CASE REPORT
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The role of c-MYC expression in the diagnostic and clinical confirmation of radiation-induced angiosarcoma

Uloga ekspresije c-MYC u dijagnozi i kliničkoj potvrdi angiosarkoma izazvanog zračenjem

Luka Jovanović*†, Predrag Filipović*†, Jelena Dedović Stojaković*†, Zorka Milovanović‡, Milica Labudović Borović†, Jasminka Tanasijević§, Predrag Petrašinović*†, Tamara Marinković*†, Vesna Plešinac Karapandžić*†

Institute for Oncology and Radiology of Serbia, *Department of Radiation Oncology, ‡Department of Pathology, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; §General Hospital Studenica, Kraljevo, Serbia

Abstract

Introduction. Angiosarcomas (ASs) arising from vascular tissue, account for 3.3% of all sarcomas and have a poor prognosis. Radiation-induced AS is a rare late complication of radiotherapy (RT) treatment and is characterized by a gene expression profile such as amplification of the MYC oncogene, by which we can distinguish primary from secondary induced tumor. **Case report.** For a 77-year-old female patient with early-stage endometrial adenocarcinoma, a radical hysterectomy with bilateral salpingo-oophorectomy was initially done. According to pathological risk factors, the postoperative external beam conformal RT (CRT) of the pelvis was administered with concomitant brachytherapy. Six years after the treatment, on the anterior abdominal wall, in the region of the postoperative irradiation field and surgical scar, an infiltrative AS of the skin and subcutaneous adipose tissue was histologically confirmed. The patient received six cycles of mono-adriamycin chemotherapy with verified partial regression. Additional immunohistochemical analysis (IHC) of c-MYC, Ki67, and CD34 expression showed a high proliferative index (Ki67 around 60%) and c-MYC positivity indicating the molecular pattern of radiation-induced AS. Furthermore, the high proliferative index could explain the positive response to chemotherapy. **Conclusion.** The novel postoperative RT techniques provide better survival and local control in risk-endometrial cancer groups with a decrease in irradiation complications. These patients with longer survival are at a higher risk of developing radiation-induced tumors as late side-effects of RT. When assessing the probability of radiation-induced AS, IHC analysis of c-MYC expression could distinguish secondary from other AS if Cahan's criteria are fulfilled.

Key words:
adenocarcinoma; genetics; immunohistochemistry; neoplasms, radiation-induced; radiotherapy, adjuvant; risk assessment; sarcoma; survival.

Apstrakt

Uvod. Angiosarkomi (AS) koji nastaju iz vaskularnog tkiva, čine 3,3% svih sarkoma i imaju lošu prognozu. Zračenjem indukovani AS su retka kasna komplikacija lečenja radioterapijom (RT), a karakterišu se posebnim profilom genske ekspresije, poput amplifikacije c-MYC onkogena, pomoću kojeg možemo razlikovati primarni od sekundarno indukovanog tumora. **Prikaz bolesnika.** Bolesnici staroj 77 godina, sa adenokarcinomom endometrijuma u ranom stadijumu, inicijalno je urađena radikalna histerektomija sa bilateralnom adneksektomijom. Prema patohistološkim faktorima rizika, indikovana je postoperativna konformalna RT (CRT) karlice, uz brahiterapiju. Šest godina nakon tretmana na prednjem trbušnom zidu, u regiji postoperativnog ožiljka i zračnog polja, histološki je potvrđen infiltrativni AS kože i potkožnog masnog tkiva. Bolesnica je primila šest ciklusa hemioterapije mono-adriamicinom, sa verifikovanom delimičnom regresijom. Dodatna imunohistohemijska analiza (IHH) ekspresije c-MYC, Ki67 i CD34 pokazala je visok proliferativni indeks (Ki67 oko 60%) i pozitivnost c-MYC što je ukazivalo na molekulski obrazac AS izazvanog zračenjem. Visoki proliferativni indeks mogao je objasniti dobar odgovor na hemioterapiju. **Zaključak.** Nove tehnike RT omogućavaju bolje preživljavanje i lokalnu kontrolu bolesnica sa karcinomom endometrijuma visokog rizika. Međutim, produženo preživljavanje tih bolesnica stavlja ih u povišen rizik od razvoja tumora izazvanih radijacijom kao i drugih kasnih efekata RT.

Correspondence to: Luka Jovanović, Institute for Oncology and Radiology of Serbia, Department of Radiation Oncology, Pasterova 14, 11 000 Belgrade, Serbia. E-mail: lukabgd01137@gmail.com

Ukoliko su ispunjeni Cahan-ovi kriterijumi, dodatne IHH analize ekspresije c-MYC mogu pomoći u razlikovanju sekundarnih od ostalih AS, kada se procenjuje mogućnost pojave radijaciom indukovanog AS.

Ključne reči:
adenokarcinom; genetika; imunohistohemija; neoplazme, radijacijom uzrokovane; radioterapija, adjuvantna; rizik, procena; sarkomi; preživljavanje.

Introduction

Angiosarcoma (AS) is a rarely occurring malignancy that arises from vascular tissue and has a poor prognosis. The incidence of all soft tissue sarcomas in Europe ranges from 3.3 per 100,000 in Eastern Europe to 4.7 per 100,000 in Northern Europe, and it is reported that 3.3% of all sarcomas are ASs [1]. By site, ASs are divided as follows: soft tissue AS, bone AS, and cutaneous AS. Furthermore, cutaneous ASs are divided as scalp and face AS, AS in the contest of lymphedema (Stewart-Treves syndrome), epithelioid AS, and radiation-induced AS [2].

AS is characterized by diverse but recurrent chromosomal abnormalities and mutations of genes involved in angiogenesis and endothelial cell receptors [3]. The patterns of mutation are so distinct that they could distinguish secondary, mostly radiation-induced ASs from primary tumors [4].

The first set of criteria used for the diagnosis of radiation-induced malignancy (RIM) was established by Cahan et al. [5] in 1948. Today, modified Cahan's [5] criteria are used to encompass the following: RIM must arise within the boundaries of the irradiation field; duration of the latent period between proposed induced malignancy and previous irradiation must be greater than 4 years; primary malignancy and induced malignancy must be biopsied and of different histology; the tissue that arises from the induced malignancy must be metabolically and genetically normal before irradiation.

From a molecular point of view, around 100 genes are deregulated during secondary AS development, including upregulation of MYC, KIT, and RET genes, as well as concomitant upregulation of MYC and FLT4 and downregulation of CDKN2C gene. Similar genetic patterns are present in other radiation-induced tumors, suggesting the distinct tumorigenic mechanism of radiation [6].

In our study, we analyzed the clinical problem of distinguishing the primary from secondary-radiation induced AS by immunohistochemical (IHC) analysis of c-MYC and other markers expression in the case of a patient with endometrial cancer treated with adjuvant radiotherapy.

Case report

The patient was 77 years old with no prior or family history of malignancy. Initial staging workups revealed endometrial adenocarcinoma of stage IC according to the Union International Cancer Control (UICC) and International Federation of Gynecology and Obstetrics (FIGO). According to the protocol, the patient underwent radical hysterectomy with bilateral salpingo-oophorectomy in November 2011 at the Military Medical Academy (MMA) in Belgrade. Histopathology (HP) findings confirmed endometrial adenocarcinoma FIGO IC (pT1c), infiltrating the uterine wall to a depth of more than 1/2 myometrium.

According to the multidisciplinary board decision, adjuvant conformal external beam RT (CRT) of the pelvis was administered. A total dose of 45 Gy in 25 fractions was given (5 days *per* week) at the MMA Radiotherapy Department, while three applications of intracavitary brachytherapy were performed at the Institute for Oncology and Radiology of Serbia in the period from January to March 2013. Treatment-related early toxicity during RT treatment included diarrheas and tenesmus, which were successfully medically treated.

In March 2019, a patient underwent surgical excision of clinically suspicious multiple cutaneous tumors in the region of the surgery scar and irradiated area of the anterior abdominal wall in the General Hospital in Kraljevo. Initial HP findings were characterized as mesenchymal malignancy. Further IHC analysis revealed infiltrative AS of skin and subcutaneous fat tissue with R1 posterior resection margin.

Further diagnostic workup, which included magnetic resonance imaging (MRI) of the abdomen, detected a soft tissue tumor of the anterior abdominal wall with no further spreading. The patient underwent six cycles of systemic chemotherapy, including mono-adriamycin (75 mg/m^2), with a good partial response (Figure 1).

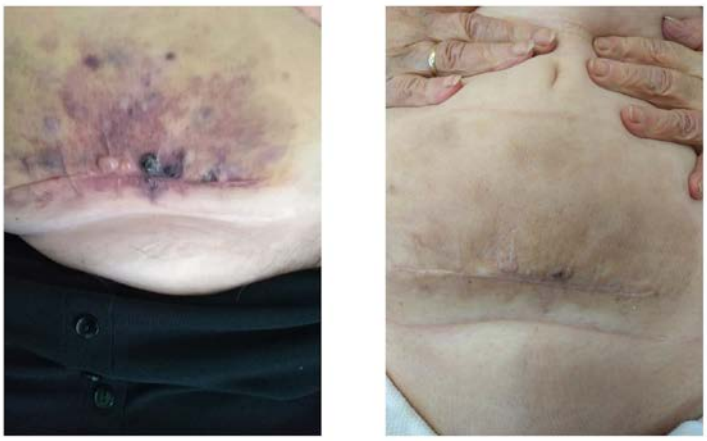


Fig. 1 – Clinical presentation of angiosarcoma in our patient: initially, before chemotherapy with mono-ADM (left) and after chemotherapy (right).

Specimen of skin measuring 105 mm × 40 mm × 30 mm was sent. On the cross-section, we revealed fields of hemorrhage in subcutis.

Sections of 4 μm thickness were sampled. Standard histological analysis was performed using standard hematoxylin and eosin staining. Sections revealed a tumor in the deep portion of the dermis and fat tissue. The tumor consisted of malignant fusiform cells arranged in pseudovascular channels. Neoplastic cells had hyperchromatic *nuclei* and numerous mitotic figures.

The same blocks of tissue, previously prepared for classical pathohistology, were used for IHC analysis.

We performed IHC staining for CD34 (Ventana, RTU, clone QBEnd/10), Ki67 (Ventana, RTU, clone 30-9, RTU), and c-MYC (clone 9E10.3).

Staining for CD34 and Ki67 was performed in immunostainer BenchMark GX, Ventana. C-MYC was stained manually. For c-MYC identification, the demasking procedure was used as the first step in the IHC procedure. For antigen unmasking, a 10 mM citrate buffer (pH6) was used for 21 min in a microwave oven at the maximum power of 800 W. The sections were then washed with TBS [TRIS (hydroxymethyl) aminomethane buffer saline] and incubated with the primary c-MYC monoclonal antibody (Thermo Fisher Scientific Invitrogen, MA5-12080, clone 9E10.3) diluted at 1 : 50 ratio. The sections were treated using the commercial Thermo Scientific UltraVision Quanto Detection System HRP DAB (TL-060-QHD). Immunoreactions were subsequently developed using DAB (diaminobenzidine) as a chromogen. The sections were counterstained with Mayer's hematoxylin.

The quality and the specificity of the developed immunoreactions were controlled by negative controls performed by omitting the primary antibody and applying TBS instead.

CD34 immunoreactivity showed the vascular origin of the tumor. *Nuclei* of malignant cells had a high proliferative index (Ki67 around 60%). C-MYC protein expression was shown in numerous positive tumor cells (Figure 2).

Discussion

Secondary AS occurs as radiation-induced but also in patients with chronic lymphoedema due to prior lymphadenectomy (after breast surgery) or in patients that have chronically altered lymph drainage for other reasons (i.e., Stewart-Treves syndrome) [7, 8].

Radiation-induced secondary malignancies are rare but important late side effects of RT and have an impact on optimal treatment decision-making, especially with expected survival longer than 5 years.

The adverse effects (AEs) of radiation vary depending on the technique and dose applied, and they are generally divided into early and late AEs. Early toxic effects of radiation on healthy tissue are due to acute inflammation (radiation co-

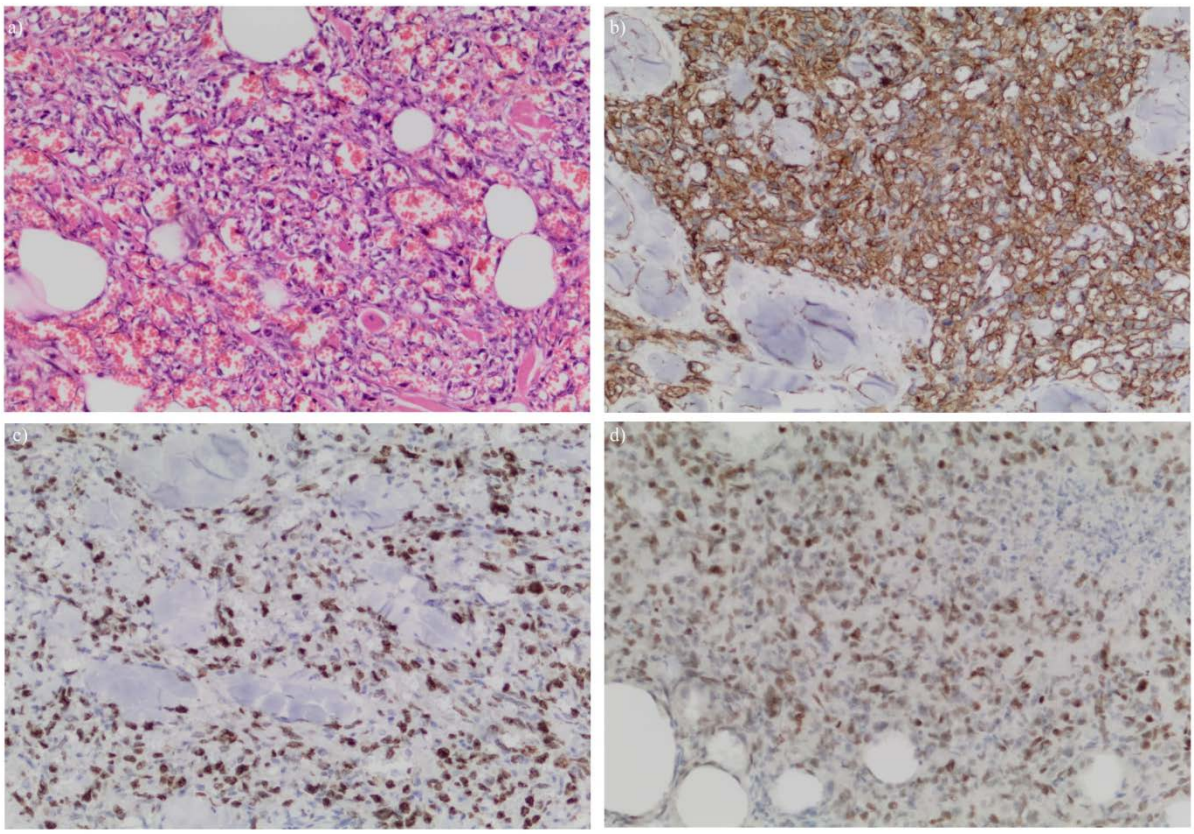


Fig. 2 – Pathohistology (PH) and immunohistochemical (IHC) analysis:
a) neoplastic cells arranged in poorly formed vascular spaces [hematoxylin and eosin (HE), ×400];
b) CD34 immunostaining-positive tumor cells (×400); c) Ki67 immunostaining – high proliferative index (×400); d) c-MYC immunostaining-numerous positive tumor cells (×400).

litis, cystitis, radio-dermatitis, etc.). On the other hand, late AEs are caused by microvascular damage, chronic inflammation, and radiation-induced genetic instability. Whereas early AEs are reversible and have a good prognosis when treated medically, late effects are permanent and generally are less responsive to medications or lifestyle changes.

The development of contemporary RT techniques such as CRT (4 field box-technique) and intensity-modulated RT (IMRT) have improved target dose coverage and reduced early and late treatment toxicities [9]. Some studies, however, found no reduction of gastrointestinal and genitourinary toxicities of radiation when CRT is used and even suggested a positive correlation between the development of early and late toxicities in organs that receive a relatively high dose of radiation during the CRT treatment [10–12].

Nevertheless, contemporary radiation techniques (CRT and IMRT) made escalation of treatment dose possible, which increased the number of long-term cancer survivors at increased risk of developing late AEs of RT, including RIM [13].

Endometrial carcinoma is one of the most common gynecologic malignancies worldwide, with standard treatment protocol in early operable stages, which includes radical hysterectomy followed by adjuvant RT if postoperative histology assesses risk factors for local recurrence (high-grade carcinomas or deep myometrial and cervical stroma invasion) [14, 15]. Adjuvant RT provides a significant improvement in local control and disease-free survival after 5 years of 90% for intermediate-risk patients and around 80% for high-risk patients (high-grade tumors or myometrial invasion) [16]. In our patient, the adjuvant RT was performed due to a microscopic invasion of more than half of the myometrium to achieve better local control (according to hospital protocol at the period).

Modern CRT allows more precise irradiation of a targeted volume, with a sparing effect on normal tissue. However, with these techniques, a larger volume of normal tissue is irradiated with a lower dose. RIM arises mainly in the irradiated tissue or the nearby tissues due to collateral radiation exposure [17, 18]. A large cohort study by Chaturvedi et al. [19] has shown that after radiation treatment of gynecological malignancies (external beam RT and brachytherapy), there is a secondary malignancy incidence increase of 12% compared with the cohort that did not receive radiation treatment. RIM was detected with a median follow-up of 12.2 years. The most common RIM observed were anal, colorectal, and gynecological malignancies [19]. Concerning endometrial carcinoma treatment, (PORTEC)-1 trial showed that 22% of the patients that received RT developed secondary neoplasm after 15 years, while 16% of non-irradiated patients developed secondary neoplasms [20].

In several clinical series, comparing c-MYC gene amplification and expression between secondary AS of the skin and primary AS showed that c-MYC expression is statistically significantly more prevalent in secondary AS [21, 22]. Moreover, a c-MYC expression is present in secondary AS associated with chronic lymphedema (Stewart-Treves sy) [23]. Nevertheless, in a minority of primary skin ASs, the c-MYC expression could be found too, as well as in primary AS of other sites [24].

The study by Styring et al. [6] underlined the role of MYC, KIT, and RET genes upregulation in the pathogenesis of radiation-induced AS and its diagnostic application as the basis for the therapeutic use of kinase inhibitors in these sarcomas. This study also found that over 100 genes are significantly deregulated between primary and secondary ASs, for example, the upregulation of FLT4, a tyrosine kinase receptor for vascular endothelial growth factor (VEGF), and other vascular-specific receptor tyrosine kinases such as TIE1, KDR, and FLT1. This somatic mutation pattern could be of diagnostic importance since it is common in radiation-induced sarcomas and other RIM.

In the end, it should be noted that the overall prognosis of radiation-induced AS is poor [25]. Although surgical treatment is the therapy of choice in other sarcomas, it seems that re-occurrence of the AS, including radiation-induced ones, is high, even when radical excision is made [25]. This could be explained by the multifocality of the tumor, so a truly negative resection margin is hard to be achieved [26]. Hematogenic spread in the lungs, pleura, and bone, as well as spread to regional lymph nodes, is possible [27, 28]. At the time, doxorubicin-based chemotherapy remained the standard treatment for metastatic or unresectable AS, but other chemotherapeutic agents such as taxanes showed activity against AS [29]. Overall response rates to chemotherapy are variable from 20% to 60% [30]. In our patient, a high proliferative index (Ki67 around 60%) and c-MYC positivity could explain a positive response to chemotherapy.

Target therapy could be a potential new approach in the treatment of radiation-induced AS, such as tyrosine kinase inhibitors sorafenib, brivanib, and sirolimus, as well as KIT inhibitor imatinib, anti-VEGF antibody bevacizumab, and thalidomide but the experience is still limited, and further studies are necessary [26].

Conclusion

The development of novel RT techniques provided longer survival and better local control of patients with high-risk endometrial cancer. Longer survival of these patients put them at a higher risk of developing RIM and other late side-effects of RT. When assessing the probability of radiation-induced AS, IHH finding of c-MYC expression could help distinguish secondary from other ASs if Cahan‘s criteria are fulfilled. Additional IHH analysis of other molecular markers such as KIT or RET kinases and VEGF expression could be of diagnostic and therapeutic importance in the era of target therapy in oncology.

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