT. Ta metilacija ločuje bolnike z dolgotrajnim preživetjem od neizbrane skupine bolnikov z GBM. Za povečanje zanesljivosti metodologije določanja statusa metilacije MGMT je potrebna standardizacija postopka verižne polimerazne reakcije specifične za metilacijo.

Radiol Oncol 2010; 44(2): 121-123.
10.2478/v10019-010-0004-1

Klinični primer zasevka raka danke v penis

Yildirim M, Coskun A, Pürten M, Oztekin O, Ilhan E

Izhodišča. Zasevki v penis so redki, običajno pa jih povzročajo genitourinarni in kolorektalni raki.

Prikaz primera. Prikazujemo 77-letnega bolnika z zasevkoma raka danke v penis. Na kliniko je bil napoten zaradi razjede na penisu, semierektilnega penisa in motenj uriniranja. Slikovne preiskave so pokazale vozličasto spremembo v glansu penisa in številne kostne zasevke. Na zdravljenje s kemoterapijo ni odgovoril in ocenili smo, da je napoved poteka bolezni slaba.

Zaključki. Slikovne preiskave in biopsija so razjasnili diagnozo bolezni, zdravljenje takšnih bolnikov pa ni dovolj uspešno, kot kaže tudi primer prikazanega bolnika.

Radiol Oncol 2010; 44(2): 124-130.
doi: 10.2478/v10019-010-0017-9

Elektronska metoda za zagotavljanje kakovosti pri obsevanju IMRT s povezovanjem megavoltnih elektronskih slikovnih naprav z načrtovalnim sistemom, sistemom zabeležbe in overitve ter obsevalnimi napravami

Bailey DW, Kumaraswamy L, Podgorsak MB

Izhodišča. Namen raziskave je bil uvajanje elektronske metode za zagotavljanje kakovosti pri obsevanju IMRT, to je obsevanju z modulirano intenziteto žarkovnega snopa. Uporabili smo megavoltne elektronske slikovne naprave iz amorfnega silicija in jih povezali z načrtovalnim sistemom, sistemom zabeležbe in overitve (R&V) ter z obsevalnimi napravami neodvisnih proizvajalcev.

Metode. Z načrtovalnim sistemom smo na podlagi dejanskega obsevalnega načrta ustvarili verifikacijski obsevalni načrt. Obsevalna polja smo prenesli na sistem R&V, nato smo jih obsevali in pri tem kontolirali kakovost (način QA) z iztegnjeno elektronsko portalno slikovno napravo. Zajete dozimetrične slike smo avtomatično shranili v formatu DICOM-RT na trdi disk računalnika, ki je vodil obsevalno napravo. Slike relativne doze smo nato poslali na sistem R&V. Slike z informacijo o absolutni dozi smo prenesli z računalnika, ki je vodil obsevalno napravo, na načrtovalni sistem in uvedli v verifikacijski obsevalni načrt za nadaljnjo analizo. Zajete slike z ekrana smo uvedli v sistem R&V v obliki elektronske datoteke (npr. PDF). Pri tem smo lahko na ekranu videli primerjavo uporabe analize gama in tudi primerjavo izodoznih črt. To smo naredili pred pričetkom obsevanja bolnika. Tudi sliko relativne doze, ki jo je izračunal načrtovalni sistem, smo lahko poslali na sistem R&V in jo na ta način primerjali s sliko relativne doze, ki smo jo napravili z elektronsko portalno slikovno napravo.

Rezultati. Na našem oddelku načrtovalni sistem, sistem R&V ter obsevalne naprave niso integrirani. Kljub temu smo uspeli zagotoviti kakovost obsevanja IMRT brez beleženja na papir in na film. V sistemu R&V smo bolnikovim podatkom pripeli ustrezne dokumente v elektronski obliki. Na ta način smo omogočili bolj učinkovito analizo in overovitev. Sliki, ki sta kazali izračunano in izmerjeno relativno dozno porazdelitev, smo lahko v elektronski obliki primerjali v sistemu R&V. Tako smo lahko analizirali razlike med njima in zagotovili, da bolnika obsevamo s pravilno dozo.

Zaključki. Pokazali smo, da je elektronsko zagotavljanje kakovosti obsevanja IMRT mogoče, tudi kadar planirni sistem ni integriran s sistemom R&V in obsevalnimi napravami.

Radiol Oncol 2010; 44(2): 131-134.
doi: 10.2478/v10019-010-0019-7

Učinek 905 MHz mikrovalovnega sevanja na rast kolonij kvasovk *Saccharomyces cerevisiae* sevov FF18733, FF1481 in D7

Vrhovac I, Hraščn R, Franekić J

Izhodišča. Namen raziskave je bil proučiti učinek nizkoradiofrekvenčnega valovanja (RF/MW), ki ga sevajo mobilni telefoni, na rast kvasovk *Saccharomyces cerevisiae*.

Materiali in metode. Sevi *S. cerevisiae* FF18733 (divji tip), FF1481 (mutacija rad1) in D7 (običajno uporabljen za detekcijo recipročne in nerecipročne mitotične rekombinacije) so bili izpostavljeni 905 MHz elektromagnetnemu polju ki je zelo podoben signalu pri globalnem sistemu prenosnih komunikacij (Global System for Mobile Communication, GSM) uporabljenem v prenosnih telefonih pri specifični absorpciji (SAR) 0.12 W/kg.

Rezultati. Ko smo izpostavili sev FF18733 *S. Cerevisiae* 15-, 30- in 60-minutnemu obsevanju RF/MW, nismo statistično značilno vplivali na rast kvasovk v primnerjavi s kontrolnimi neobsevanimi kvasovkami. Ko pa smo obsevali seve FF1481 in D7, smo statistično značilno vplivalo na rast kvasovk pri vseh dolžinah obsevanja. Pokazala se je razlika v občutljivosti, sev FF1481 je bil bolj občutljiv na obsevanje RF/MW kot sev D7.

Zaključki. Rezultati nakazujejo, da lahko pri nizkem SAR pulzno obsevanje RF/MW zavira rast različnih sevov *S. cerevisiae*.

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Notices submitted for publication should contain a mailing address, phone and/or fax number and/or e-mail of a Contact person or department.

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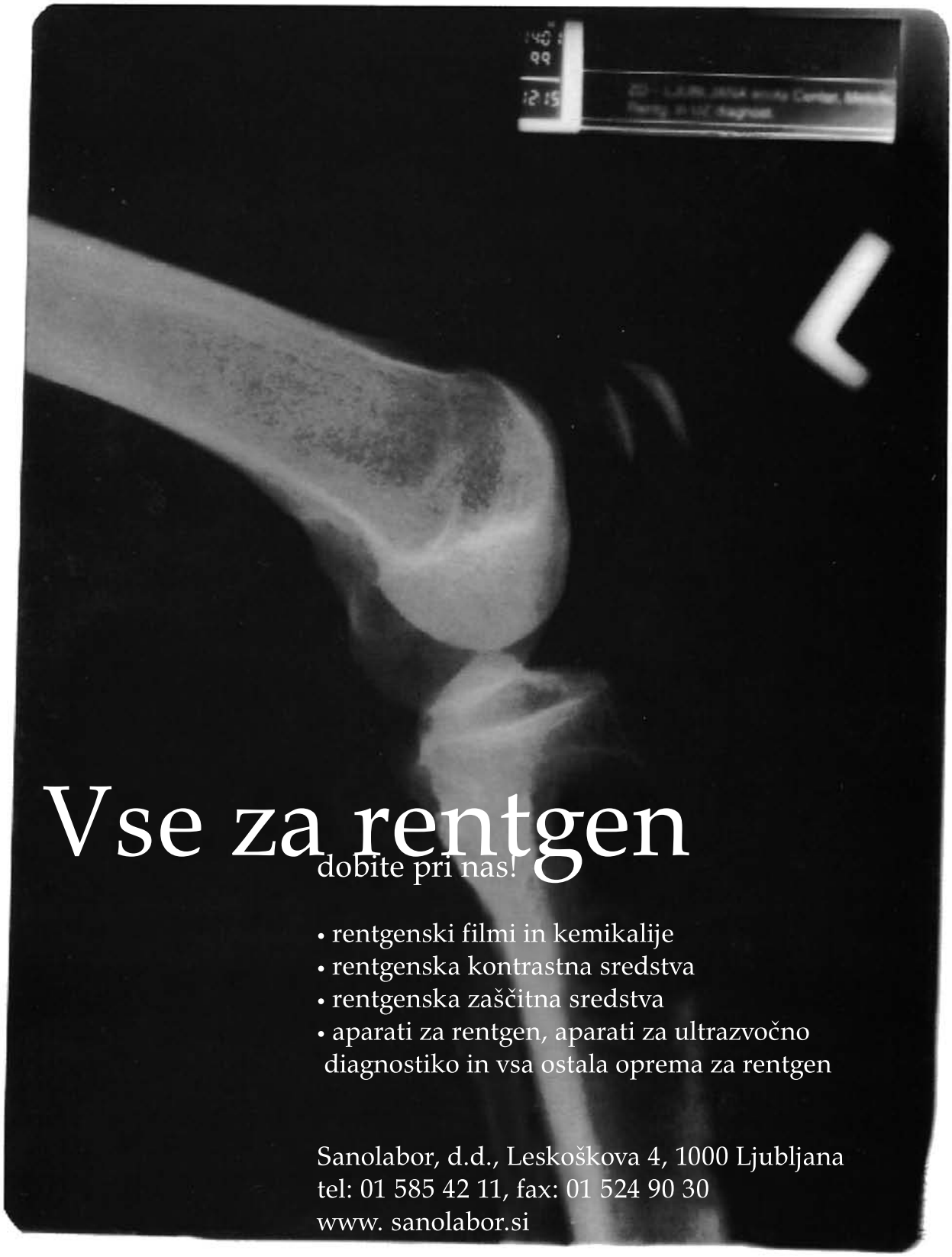

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Izredno učinkovito zdravljenje prvega reda pri nedrobnoceličnem pljučnem raku z mutacijo EGFR

Iressa je prva in edina tarčna monoterapija, ki dokazano podaljša preživetje brez napredovanja bolezni v primerjavi z dvojno kemoterapijo kot zdravljenje prvega reda pri bolnikih z napredovalim nedrobnoceličnim pljučnim rakom z mutacijo EGFR. [1]

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

1. Povzetek glavih značilnosti zdravila Iressa (gefitinib). Junij 2009.

Sestava: Filmsko obložene tablete vsebujejo 250 mg gefitiniba. **Indikacije:** zdravljenje odraslih bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim pljučnim rakom z aktivacijskimi mutacijami EGFR-TK **Odmerjanje in način uporabe:** Zdravljenje z gefitinibom mora uvesti in nadzorovati zdravnik, ki ima izkušnje z uporabo zdravil proti raku. Priporočeno odmerjanje zdravila IRESSA je ena 250-mg tableta enkrat na dan. Tableto je mogoče vzeti s hrano ali brez nje, vsak dan ob približno istem času. **Kontraindikacije:** preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov, dojenje **Opozorila in previdnostni ukrepi:** Pri 1,3 % bolnikov, ki so dobivali gefitinib, so opažali intersticijsko bolezen pljuč (IBP). Ta se lahko pojavi akutno in je bila v nekaterih primerih smrtna. Če se bolniku poslabšajo dihalni simptomi, npr. dispneja, kašelj in zvišana telesna temperatura, morate zdravljenje z zdravilom IRESSA prekiniti in bolnika takoj preiskati. če je potrjena IBP, morate terapijo z zdravilom IRESSA končati in bolnika ustrezno zdraviti. čeprav so bile nepravilnosti testov jetrnih funkcij pogoste, so jih redko zabeležili kot hepatitis. Zato so priporočljive redne kontrole delovanja jeter. V primeru blagih do zmernih sprememb v delovanju jeter je treba zdravilo IRESSA uporabljati previdno. Če so spremembe hude, pride v poštev prekinitev zdravljenja. Zdravilo IRESSA vsebuje laktozo. Bolniki z redko dedno intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze2galaktoze ne smejo jemati tega zdravila. Bolnikom naročite, da morajo takoj poiskati zdravniško pomoč, če se jim pojavijo kakršnikoli očesni simptomi, huda ali dolgotrajna driska, navzea, bruhanje ali anoreksija, ker lahko vse te posredno povzročijo dehidracijo. **Medsebojno delovanje zdravile:** Induktorji CYP3A4 lahko povečajo presnovo gefitiniba in zmanjšajo njegovo koncentracijo v plazmi. Zato lahko sočasna uporaba induktorjev CYP3A4 (npr. fenitoina, karbamazepina, rifampicina, barbituratov ali zeliščnih pripravkov, ki vsebujejo šentjanževko/Hypericum perforatum) zmanjša učinkovitost zdravljenja in se ji je treba izogniti. Pri posameznih bolnikih, ki imajo genotip slabih metabolizatorjev s CYP2D6, lahko zdravljenje z močnim zaviralcem CYP3A4 poveča koncentracijo gefitiniba v plazmi. Na začetku zdravljenja z zaviralcem CYP3A4 je treba bolnike natančno kontrolirati glede neželenih učinkov gefitiniba. Pri nekaterih bolnikih, ki so jemali varfarin skupaj z gefitinibom, so se pojavili zvišanje internacionalnega normaliziranega razmerja (INR) in/ali krvavitve. Bolnike, ki sočasno jemljejo varfarin in gefitinib, morate redno kontrolirati glede sprememb protrombinskega časa (PČ) ali INR. Zdravila, ki občutno in dolgotrajno zvišajo pH v želodcu npr. zaviralci protonske črpalke in antagonisti H2, lahko zmanjšajo biološko uporabnost gefitiniba in njegovo koncentracijo v plazmi in tako zmanjšajo učinkovitost. Redno jemanje antacidov, uporabljenih blizu časa jemanja zdravila IRESSA, ima lahko podoben učinek. **Neželeni učinki:** V kumulativnem naboru podatkov kliničnih preskušanj III. faze so bili najpogosteje opisani neželeni učinki, ki so se pojavili pri več kot 20 % bolnikov, driska in kožne reakcije (vključno z izpuščajem, aknami, suho kožo in srbenjem). Neželeni učinki se ponavadi pojavijo prvi mesec zdravljenja in so praviloma reverzibilni. Ostali pogostejši neželeni učinki so: anoreksija, konjunktivitis, blefaritis in suho oko, krvavitev, npr. epistaksa in hematurija, intersticijska bolezen pljuč (1,3 %), navzea, bruhanje, stomatitis, dehidracija, suha usta, nepravilnosti testov jetrnih funkcij, bolezni nohtov, alopecija, asimptomatično laboratorijsko zvišanje kreatinina v krvi, proteinurija, astenija, pireksija. **Vrsta in vsebina ovojnine:** škatla s 30 tabletami po 250 mg gefitiniba **Način izdajanja zdravila:** samo na recept **Datum priprave besedila:** junij 2009 **Imetnik dovoljenja za promet:** AstraZeneca AB, S-151 85, Sodertalje, Švedska Pred predpisovanjem, prosimo, preberite celoten povzetek glavnih značilnosti zdravila. **Dodatne informacije so na voljo pri:** AstraZeneca UK Limited, Podružnica v Sloveniji, Verovškova 55, 1000 Ljubljana, telefon: 01/51 35 600.

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- absolutno število nevtrofilcev (ANC – Absolute Neutrophil Count) ≥ 1,5 x 109/l;
- število trombocitov ≥ 100 x 109/l;
- skupna merila toksičnosti (SMT) za nehematološko toksičnost ≤ 1. stopnje (z izjemo alopecije, navzee in bruhanja).

Med zdravljenjem morate pri bolniku enkrat na teden pregledati celotno krvno sliko.

Faza monoterapije Štiri tedne po zaključku faze sočasnega zdravljenja s TMZ in RT naj bolnik jemlje TMZ do 6 ciklov monoterapije. V 1. ciklu (monoterapije) je odmerek zdravila 150 mg/m2 enkrat na dan 5 dni, temu pa naj sledi 23 dni brez terapije. Na začetku 2. cikla odmerek povečajte na 200 mg/m2, če je SMT za nehematološko toksičnost za 1. cikel stopnje ≤ 2 (z izjemo alopecije, slabosti in bruhanja), absolutno število nevtrofilcev (ANC) ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Če odmerka niste povečali v 2. ciklu, ga v naslednjih ciklih ne smete povečevati. Ko pa odmerek enkrat povečate, naj ostane na ravni 200 mg/m2 na dan v prvih 5 dneh vsakega naslednjega cikla, razen če nastopi toksičnost. Zmanjšanje odmerka in ukinitev zdravila med fazo monoterapije opravite, kot je opisano v preglednicah 2 in 3. Med zdravljenjem morate 22. dan pregledati celotno krvno sliko (21 dni po prvem odmerku TMZ). **Odrasli in pediatrični bolniki, stari 3 leta ali več, s ponavljajočim se ali napredujočim malignim gliomom:**Posamezen cikel zdravljenja traja 28 dni. Bolniki, ki še niso bili zdravljeni s kemoterapijo, naj jemljejo TMZ v odmerku 200 mg/m2 enkrat na dan prvih 5 dni, temu pa naj sledi 23-dnevni premor (skupaj 28 dni). Pri bolnikih, ki so že bili zdravljeni s kemoterapijo, je začetni odmerek 150 mg/m2 enkrat na dan, v drugem ciklu pa se poveča na 200 mg/m2 enkrat na dan 5 dni, če ni bilo hematoloških toksičnih učinkov. **Kontraindikacije:** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Preobčutljivost za dakarbazin (DTIC). **Posebna opozorila in previdnostni ukrepi: Pljučnica, ki jo povzroča Pneumocystis carinii** Pilotno preskušanje podaljšane 42-dnevne sheme zdravljenja je pokazalo, da pri bolnikih, ki so sočasno prejemali TMZ in RT, obstaja še posebej veliko tveganje za nastanek pljučnice zaradi okužbe s Pneumocystis carinii (PCP). **Malignosti** Zelo redko so poročali tudi o primerih mielodisplastičnega sindroma in sekundarnih malignostih, vključno z mieloidno levkemijo. Antiemetično zdravljenje Navzea in bruhanje sta pogosto povezana z zdravljenjem s TMZ. **Antiemetično zdravljenje** se lahko da pred uporabo TMZ ali po njej. **Odrasli bolniki z novo diagnosticiranim multiformnim glioblastomom** Antiemetična profilaksa je priporočljiva pred začetnim odmerkom sočasne faze in je močno priporočljiva med fazo monoterapije. **Ponavljajoči se ali napredujoči maligni gliom** Pri bolnikih, ki so močno bruhali (stopnja 3 ali 4) v prejšnjih ciklih zdravljenja, je potrebno antiemetično zdravljenje. **Laboratorijske vrednosti** Pred jemanjem zdravila morata biti izpolnjena naslednja pogoja za laboratorijske izvide: ANC ≥ 1,5 x 109/l in število trombocitov ≥ 100 x 109/l. Na 22. dan (21 dni po prvem odmerku) ali v roku 48 ur od navedenega dne, morate pregledati celotno krvno sliko in jo nato spremljati vsak teden, dokler ni ANC > 1,5 x 109/l in število trombocitov > 100 x 109/l. Če med katerimkoli ciklom ANC pade na < 1,0 x 109/l ali število trombocitov na < 50 x 109/l, morate odmerek zdravila v naslednjem ciklu zmanjšati za eno stopnjo (glejte poglavje 4.2). Stopnje odmerka so 100 mg/m2, 150 mg/m2 in 200 mg/m2. Najmanjši priporočeni odmerek je 100 mg/m2. **Pediatrična uporaba** Kliničnih izkušenj z uporabo TMZ pri otrocih, mlajših od 3 let, ni. Izkušnje z uporabo tega zdravila pri starejših otrocih in mladostnikih so zelo omejene. **Starejši bolniki (stari > 70 let)** Videti je, da je pri starejših bolnikih tveganje za nevtropenijo ali trombocitopenijo večje, kot pri mlajših. Zato je pri uporabi zdravila TMZ pri starejših bolnikih potrebna posebna previdnost. **Moški bolniki** Moškim, ki se zdravijo s TMZ je treba svetovati, naj ne zaplodijo otroka še šest mesecev po prejetem zadnjem odmerku in naj se pred zdravljenjem posvetujejo o možnostih za shranitev zmrznjene sperme. **Natrij** To zdravilo vsebuje 2,4 mmol natrija na vialo. To je treba upoštevati pri bolnikih na nadzorovani dieti z malo natrija. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:** Študije medsebojnega delovanja so izvedli le pri odraslih. V ločeni študiji 1. faze, sočasna uporaba TMZ in ranitidina ni povzročila spremembe obsega absorpcije temozolomida ali izpostavljenosti njegovem aktivnem presnovku monometiltriazenoimidazol karboksamidu (MTIK). Analiza populacijske farmakokinetike v preskušanjih 2. faze je pokazala, da sočasna uporaba deksametazona, proklorperazina, fenitoina, karbamazepina, ondansetrona, antagonistov receptorjev H2 ali fenobarbitala ne spremeni očistka TMZ. Sočasno jemanje z valprojsko kislino je bilo povezano z majhnim, a statistično pomembnim zmanjšanjem očistka TMZ. Študij za določitev učinka TMZ na presnovo ali izločanje drugih zdravil niso izvedli. Ker pa se TMZ ne presnavlja v jetrih in se na beljakovine veže le v majhni meri, je malo verjetno, da bi vplival na farmakokinetiko drugih zdravil. Uporaba TMZ v kombinaciji z drugimi mielosupresivnimi učinkovinami lahko poveča verjetnost mielosupresije. **Neželeni učinki:** Pri bolnikih, ki se zdravijo s TMZ v kombinaciji z RT ali monoterapijo po RT zaradi novo diagnosticiranega multiformnega glioblastoma ali z monoterapijo pri bolnikih s ponavljajočim se ali napredujočim gliomom, so bili zelo pogosti neželeni učinki podobni; slabost, bruhanje, zaprtje, neješčnost, glavobol in utrujenost. Pri bolnikih z novo diagnosticiranim glioblastomom multiforme na monoterapiji so zelo pogosto poročali o konvulzijah, medtem ko je bil izpuščaj opisan zelo pogosto pri bolnikih z novo diagnosticiranim multiformnim glioblastomom, ki so prejemali TMZ sočasno z RT, ter pri tistih, ki so zdravilo prejemali v obliki monoterapije, pogosto pa pri tistih s ponavljajočim se gliomom. Pri obeh indikacijah so o večini hematoloških neželenih reakcij poročali pogosto ali zelo pogosto. **Imetnik dovoljenja za promet:** Schering-Plough Europe, Rue de Stalle 73, Bruselj Belgija **Način in režim izdaje zdravila: Zdravilo Temodal 20 mg, 100 mg, 140mg, 180 mg, 250 mg** se izdaja na recept (Rp/Spec), Temodal 2,5 mg/ml prašek za raztopino za infundiranje pa je namenjeno uporabi samo v bolnišnicah (H). **Datum priprave informacije:** februar 2010

Literatura: 1 Povzetek temeljnih značilnosti zdravila Temodal 2 Stupp R, et. al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised III study: 5-year analysis of the EORTC-NCIC trial

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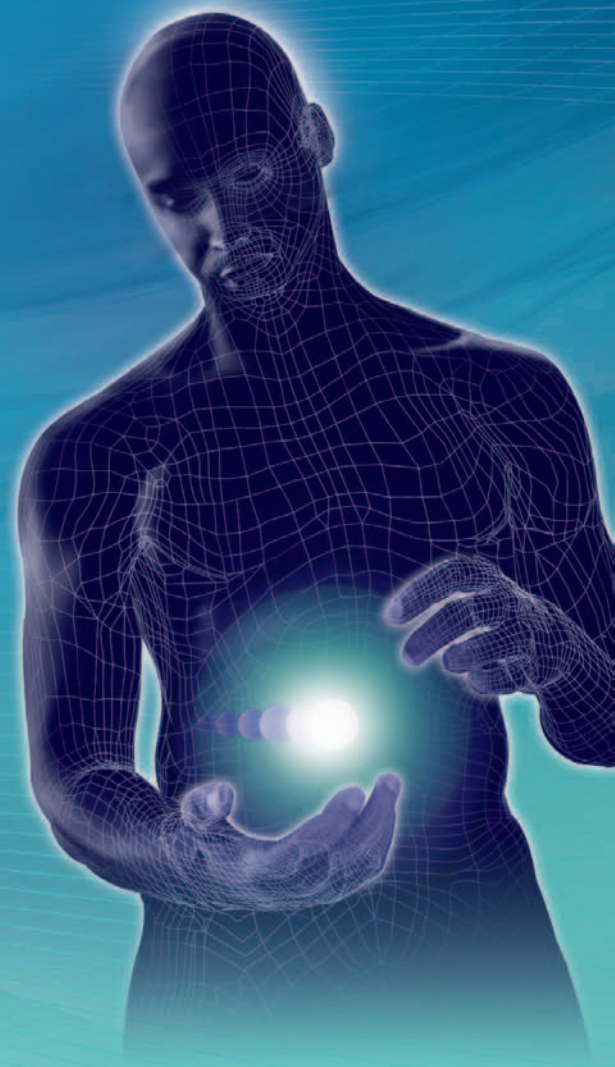

Resnični napredek

Pomembno izboljšanje preživetja potrjeno tudi ob daljšem spremljanju bolnikov.[2]

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Samo za strokovno javnost.

Ime zdravila: Tarceva 25 mg/100 mg/150 mg filmsko obložene tablete

Kakovostna in količinska sestava: Ena filmsko obložena tableta vsebuje 25 mg, 100 mg ali 150 mg erlotiniba (v obliki erlotinibijevega klorida).

Terapevtske indikacije: Nedrobnocelični rak pljuč: Zdravilo Tarceva je indicirano za samostojno vzdrževalno zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč s stabilno boleznijo po 4 ciklih standardne kemoterapije na osnovi platine v prvi liniji zdravljenja. Zdravilo Tarceva je indicirano tudi za zdravljenje bolnikov z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč po neuspehu vsaj ene predhodne kemoterapije. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja ali drugih klinično pomembnih učinkov zdravljenja niso dokazali pri bolnikih z EGFR-negativnimi tumorji. Rak trebušne slinavke: Zdravilo Tarceva je v kombinaciji z gemcitabinom indicirano za zdravljenje bolnikov z metastatskim rakom trebušne slinavke. Pri predpisovanju zdravila Tarceva je treba upoštevati dejavnike, povezane s podaljšanim preživetjem. Koristnega vpliva na podaljšanje preživetja niso dokazali za bolnike z lokalno napredovalo boleznijo.

Odmerjanje in način uporabe: Zdravljenje z zdravilom Tarceva mora nadzorovati zdravnik z izkušnjami pri zdravljenju raka. Zdravilo Tarceva vzamemo najmanj eno uro pred zaužitjem hrane ali dve uri po tem. Kadar je potrebno odmerek prilagoditi, ga zmanjšujemo v korakih po 50 mg. Pri sočasnem jemanju substratov in modulatorjev CYP3A4 bo morda potrebna prilagoditev odmerka. Pri dajanju zdravila Tarceva bolnikom z jetrno okvaro je potrebna previdnost. Če se pojavijo hudi neželeni učinki, pride v poštev zmanjšanje odmerka ali prekinitev zdravljenja z zdravilom Tarceva. Uporaba zdravila Tarceva pri bolnikih s hudo jetrno ali ledvično okvaro ter pri otrocih ni priporočljiva. Bolnikom kadilcem je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih manjše kot pri nekadilcih. Nedrobnocelični rak pljuč: Priporočeni dnevni odmerek zdravila Tarceva je 150 mg. Rak trebušne slinavke: Priporočeni dnevni odmerek zdravila Tarceva je 100 mg, v kombinaciji z gemcitabinom. Pri bolnikih, pri katerih se kožni izpuščaj v prvih 4 do 8 tednih zdravljenja ne pojavi, je treba ponovno pretehtati nadaljnje zdravljenje z zdravilom Tarceva.

Kontraindikacije: Huda preobčutljivost za erlotinib ali katero koli pomožno snov.

Posebna opozorila in previdnostni ukrepi: Močni induktorji CYP3A4 lahko zmanjšajo učinkovitost erlotiniba, močni zaviralci CYP3A4 pa lahko povečajo toksičnost. Sočasnemu zdravljenju s temi zdravili se je treba izogibati. Bolnikom, ki kadijo, je treba svetovati, naj prenehajo kaditi, saj so plazemske koncentracije erlotiniba pri kadilcih zmanjšane v primerjavi s plazemskimi koncentracijami pri nekadilcih. Verjetno je, da je velikost zmanjšanja klinično pomembna. Pri bolnikih, pri katerih se akutno pojavijo novi in/ali poslabšajo nepojasnjeni pljučni simptomi, kot so dispneja, kašelj in vročina, je zdravljenje z zdravilom Tarceva treba prekiniti, dokler ni znana diagnoza. Bolnike, ki se sočasno zdravijo z erlotinibom in gemcitabinom, je treba skrbno spremljati zaradi možnosti pojava toksičnosti, podobni intersticijski pljučni bolezni. Če je ugotovljena intersticijska pljučna bolezen, zdravilo Tarceva ukinemo in uvedemo ustrezno zdravljenje. Pri približno polovici bolnikov, ki so se zdravili z zdravilom Tarceva, se je pojavila driska. Zmerno do hudo drisko zdravimo z loperamidom. V nekaterih primerih bo morda potrebno zmanjšanje odmerka. V primeru hude ali dolgotrajne driske, navzeje, anoreksije ali bruhanja, povezanih z dehidracijo, je zdravljenje z zdravilom Tarceva treba prekiniti in dehidracijo ustrezno zdraviti. O hipokaliemiji in ledvični odpovedi so poročali redko. Posebno pri bolnikih z dejavniki tveganja (sočasno jemanje drugih zdravil, simptomi, bolezni ali drugi dejavniki, vključno z visoko starostjo) moramo, če je driska huda ali dolgotrajna oziroma vodi v dehidracijo, zdravljenje z zdravilom Tarceva prekiniti in bolnikom zagotoviti intenzivno intravensko rehidracijo. Dodatno je treba pri bolnikih s prisotnim tveganjem za razvoj dehidracije spremljati ledvično delovanje in serumske elektrolite, vključno s kalijem. Pri uporabi zdravila Tarceva so poročali o redkih primerih jetrne odpovedi. K njenemu nastanku je lahko pripomogla predhodno obstoječa jetrna bolezen ali sočasno jemanje hepatotoksičnih zdravil. Pri teh bolnikih je treba zato premisliti o rednem spremljanju jetrnega delovanja. Dajanje zdravila Tarceva je treba prekiniti, če so spremembe jetrnega delovanja hude. Bolniki, ki prejemajo zdravilo Tarceva, imajo večje tveganje za razvoj perforacij v prebavilih, ki so jih opazili občasno. Pri bolnikih, ki sočasno prejemajo zdravila, ki zavirajo angiogenezo, kortikosteroide, nesteroidna protivnetna zdravila (NSAID) in/ali kemoterapijo na osnovi taksanov, ali so v preteklosti imeli peptični ulkus ali divertikularno bolezen, je tveganje večje. Če pride do tega, je treba zdravljenje z zdravilom Tarceva dokončno ukiniti. Poročali so o primerih kožnih bolezni z mehurji in luščenjem kože, vključno z zelo redkimi primeri, ki so nakazovali na Stevens-Johnsonov sindrom/toksično epidermalno nekrolizo in so bili v nekaterih primerih smrtni. Zdravljenje z zdravilom Tarceva je treba prekiniti ali ukiniti, če se pri bolniku pojavijo hude oblike mehurjev ali luščenja kože. Zelo redko so poročali o primerih perforacije ali ulceracije roženice; opazili so tudi druge očesne bolezni. Zdravljenje z zdravilom Tarceva je treba prekiniti ali ukiniti, če se pri bolnikih pojavijo akutne očesne bolezni, kot je bolečina v očeh, ali se le-te poslabšajo. Tablete vsebujejo laktozo in jih ne smemo dajati bolnikom z redkimi dednimi stanji: intoleranco za galaktozo, laponsko obliko zmanjšane aktivnosti laktaze ali malabsorpcijo glukoze/galaktoze.

Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:
Erlotinib se pri ljudeh presnavlja v jetrih z jetrnimi citokromi, primarno s CYP3A4 in v manjši meri s CYP1A2. Presnova erlotiniba zunaj jeter poteka s CYP3A4 v črevesju, CYP1A1 v pljučih in CYP1B1 v tumorskih tkivih. Z zdravilnimi učinkovinami, ki se presnavljajo s temi encimi, jih zavirajo ali pa so njihovi induktorji, lahko pride do interakcij. Erlotinib je srednje močan zaviralec CYP3A4 in CYP2C8, kot tudi močan zaviralec glukuronidacije z UGT1A1 *in vitro*. Pri kombinaciji ciprofloksacina ali močnega *zaviralca CYP1A2* (npr. fluvoksamina) z erlotinibom je potrebna previdnost. V primeru pojava neželenih dogodkov, povezanih z erlotinibom, lahko odmerek erlotiniba zmanjšamo. Predhodno ali sočasno zdravljenje z zdravilom Tarceva ni spremenilo očistka prototipov *substratov CYP3A4*, midazolama in eritromicina. Inhibicija glukoronidacije lahko povzroči interakcije z zdravili, ki so *substrati UGT1A1* in se izločajo samo po tej poti. Močni *zaviralci aktivnosti CYP3A4* zmanjšajo presnovo erlotiniba in zvečajo koncentracije erlotiniba v plazmi. Pri sočasnem jemanju erlotiniba in močnih zaviralcev CYP3A4 je zato potrebna previdnost. Če je treba, odmerek erlotiniba zmanjšamo, še posebno pri pojavu toksičnosti. Močni *spodbujevalci aktivnosti CYP3A4* zvečajo presnovo erlotiniba in pomembno zmanjšajo plazemske koncentracije erlotiniba. Sočasnemu dajanju zdravila Tarceva in induktorjev CYP3A4 se je treba izogibati. Pri bolnikih, ki potrebujejo sočasno zdravljenje z zdravilom Tarceva in močnim induktorjem CYP3A4, je treba premisliti o povečanju odmerka do 300 mg ob skrbnem spremljanju njihove varnosti. Zmanjšana izpostavljenost se lahko pojavi tudi z drugimi induktorji, kot so fenitoin, karbamazepin, barbiturati ali šentjanževka. Če te zdravilne učinkovine kombiniramo z erlotinibom, je potrebna previdnost. Kadar je mogoče, je treba razmisliti o drugih načinih zdravljenja, ki ne vključujejo močnega spodbujanja aktivnosti CYP3A4. Bolnikom, ki jemljejo varfarin ali druge *kumarinske antikoagulante*, je treba redno kontrolirati protrombinski čas ali INR. Sočasna uporaba *zaviralcev P-glikoproteina*, kot sta ciklosporin in verapamil, lahko vodi v spremenjeno porazdelitev in/ali spremenjeno izločanje erlotiniba. Za erlotinib je značilno zmanjšanje topnosti pri pH nad 5. *Zdravila, ki spremenijo pH v zgornjem delu prebavil*, lahko spremenijo topnost erlotiniba in posledično njegovo biološko uporabnost. Učinka antacidov na absorpcijo erlotiniba niso proučevali, vendar je ta lahko zmanjšana, kar vodi v nižje plazemske koncentracije. Kombinaciji erlotiniba in zaviralca protonske črpalke se je treba izogibati. Če menimo, da je uporaba antacidov med zdravljenjem z zdravilom Tarceva potrebna, jih je treba jemati najmanj 4 ure pred ali 2 uri po dnevnem odmerku zdravila Tarceva. Če razmišljamo o uporabi ranitidina, moramo zdravili jemati ločeno: zdravilo Tarceva je treba vzeti najmanj 2 uri pred ali 10 ur po odmerku ranitidina. V študiji faze Ib ni bilo pomembnih učinkov *gemcitabina* na farmakokinetiko erlotiniba, prav tako ni bilo pomembnih učinkov erlotiniba na farmakokinetiko gemcitabina. Erlotinib poveča koncentracijo platine. Pomembnih učinkov *karboplatina* ali paklitaksela na farmakokinetiko erlotiniba ni bilo. *Kapecitabin* lahko poveča koncentracijo erlotiniba. Pomembnih učinkov erlotiniba na farmakokinetiko kapecitabina ni bilo.

Neželeni učinki: *Zelo pogosti neželeni učinki* so kožni izpuščaj in driska, kot tudi utrujenost, anoreksija, dispneja, kašelj, okužba, navzeja, bruhanje, stomatitis, bolečina v trebuhu, pruritus, suha koža, suhi keratokonjunktivitis, konjunktivitis, zmanjšanje telesne mase, depresija, glavobol, nevropatija, dispepsija, flatulenca, alopecija, okorelost, pireksija. *Pogosti neželeni učinki* so gastrointestinalne krvavitve, krvavitev iz nosu, nenormalnosti testov jetrne funkcije, keratitis, zanohtnica. *Redko* so poročali o jetrni odpovedi. *Občasno* pa o perforacijah v prebavilih, poraščenosti moškega tipa pri ženskah, spremembah obrvi, krhkih nohtih, odstopanju nohtov od kože, blagih reakcijah na koži (npr. hiperpigmentacija), spremembah trepalnic, resni intersticijski pljučni bolezni, vključno s smrtnimi primeri. *Zelo redko* so poročali o primerih, ki so nakazovali na Stevens-Johnsonov sindrom/toksično epidermalno nekrolizo in so bili v nekaterih primerih smrtni, ter o ulceracijah in perforacijah roženice.

Režim izdaje zdravila: H/Rp.

Imetnik dovoljenja za promet: Roche Registration Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, AL7 1TW, Velika Britanija.

Verzija: 1.0/10.

Informacija pripravljena: maj 2010.

033-10-TAR

DODATNE INFORMACIJE SO NA VOLJO PRI:
Roche farmacevtska družba d.o.o.
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Povzetek glavnih značilnosti zdravila
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ČAS ZA ŽIVLJENJE.

DOKAZANO PODALJŠA PREŽIVETJE PRI BOLNIKIH:

- z lokalno napredovalim ali metastatskim nedrobnoceličnim rakom pljuč[1]
- z metastatskim rakom trebušne slinavke[1]

[1] Povzetek glavnih značilnosti zdravila TARCEVA, www.ema.europa.eu

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SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA Epufen 12,5, 25, 50, 100 in 150 mikrogramov/uro transdermalni obliži SESTAVA: 1 transdermalni obliž vsebuje 2,89 mg, 5,78 mg 11,56 mg, 23,12 mg ali 34,65 mg fentanila. **TERAPEVTSKE INDIKACIJE:** Huda kronična bolečina, ki se lahko ustrezno zdravi le z opioidnimi analgetiki. **ODMERJANJE IN NAČIN UPORABE:** Odmerjanje je treba individualno prilagoditi ter ga po vsaki uporabi redno oceniti. Izbira začetnega odmerka: velikost odmerka fentanila je odvisna od predhodne uporabe opioidov, kjer se upošteva možnost pojava tolerance, sočasnega zdravljenja, bolnikovega splošnega zdravstvenega stanja in stopnje resnosti obolenja. Pri bolnikih, ki pred tem niso dobivali močnih opioidov, začetni odmerek ne sme preseči 12,5-25 mikrogramov na uro. Zamenjava opioidnega zdravljenja: pri zamenjavi peroralnih ali parenteralnih opioidov s fentanilom je treba začetni odmerek izračunati na osnovi količine analgetika, ki je bila potrebna v zadnjih 24 urah, jo pretvoriti v odgovarjajoči odmerek morfina s pomočjo razpredelnice in nato preračunati ustrezen odmerek fentanila, spet s pomočjo razpredelnice (glejte SmPC). Prvih 12 ur po prehodu na transdermalni obliž Epufen bolnik še vedno dobiva predhodni analgetik v enakem odmerku kot prej; v naslednjih 12 urah se ta analgetik daje po potrebi. Titracija odmerka in vzdrževalno zdravljenje: obliž je treba zamenjati vsakih 72 ur. Odmerek je treba titrirati individualno, dokler ni dosežen analgetični učinek. Odmerek 12,5 mikrogramov/uro je primeren za titriranje odmerka v manjšem odmernem območju. Če analgezija na koncu začetnega obdobja nošenja obliža ni zadostna, se lahko odmerek po 3 dneh zveča. Možno je, da bodo bolniki potrebovali občasne dodatne odmerke kratko delujočih analgetikov (npr. morfina) za prekinitev bolečine. Sprememba ali prekinitev zdravljenja: vsaka zamenjava z drugim opioidom mora potekati postopoma, z majhnim začetnim odmerkom in počasnim zvečevanjem. Splošno veljavno pravilo je postopna ustavitev opioidne analgezije, da bi preprečili odtegnitvene simptome, kot so navzeja, bruhanje, diareja, anksioznost in mišični tremor. Uporaba pri starejših bolnikih: starejše in oslabljene bolnike je treba skrbno opazovati zaradi simptomov prevelikega odmerjanja ter odmerek po potrebi zmanjšati. Uporaba pri otrocih: transdermalni obliži Epufen se lahko uporabljajo le pri pediatričnih bolnikih (starih od 2 do 16 let), ki tolerirajo opioide in peroralno že dobivajo opioide v odmerku, enakovrednemu najmanj 30 mg morfina na dan. Bolnik mora prvih 12 ur po prehodu na Epufen še vedno dobivati predhodni analgetik v enakem odmerku kot prej. V naslednjih 12 urah je treba ta analgetik dajati odvisno od kliničnih potreb. Titracija odmerka in vzdrževalno zdravljenje: če je analgetični učinek Epufena prešibak, je treba bolniku dodati morfin ali drugi opioid s kratkim delovanjem. Odvisno od dodatnih potreb po analgeziji in jakosti bolečine pri otroku se lahko uporabi več obližev. Odmerek je treba prilagajati korakoma, po 12,5 mikrogramov/uro. Uporaba pri bolnikih z jetrno ali ledvično okvaro: Zaradi možnosti pojava simptomov prevelikega odmerjanja je treba te bolnike skrbno spremljati in odmerek ustrezno zmanjšati. Uporaba pri bolnikih s povečano telesno temperaturo: Pri teh bolnikih bo morda treba prilagoditi odmerek. **Način uporabe:** transdermalni obliž Epufen je treba takoj po odprtju vrečke nalepiti na nerazdraženo, neobsevano kožo, na ravno površino prsnega koša, zgornjega dela hrbta ali nadlakti. Po odstranitvi zaščitne plasti je treba obliž trdno pritrditi na izbrano mesto in z dlanjo pritiskati približno 30 sekund, da se obliž popolnoma nalepi, še zlasti na robovih. Uporaba pri otrocih: pri mlajših otrocih je obliž priporočljivo nalepiti na zgornji del hrbta, ker je manjša verjetnost, da bi otrok odstranil obliž. Transdermalnega obliža se ne sme deliti, ker podatkov o tem ni na voljo. **KONTRAINDIKACIJE:** Preobčutljivost za zdravilno učinkovino, hidrogenirano kolofonijo, sojo, arašide ali katerokoli pomožno snov. Akutna ali pooperativna bolečina, ko v kratkem časovnem obdobju ni možno titriranje odmerka in obstaja verjetnost za življenjsko ogrožajočo respiratorno depresijo. Huda okvara osrednjega živčnega sistema. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** Zaradi razpolovne dobe fentanila je treba bolnika v primeru pojava neželenega učinka opazovati še 24 ur po odstranitvi obliža. Pri nekaterih bolnikih, ki uporabljajo transdermalni obliž Epufen, se lahko pojavi respiratorna depresija. Epufen je treba previdno dajati: bolnikom s kronično pljučno boleznijo, zvišanim intrakranialnim tlakom, možganskim tumorjem, boleznimi srca, jeter in ledvic, tistim z zvišano telesno temperaturo, pri starejših bolnikih in otrocih, bolnikih z miastenijo gravis. Odvisnost od zdravila: kot posledica ponavljajoče se uporabe se lahko razvijeta toleranca na učinkovino ter psihična in/ali fizična odvisnost od nje. Ostali: lahko se pojavijo neepileptične (mio)klonične reakcije. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** Derivati barbiturne kisline, opioidi, anksiolitiki in pomirjevala, hipnotiki, splošni anestetiki, fenotiazini, mišični relaksanti, sedativni antihistaminiki in alkoholne pijače, zaviralci MAO, itrakonazol, ritonavir, ketokonazol, nekateri makrolidni antibiotiki, pentazocin, buprenorfin. **VPLIV NA SPOSOBNOST VOŽNJE IN UPRAVLJANJA S STROJI:** Zdravilo ima močan vpliv na sposobnost vožnje in upravljanja s stroji. **NEŽELENI UČINKI:** Najbolj resen neželen učinek fentanila je respiratorna depresija. Zelo pogosti (≥ 1/10): dremavost, glavobol, navzeja, bruhanje, zaprtje, znojenje, srbenje, somnolenca. Pogosti (≥ 1/100 do < 1/10): kserostomija, dispepsija, reakcije na koži na mestu aplikacije, sedacija, zmedenost, depresija, tesnoba, živčna napetost, halucinacije, zmanjšan apetit. Občasni (≥ 1/1000 do < 1/100): tahikardija, bradikardija, tremor, parestezija, motnje govora, dispneja, hipoventilacija, diareja, zastajanje urina, izpuščaj, rdečina, hipertenzija, hipotenzija, evforija, amnezija, nespečnost, vznemirljivost. Nekateri od naštetih neželenih učinkov so lahko posledica osnovne bolezni ali drugih zdravljenj. Drugi neželeni učinki: odpornost, fizična in psihična odvisnost se lahko razvijejo med dolgotrajno uporabo fentanila. Pri nekaterih bolnikih se lahko pojavijo odtegnitveni simptomi, ko zamenjajo prejšnje opiodne analgetike s transdermalnim obližem s fentanilom ali po nenadni prekinitvi zdravljenja. **NAČIN IZDAJE:** Samo na zdravniški recept. **OPREMA:** Škatle s 5 transdermalnimi obliži. **IMETNIK DOVOLJENJA ZA PROMET:** Lek farmacevtska družba, d.d., Verovškova 57, Ljubljana, Slovenija **INFORMACIJA PRIPRAVLJENA:** avgust 2009

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Korekcijska faza: 50 i.e./kg 3 x tedensko. Odmerek prilagajamo postopno, z vsaj štiritedenskimi časovnimi presledki za 25 i.e./kg 3 x tedensko. Vzdrževalna faza: priporočen skupni tedenski odmerek je od 75 do 300 i.e./kg. ***Odrasli bolniki z zmanjšanim ledvičnim delovanjem, ki se še ne zdravijo z dializo:*** začetni odmerek je 50 i.e./kg s.c. 3 x tedensko. Odmerek prilagajamo postopno, z vsaj štiritedenskimi časovnimi presledki za 25 i.e./kg 3 x tedensko. Vzdrževalni odmerek je od 17 do 33 i.e./kg 3 x tedensko, največji tedenski odmerek ne sme presegati 200 i.e./kg 3 x tedensko. ***Odrasli bolniki na peritonealni dializi:*** Korekcijska faza: 50 i.e./kg s.c. 2 x tedensko.Vzdrževalni odmerek je od 25 do 50 i.e./kg 2 x tedensko. ***Odrasli bolniki z rakom s simptomatsko anemijo, ki se zdravijo s kemoterapijo:*** Bolnike z anemijo zdravimo do ciljne koncentracije Hb 100-120 g/l, Hb pa ne sme preseči 120 g/l. Začetni odmerek je 150 i.e./kg s.c. 3 x tedensko ali 450 i.e./kg s.c. 1 x tedensko. ***Odrasli kirurški bolniki, vključeni v program avtologlnega zbiranja krvi za avtotransfuzijo:*** 600 i.e./kg i.v., 2-krat na teden v obdobju treh tednov pred kirurškim posegom. Odrasli kirurški bolniki, ki niso vključeni v program avtologlnega zbiranja krvi za avtotransfuzijo: 600 i.e./kg, s.c., enkrat tedensko v obdobju treh tednov pred kirurškim posegom in na dan kirurškega posega. **Kontraindikacije:** čista aplazija rdečih krvnih celic (PRCA), nenadzorovana arterijska hipertenzija, kontraindikacije povezane s programom avtologlnega zbiranja krvi, preobčutljivost za katerokoli sestavino zdravila, bolniki, pri katerih je predviden večji elektiven kirurški poseg in niso vključeni v program avtologlnega zbiranja krvi s hudo koronarno, cerebrovaskularno, karotidno ali periferno arterijsko bolezen ali so nedavno preboleli miokardni infarkt ali cerebrovaskularni dogodek, bolniki, ki ne morejo prejemati ustrezne antitrombotične profilakse. **Posebna opozorila in previdnostni ukrepi:** Med zdravljenjem moramo spremljati in nadzorovati krvni tlak, če ga ne moremo urediti, moramo zdravljenje prekiniti. Potrebna je previdna uporaba zdravila pri bolnikih z epilepsijo in kronično boleznijo jeter. Prvih osem tednov zdravljenja priporočamo redno spremljanje števila trombocitov. Za optimalen odgovor na zdravljenje, je treba zagotoviti ustrezne zaloge železa. Po več mesecih ali letih zdravljenja s subkutano apliciranim zdravilom so redko poročali o PRCA, povzročeni s protitelesi. Če sumimo PRCA moramo zdravljenje takoj prekiniti. Zaradi verjetnosti navzkrižne reakcije s protitelesi, bolniku ne smemo dati drugega epoetina in mu moramo zagotoviti ustrezno zdravljenje. Pri ocenjevanju ustreznosti odmerka pri bolnikih z rakom, ki prejemajo kemoterapijo, moramo upoštevati, da minejo 2-3 tedni od začetka zdravljenja do pojava eritrocitov, nastalih pod njegovim vplivom v krvi. Kot pri vseh rastnih faktorjih obstaja verjetnost, da bi lahko spodbujali razvoj katere koli vrste rakave bolezni. Pri bolnikih, pri katerih je predviden večji elektivni ortopedski kirurški poseg, je treba ugotoviti vzrok za anemijo in ga odpraviti pred začetkom zdravljenja. Pri bolnikih s kroničnim ledvičnim odpovedovanjem je potrebna previdnost. **Interakcije:** Ni dokazov, da zdravljenje z epoetinom alfa vpliva na metabolizem drugih zdravil. Ker se ciklosporin veže na eritrocite, obstaja možnost interakcije med zdraviloma. **Neželeni učinki:** trombocitemija, PRCA, anafilaktična reakcija, hipersenzitivnost, krči, glavobol, cerebralna krvavitev, cerebrovaskularni dogodki, hipertenzivna encefalopatija, tranzitorna ishemična ataka, hipertenzija, tromboze, pljučna embolija, navzea, diareja, bruhanje, izpuščaj, angionevrotični edem, urtikarija, artralgija, mialgija, porfirija, pireksija, gripi podobni simptomi, neučinkovitost zdravila, periferni edem, reakcija na mestu injiciranja, tromboza žilnega pristopa. **Imetnik dovoljenja za promet:** Johnson & Johnson d.o.o. Šmartinska 53, 1000 Ljubljana **Režim izdajanja zdravila:** H/Rp. **Datum revizije:** 11. 12. 2009.

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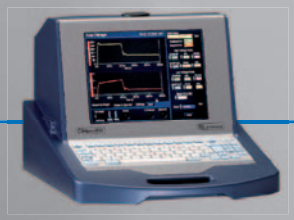

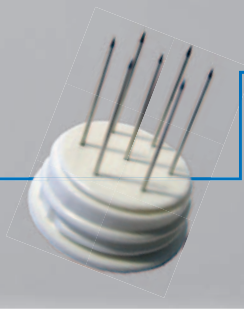

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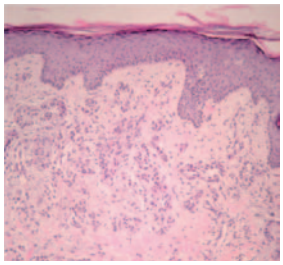

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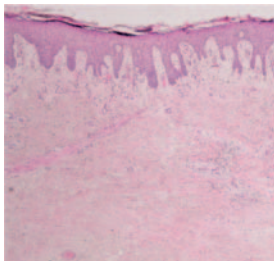

Quaglino P, Annals Of Surgical Oncology. 15 (8): 2215-2222. 2008

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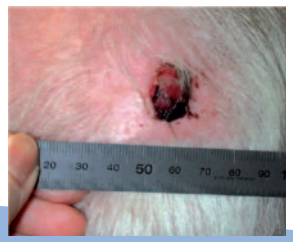

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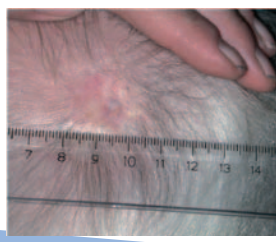

Gehl J, EJC Supplements, Volume 4, N° 11: 35-37, 2006

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Introduction should state the purpose of the article and summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Materials and methods should provide enough information to enable experiments to be repeated. New methods should be described in detail. Reports on human and animal subjects should include a statement that ethical approval of the study was obtained.

Results should be presented clearly and concisely without repeating the data in the figures and tables. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

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Dent RAG, Cole P. *In vitro* maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981; **43:** 486-95.

Chapman S, Nakielny R. *A guide to radiological procedures.* London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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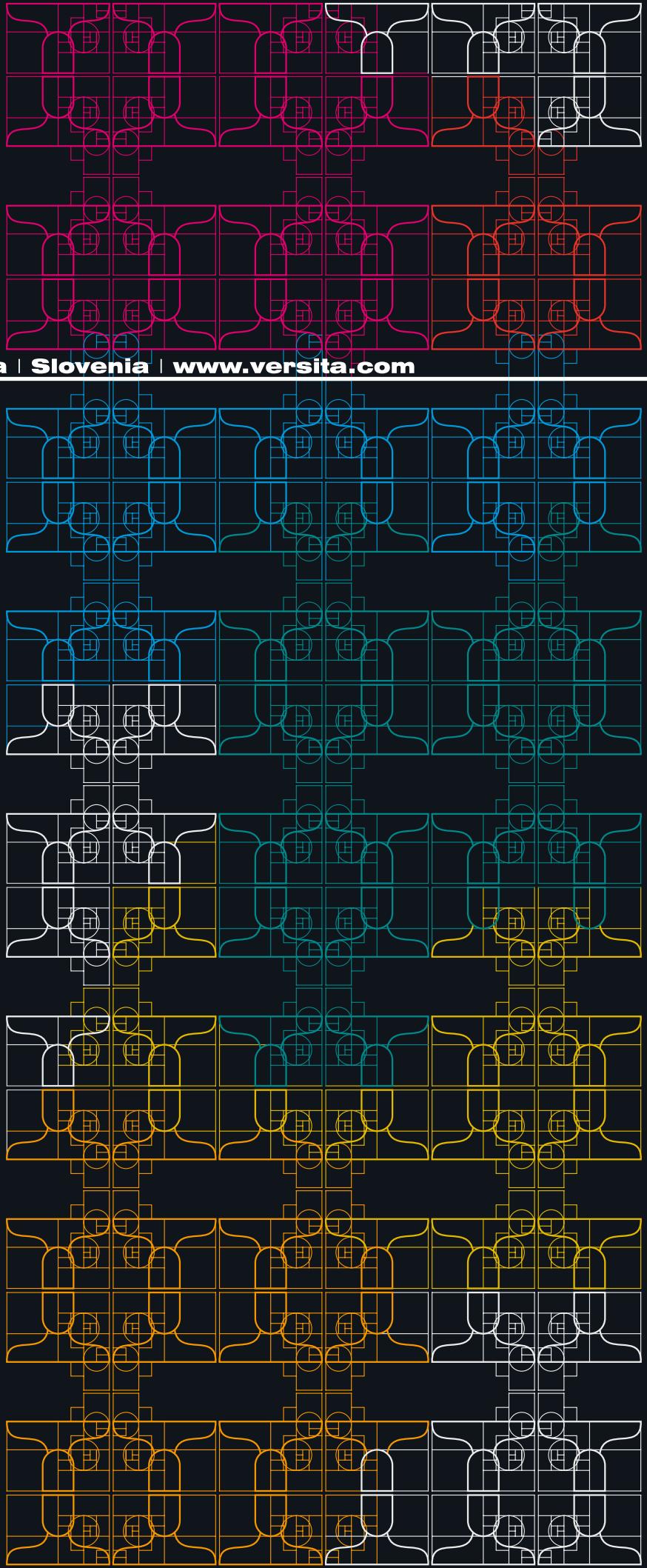

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