Conceição Maria Fraga Guedes

ESTUDO CLÍNICO-PATOLÓGICO, IMUNO-HISTOQUÍMICO E MOLECULAR (AMPLIFICAÇÃO DO GENE *MYC*) EM ANGIOSSARCOMAS E LESÕES VASCULARES ATÍPICAS DA MAMA

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À minha família, presente de Deus

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“Nem olhos viram, nem ouvidos ouviram, nem jamais penetrou em coração humano o que Deus tem preparado para aqueles que o amam”.

Bíblia Sagrada, 1Cor 2:9

RESUMO

Introdução: Angiossarcomas (AS) primários (ASP) e secundários (ASS) da mama são raros, agressivos, com alta morbi-mortalidade. Lesões vasculares atípicas (LVA) são proliferações vasculares que se desenvolvem no sítio prévio de irradiação, em geral, com evolução benigna. AS de baixo grau e LVA compartilham semelhanças morfológicas, porém sem critérios específicos para distinção inequívoca entre estas lesões. A amplificação e expressão do *MYC* têm sido indicadas como uma ferramenta diagnóstica na distinção das lesões vasculares da mama induzidas por radiação.

Objetivos: Os objetivos do presente estudo foram avaliar as características clinicopatológicas, imuno-histoquímicas e evolutivas de uma série de casos de LVA da mama; investigar a amplificação e superexpressão do *MYC* em uma série de LVA e AS da mama; verificar o papel diagnóstico e se o *MYC* está envolvido na patogênese e prognóstico de LVA e AS.

Material e método: Foram identificados 30 casos de LVA e 49 de AS da mama diagnosticados entre 1999 a 2014. Fez-se estudo imuno-histoquímico (IHQ) para CD31, D2-40, CD105 e Ki-67 em todos os casos de LVA. As LVA foram classificadas em tipo-linfático, LVA-TL (D2-40 positivo), ou tipo vascular, LVA-TV (D2-40 negativo). Analisamos a amplificação do gene *MYC* e sua expressão proteica pelas técnicas de hibridização *in situ* por fluorescência (FISH) e IHQ, respectivamente, em LVA, ASP e ASS da mama. Dados sobre a evolução clínica das pacientes foram coletados e realizadas análises de sobrevida, correlacionando-as com a amplificação do *MYC*.

Resultados: Vinte e dois casos de LVA foram classificados com LVA-TL, seis casos como LVA-TV, e o D2-40 não foi testado em 2 casos. Três casos com margens cirúrgicas positivas para LVA evoluíram desfavoravelmente: um caso apresentou recidiva local de LVA 32 meses após o diagnóstico inicial, e outras duas pacientes, uma como LVA-TL e a outra como LVA-TV, progrediram para AS de alto-grau, 19 e 89 meses depois, respectivamente. Quarenta e nove pacientes tiveram o diagnóstico de AS da mama. Trinta e sete pacientes tinham ASS e 12 pacientes tinham ASP. Vinte de 37 casos de ASS (54%) mostraram amplificação do *MYC*. Não foi observada amplificação do *MYC* em nenhum caso de LVA ou de ASP. A concordância entre os resultados do FISH e IHQ das LVA, ASP e ASS foi de 100%. Análises de sobrevida mostraram que ASS com *MYC* amplificado apresentaram pior sobrevida global quando comparados com casos sem amplificação (P=0,035). Foi identificada tendência não-significativa a pior sobrevida livre de doença nos casos de ASS com amplificação do *MYC* em relação aos casos sem amplificação (P=0,155).

Conclusões: Não identificamos nenhum marcador imuno-histoquímico específico para a distinção entre LVA e AS de baixo-grau. O subtipo de LVA, baseado na expressão do D2-40, não teve papel discriminador na evolução das LVA, pois observamos o mesmo risco de

progressão para AS nos dois tipos de LVA. Margens comprometidas associaram-se a evolução clínica desfavorável. Recomendamos excisão completa das LVA e seguimento clínico periódico, até que a história natural destas lesões seja esclarecida. A amplificação do *MYC* é um marcador de alta especificidade e baixa sensibilidade para ASS e está associada a prognóstico desfavorável em ASS. As alterações genéticas e moleculares envolvidas na patogênese dos AS e LVA permanecem obscuras; novos estudos são necessários para melhor conhecimento da patogênese e evolução destas lesões.

Palavras-chave: angiossarcoma; câncer de mama; neoplasias induzidas por radiação; lesões vasculares atípicas; radioterapia; proliferações vasculares da mama; anormalidades induzidas por radiação; sobrevida livre de doença; prognóstico.

ABSTRACT

Introduction: Angiosarcomas (AS) of the breast, primary (PAS) or secondary (SAS) to radiotherapy, are rare and aggressive, with significant risk of morbidity and mortality. Atypical vascular lesions (AVL) are vascular proliferations that develop within previously irradiated skin usually with a benign clinical course. Low-grade AS and AVL share morphological similarities, and, to date, there are no specific criteria to distinguish them. Recently, *MYC* amplification and overexpression have been pointed as an important diagnostic tool to distinguish post-radiation cutaneous vascular lesions of the breast.

Purpose: We evaluated clinicopathological, immunohistochemical, and evolutive characteristics of a series of AVL of the breast. We also investigated and further compared the presence of *MYC* amplification and overexpression in a series of AVL, PAS and SAS to verify whether *MYC* is a useful diagnostic tool, and if it is implicated in the pathogenesis and prognosis of radiation-induced vascular proliferations.

Methods: We selected 30 cases of AVL and 49 cases of AS of the breast diagnosed between 1999 and 2014. Immunohistochemical study for CD31, D2-40, CD105, and Ki-67 was performed in all AVL cases. AVL were classified based on D2-40 expression: lymphatic type, LT-AVL, (D2-40 positive) or vascular type, VT-AVL (D2-40 negative). We analyzed *MYC* amplification and protein expression by fluorescent *in situ* hybridization (FISH) and IHC in AVL, PAS and SAS. Follow-up data were collected, and survival analyses were performed, comparing them with MYC amplification.

Results: Twenty-two AVL were classified as LT, six cases were classified as VT, and D2-40 was not tested in 2 cases. In three cases when margin was compromised, the patients presented with unfavorable outcomes: one patient had local recurrence of AVL 32 months after the diagnosis of AVL, and the other two cases progressed to high-grade AS 19 and 89 months later: one case was LT-AVL, and the other was VT-AVL, respectively. Of the 49 patients diagnosed with breast AS, thirty-seven patients had SAS, and twelve patients had PAS. Between 37 patients with SAS, twenty cases showed high-level *MYC* amplification and protein overexpression (54%). None of PAS or AVL cases showed *MYC* amplification or protein expression. Concordance between *MYC* amplification (FISH) and protein expression (IHC) was 100% in AVL, PAS, and SAS. Survival analysis of the SAS patients demonstrates that cases with *MYC* amplification had a significantly worse overall survival compared with cases without MYC amplification (P=0.035). There was a non-significant trend toward a poor disease-free survival between cases with and without *MYC* amplification (P=0,155)

Conclusions: Our study could not identify a specific immunohistochemical marker to distinguish AVL from low-grade AS. We cast doubt on the importance of classifying AVL according to D2-40 expression, since we found the same risk for malignant progression on both types of AVL. Since compromised surgical margins were associated with unfavorable outcomes in AVL, we recommend complete excision and close follow-up of patients until the natural history of the AVL is better explained. *MYC* amplification is a highly specific but

poorly sensitive marker for SAS, and was associated with adverse prognosis. The genetic and molecular aberrations involved in AS and AVL tumorigenesis remain poorly understood, and further studies are necessary to better understand the pathogenesis and progression of these lesions.

Keywords:
angiosarcoma; breast; breast cancer; post-radiation angiosarcoma; atypical vascular lesions; radiotherapy; vascular proliferation; disease free survival; prognosis angiosarcoma; post-irradiation; MYC; cutaneous; FISH.

SUMÁRIO

1. INTRODUÇÃO

O suprimento vascular normal da mama, assim como das demais estruturas subcutâneas, compreende artérias, capilares, veias e vasos linfáticos, presentes no tecido adiposo, estroma fibrocolagenoso e estruturas nervosas dispersas pelo tecido. Proliferações vasculares da mama clinicamente evidentes são diagnósticos raros e constituem um amplo espectro de lesões que vão desde lesões benignas como hemangiomas e lesões vasculares atípicas, transitando por lesões como hemangiopericitomas, hemangioedoteliomas, de baixo potencial maligno, a angiossarcomas mamários de alta agressividade (BRODIE; PROVENZANO, 2008).

Angiossarcomas são tumores raros e muito agressivos de origem endotelial e respondem por 2-3% de todos os sarcomas de partes moles em adultos (PENEL et al., 2011; HUNG et al., 2013). Todavia, mais de 44% dos angiossarcomas ocorrem na mama (COZEN et al., 1999). Angiossarcomas mamários podem ser divididos em duas categorias maiores: primária e secundária (relacionada à radioterapia prévia ou linfedema crônico). A despeito de ambos compartilharem características histopatológicas semelhantes e apresentarem prognóstico reservado, há diferenças marcantes entre estas categorias no que tange ao comportamento tumoral, ao diagnóstico diferencial e dados epidemiológicos dos pacientes acometidos pela doença (LUINI et al., 2007; GLAZEBROOK; MAGUT; REYNOLDS, 2008; BISWAS et al., 2009; FRAGA-GUEDES et al., 2012).

Angiossarcomas primários geralmente acometem pacientes na pré-menopausa, em mulheres mais jovens do que nos angiossarcomas secundários (BRODIE; PROVENZANO, 2008; BISWAS et al., 2009; FRAGA-GUEDES et al., 2012). Quanto à apresentação clínica, os AS primários costumam apresentar-se como tumoração palpável, com poucas alterações cutâneas, como descoloração da pele em apenas 34% dos casos (CHEN; KIRKEGAARD; BOCIAN, 1980; PANDEY et al., 2004; SHET et al., 2006). Os tumores secundários se iniciam na pele/derme na maioria dos pacientes (LUCAS, 2009; THARIAT et al., 2012; HUNG et al., 2013), enquanto que os primários têm origem predominantemente parenquimatosa (RAO et al., 2003; BRENN; FLETCHER, 2005; TAHIR et al., 2006). Trabalho recente aponta que os angiossarcomas secundários costumam apresentar pior prognóstico e maiores taxas de recidiva local quando comparados aos tumores primários (FRAGA-GUEDES et al., 2012). Porém, do ponto de vista histopatológico, ainda não foram definidos marcadores biológicos específicos que possam permitir a distinção exata entre as lesões primárias e secundárias (HUNG et al., 2013; SHON et al., 2014).

Em cerca de 3% dos casos, angiossarcomas de desenvolvem no contexto de síndromes genéticas predisponentes, como retinoblastoma bilateral, neurofibromatose de Recklinghausen, doença de Ollier, Xeroderma pigmentoso (FURY et al., 2005; PENEL et al., 2011). Contudo, linfedema crônico e radioterapia são os dois fatores clássicos de risco para angiossarcomas cutâneos e de partes moles. O primeiro caso descrito de angiossarcoma cutâneo da mama associado à linfedema crônico pós-mastectomia radical foi descrito em 1948 por Stewart e Treves e ficou conhecido como Síndrome de Stewart-Treves, um quadro extremamente raro (STEWART; TREVES, 1948; PENEL et al., 2011). A incidência deste angiossarcoma pós-mastectomia era estimada entre 0,07% a 0,45% (KAUFMANN; CHU; KAUFMAN, 1991), mas o advento da cirurgia conservadora no câncer de mama e das técnicas de biópsia do linfonodo sentinela provavelmente reduziram estas taxas a números ainda mais baixos (BRODIE; PROVENZANO, 2008). Contudo, a cirurgia conservadora da mama trouxe consigo um maior número de pacientes submetidas à radioterapia. Concomitantemente, os angiossarcomas associados a radioterapia vêm emergindo na literatura, com vários casos descritos até a presente data (WEST et al., 2005).

A radioterapia (RT) é um dos principais tratamentos adjuvantes do câncer de mama. Em países como a França, 60% das 300.000 pacientes diagnosticadas com câncer de mama/ano serão submetidas à RT (THARIAT et al., 2012). Os benefícios da radioterapia no câncer de mama no contexto de adjuvância já estão bem estabelecidos, sobretudo no que tange a menores taxas de recidiva locorregional e maior sobrevida livre de doença quando do seu emprego (VINH-HUNG; VERSCHRAEGEN, 2004; CLARKE et al., 2005; DARBY et al., 2011).

O risco relativo para a ocorrência de angiossarcoma após radioterapia é 6 vezes maior, mas ainda não está claro porque algumas pacientes desenvolvem tumores secundários após a irradiação e outras não o fazem. Os mecanismos patogenéticos que expliquem o surgimento dos angiossarcomas secundários à radioterapia ainda permanecem desconhecidos (ITALIANO et al., 2012; HUNG et al., 2013).

Os efeitos da radioterapia são tipicamente divididos em agudos ou precoces, que ocorrem dias a semanas após a exposição; e tardios ou crônicos, que aparecem meses a anos depois. As alterações precoces são secundárias à necrose dos queratinócitos em rápido processo de divisão celular (HELFRICH; SACHS; VOORHEES, 2008). Dilatação dos capilares e aumento da permeabilidade vascular levam ao eritema. A atividade reduzida dos folículos

pilares e glândulas sudoríparas conduzem à queda de pêlos e queratose. Após 3 a 4 semanas, calor, espessamento e edema podem ocorrer. Trombose de vasos, hemorragia, descamação, exsudação, hiperpigmentação e ulceração são frequentes (HELFRICH; SACHS; VOORHEES, 2008). Alterações cutâneas tardias incluem hialinização das fibras colágenas da derme, edema das células endoteliais, teleangiectasias e proliferação dos vasos mais profundos (MANDRELL; MEHTA; MCCLURE, 2008). Estas alterações, contudo, tendem a regredir nos 3 primeiros anos após a radioterapia. Após este período, portanto, qualquer alteração cutânea deve ser considerada suspeita e encaminhada para investigação (MANDRELL; MEHTA; MCCLURE, 2008).

Nos últimos 10 anos, um número significativo de lesões vasculares cutâneas associadas à radioterapia tem sido descrito na literatura (GENGLER et al., 2007). Entre elas, destacam-se as lesões vasculares atípicas, conhecidas como lesões benignas, e os angiossarcomas, tumores malignos associados à alta morbi-mortalidade.

Do ponto de vista histológico, os angiossarcomas cutâneos se apresentam como tumores difusamente infiltrativos, que, com frequência, invadem o tecido subcutâneo. Seu espectro histológico varia de lesões bem diferenciadas ou de baixo grau histológico a lesões pouco diferenciadas e de alto grau histológico; além disso, é comum a concomitância de diferentes graus de diferenciação celular em um mesmo tumor. Tumores de alto grau geralmente apresentam necrose e alto índice mitótico e, por vezes, podem apresentar um padrão celular epitelioide. Estes tumores que são predominantemente compostos de células epitelioides são denominados angiossarcomas epitelioides e constituem diagnóstico diferencial de carcinoma mamário (BRENN; FLETCHER, 2006).

Quando o diagnóstico destas lesões é questionável ou apenas um pequeno fragmento de biópsia está disponível para estudo, a imuno-histoquímica se torna ferramenta importante na caracterização destes tumores. Marcadores endoteliais como CD31, CD34 e fator VIII auxiliam o diagnóstico, porém as citoqueratinas podem se mostrar positivas focalmente em tumores epitelióides em 35% dos casos (MEIS-KINDBLOM; KINDBLOM, 1998; BRENN; FLETCHER, 2006; LUCAS, 2009). Em amostras de tecido maiores, um achado interessante e relativamente frequente é a presença de pequenas lesões vasculares circunscritas, muitas vezes indistinguíveis de áreas de angiossarcoma de baixo grau, que foram denominadas de lesões vasculares atípicas (LVA) por Fineberg e Rosen (FINEBERG; ROSEN, 1994). Fineberg e Rosen definiram o conceito de lesões vasculares atípicas da pele da mama após

radioterapia e, desde então, esta terminologia vem sendo adotada por diversos autores em trabalhos posteriores sobre estas lesões (DIAZ-CASCAJO et al., 1999; BRENN; FLETCHER, 2005; PATTON; DEYRUP; WEISS, 2008).

Até o momento, ainda pouco se sabe sobre a patogênese das LVA na pele irradiada. Alguns autores acreditam que as LVA resultam de uma dilatação permanente dos capilares linfáticos associada com a interrupção do fluxo linfático (REQUENA et al., 2002). Outros autores as consideram uma proliferação reativa secundária ao dano vascular promovido pela irradiação e cirurgia (FINEBERG; ROSEN, 1994; DIAZ-CASCAJO et al., 1999; SANTI et al., 2011). Sabe-se que a irradiação causa danos ao DNA, podendo resultar em deleções, rearranjos e mudanças na expressão gênica (SANTI et al., 2011).

A importância das LVA consiste no seu diagnóstico diferencial com angiossarcomas de baixo grau e nas incertezas que rondam o seu comportamento biológico. Apesar de LVA e AS ocorrerem no sítio de irradiação prévia da pele após o tratamento conservador do câncer de mama com radioterapia, há diferenças clínicas, histopatológicas e evolutivas importantes entre estas lesões.

As LVA apresentam-se como pápulas diminutas (geralmente < 5mm) na pele da mama irradiada, de coloração rósea à acastanhada (PATTON; DEYRUP; WEISS, 2008; FRAGA-GUEDES et al., 2014). Elas costumam ser multifocais, circunscritas, e incidem em pacientes com idade média de 50 anos, uma década mais cedo que os angiossarcomas (MATTOCH et al., 2007). Apesar da dose de irradiação prévia (40-60 Gy) reportada seja a mesma em ambos os tumores (GENGLER et al., 2007), o intervalo de latência entre a RT e a incidência de LVA é em média de 3 anos, enquanto que o tempo médio para a incidência de AS pós-RT é de 7 anos (BRENN; FLETCHER, 2005; 2006). Na histopatologia, as LVA apresentam-se como lesões pequenas, circunscritas, simétricas na derme superficial e frequentemente apresentam espaços vasculares dilatados (LUCAS, 2009). Em contraste com os AS, extensão para o tecido subcutâneo é infrequente, assim como a presença de múltiplas camadas endoteliais, atipia celular importante, figuras mitóticas, necrose e “lagos de sangue”, características dos AS, não estão presentes nas LVA (BRENN; FLETCHER, 2006; GENGLER et al., 2007).

Contudo, as LVAS podem apresentar características muito semelhantes aos angiossarcomas de baixo grau e, portanto, nem sempre é possível classificar facilmente uma lesão vascular pós-irradiação. Até a presente data, o papel da imuno-histoquímica no diagnóstico diferencial

destas lesões vasculares pós-irradiação não está claro (MATTOCH et al., 2007). LVA e AS mostram positividade para os marcadores endoteliais CD31, CD34 e fator VIII, assim como para o marcador de endotélio linfático D2-40 (podoplanina) (KAHN; BAILEY; MARKS, 2002; BRENN; FLETCHER, 2005; 2006; GENGLER et al., 2007).

Em contraste com os AS, as LVA apresentam um comportamento clínico benigno, e não há relato na literatura de metástases ou morte pela doença (FINEBERG; ROSEN, 1994; DIAZ-CASCAJO et al., 1999; REQUENA et al., 2002; GENGLER et al., 2007). Todavia, há trabalhos que mostraram casos de recidiva de LVA (BRENN; FLETCHER, 2005; GENGLER et al., 2007), e até mesmo de progressão para angiossarcoma cutâneo (DI TOMMASO; FABBRI, 2003; BRENN; FLETCHER, 2005; PATTON; DEYRUP; WEISS, 2008).

Patton et al tentaram analisar o risco de desenvolver AS a partir de LVA interpretando a heterogeneidade morfológica entre as LVA (PATTON; DEYRUP; WEISS, 2008). Estes autores classificaram as LVA em tipos linfático (D2-40 positivo) e vascular (D2-40 negativo), concluindo que o tipo linfático de LVA é o mais frequente e de melhor prognóstico, enquanto que o tipo vascular tenderia a apresentar atipia celular mais acentuada e maior risco de desenvolvimento de AS cutâneo subsequente. Contudo, neste trabalho, os autores observaram dois casos de progressão para AS, um partindo de uma LVA do tipo linfático, outro do tipo vascular. Conclui-se, portanto, que D2-40 não é um marcador inequívoco do comportamento evolutivo das LVA.

No contexto atual pós-genômico do câncer de mama, emerge a hipótese de que o conhecimento das transformações genéticas no amplo espectro das proliferações vasculares associadas à radioterapia da pele da mama poderá ajudar a esclarecer a patogênese das LVA e sua real relação com os AS. Santi et al. investigaram as alterações no gene supressor tumoral *TP53* em uma série de LVA e AS (SANTI et al., 2011). O *TP53* é o gene que mais apresenta mutações em uma ampla variedade de tumores. O *TP53* pode ser um alvo potencial da radiação ionizante, e a perda da sua função parece estar relacionada com a tumorigênese nos AS (SANTI et al., 2011). Alterações no *TP53* induzem superexpressão do VEGF (*vascular endotelial growth factor*), um fator relacionado angiogênese tumoral, que poderia induzir o surgimento dos AS, pelo menos em alguns casos (SANTI et al., 2011). Inativação mutacional do *TP53* foi observada em taxas similares em LVA (83%) e AS cutâneos (87,5%), e os autores concluíram que este fato corrobora com a hipótese de que AS e LVA estão biologicamente associados, como extremos de um continuum morfológico.

Todavia, estudos mais recentes que investigaram a amplificação do gene *c-myc* em AS e LVA apontam para outra direção (SHEEN; DICKSON, 2002; MANNER et al., 2010; GUO et al., 2011; MENTZEL et al., 2012). Estes trabalhos foram unânimes em afirmar que apenas os AS secundários apresentam amplificação do *MYC*, enquanto que as LVA e AS primários não apresentam esta alteração. Desta forma, corroboram com a hipótese de que estas lesões apresentariam patogêneses distintas.

Os genes *myc* são uma família de proto-oncogenes que inclui *c-myc*, *N-myc* e *L-myc*, sendo *o c-myc* o gene mais amplamente estudado. Este gene foi primeiramente identificado no início da década de 80 em estudos abordando o linfoma de Burkitt. Translocações na região do cromossomo 8 contendo este gene são achados frequentes no linfoma de Burkitt, demonstrando que o *c-myc* está implicado em sua patogênese (DALLA-FAVERA et al., 1982; TAUB et al., 1982). Desde então, expressões aberrantes deste gene têm sido apontadas em diversos tipos de tumores (NESBIT; TERSAK; PROCHOWNIK, 1999). O gene *myc* está localizado no cromossomo 8q21 e consiste em 3 exons que estão envolvidos na regulação da expressão de numerosos outros genes, particularmente os genes relacionados com divisão celular, crescimento celular e apoptose. O *c-myc* codifica um fator de transcrição que funciona na dimerização e ligação do DNA e tem um papel fundamental na proliferação, regulação, diferenciação e apoptose celulares (FELLER; MAHALINGAM, 2013). Portanto, as funções-chave deste gene são: controlar o crescimento e proliferação das células e induzir morte celular (apoptose). Desregulação do c-myc tem sido associada a um amplo espectro de cânceres em seres humanos além de estar envolvida na angiogênese (PELENGARIS; KHAN; EVAN, 2002). A desregulação do *MYC* promove a proliferação celular através da entrada inadequada da fase S para fase G1. Curiosamente, a superexpressão de *MYC in vitro* induz alterações da detenção de G1/S secundária à radiação ionizante. Estes achados sugerem que a radioterapia em pacientes com câncer de mama pode predispor a angiossarcomas com *MYC*-amplificado (GUO et al., 2011)

O papel do *MYC* na angiogênese tem importância singular em tumores vasculares, como angiossarcomas. Um dos principais eventos pró-angiogênicos mediados pelo *MYC* está relacionado à ativação do grupo miR-17-92. Este grupo miRNA, localizado no cromossomo 13q31,3, codifica 6 miRNA maduros: miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a e miR-92a-1, e é um alvo de transcrição direto do *MYC* (ITALIANO et al., 2012).

Baudino et al demonstraram que a participação do gene *c-myc* é requisitada na expressão de fatores angiogênicos-relacionados como a angiopoetina-1, angiopetina-2 e trombospondina-1, sugerindo a possibilidade do *c-myc* tratar-se de um grande regulador de fatores angiogênicos (BAUDINO et al., 2002).

Estes conhecimentos motivaram o estudo do gene *c-myc* nas proliferações vasculares da mama, e, em alguns estudos, o *c-myc* vem sendo apontado como uma importante ferramenta na diferenciação de LVA, ASP e ASS (MENTZEL et al.; MANNER et al., 2010; FELLER; MAHALINGAM, 2013; KO et al., 2014). Contudo, apesar de todos os trabalhos disponíveis na literatura até o momento não apresentarem nenhum caso de amplificação do *c-myc* em LVA, estudos mais recentes apresentaram resultados conflitantes entre os angiossarcomas. Dentre os casos de Manner et al., 55% dos ASS apresentaram amplificação do *MYC*, ou seja, 45% dos casos não apresentavam esta alteração (MANNER et al., 2010). Ginter et al., Shon et al. e Italiano et al. mostraram casos de amplificação do *MYC* em angiossarcomas primários (ITALIANO et al., 2012; GINTER et al., 2013; SHON et al., 2014) . Desta forma, a presença de amplificação do *MYC* parece excluir um diagnóstico de LVA, mas a sua ausência não definiria nenhum diagnóstico.

É válido ressaltar, entretanto, que todos os trabalhos existentes até a presente data na literatura que abordaram o papel do *MYC* em proliferações vasculares como sarcomas e LVA não se restringiram às mamas. Há importante heterogeneidade na seleção dos sarcomas (inclusão de sarcomas diferentes de angiossarcomas), dos pacientes (estudos incluíram pacientes de ambos os sexos), dos locais de acometimento dos sarcomas (membros, couro cabeludo, fígado, etc.), assim como dos fatores predisponentes para angiossarcomas secundários (inclusão concomitante de casos de angiossarcoma secundário à irradiação e secundário a linfedema crônico).

Além do valor diagnóstico do *c-myc*, seu valor prognóstico nas proliferações e tumores vasculares ainda é desconhecido (FELLER; MAHALINGAM, 2013). Em carcinomas da mama, alguns estudos mostraram o *c-myc* como um fator preditivo de progressão de doença, de tumores com maiores diâmetros e envolvimento metastático linfonodal (BERNS et al., 1992; PERTSCHUK et al., 1993; SENGUPTA; BIARNES; JORDAN, 2014) . Porém, até o momento, os poucos trabalhos que investigaram a amplificação do *MYC* em relação à sobrevida em pacientes com angiossarcomas não conseguiram mostrar nenhuma associação prognóstica (ITALIANO et al., 2012; GINTER et al., 2013; SHON et al., 2014).

Ainda não há um regime de quimioterapia específico para pacientes com angiossarcoma de mama e a resposta ao tratamento atual é considerada muito pobre. Diante da possibilidade de uma terapia alvo-específica para pacientes com a expressão gênica do *c-myc* desregulada, sua manipulação farmacológica ainda é uma esperança no tratamento de tumores tão agressivos como os angiossarcomas (DELMORE et al., 2011).

LVA e AS são condições raras. Os resultados descritos na literatura correspondem a pequenas séries de casos, geralmente representadas em estudos retrospectivos multicêntricos (VORBURGER et al., 2005). Há ainda importantes controvérsias em relação à patogênese e ao comportamento biológico destas lesões. Diante desta realidade, decidimos estudar LVA e AS em seus aspectos histopatológicos, imuno-histoquímicos e moleculares, a fim de melhor compreender a tumorigênese destas proliferações vasculares da mama.

2. OBJETIVOS

Avaliar os aspectos clínico-patológicos e imuno-histoquímicos de uma série de casos de angiossarcomas e de lesões vasculares atípicas da mama e:

a) Caracterizar os aspectos clínicos, histopatológicos e de marcadores imuno-histoquímicos em LVA;

b) Investigar e comparar a presença de amplificação do *MYC* em angiossarcomas primários e secundários e em lesões vasculares atípicas e se a amplificação gênica está implicada na patogênese das proliferações vasculares induzidas por radiação;

c) Avaliar a utilidade do FISH para amplificação do *MYC* e imuno-histoquímica para sua expressão proteica como métodos diagnósticos auxiliares na avaliação de amostras de ASS e LVA, comparando as duas técnicas.

d) Avaliar o *MYC* como fator prognóstico nos casos em que foi observada a sua amplificação.

MATERIAS E MÉTODOS, RESULTADOS E DISCUSSÃO

Serão apresentados sob a forma de artigos científicos aceitos e/ou submetidos para publicação.

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CLINICAL TRIAL

Clinicopathological and immunohistochemical study of 30 cases of post-radiation atypical vascular lesion of the breast

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Abstract Atypical vascular lesions (AVL) that occur in the field of prior radiation therapy for breast carcinoma are placed within the differential diagnosis with low grade angiosarcoma and other benign vascular lesions. Although considered a benign entity, the exact biological behavior of AVLs is not fully established because of the small number of cases reported in the literature. We aim to further characterize these lesions clinically and histopathologically, and to study their behavior. We report a series of 30 patients with AVL of the breast occurring after radiation exposure, diagnosed and treated at the European Institute of Oncology, Italy. Immunohistochemical study was performed in all cases, using CD31, D2-40, CD105, and Ki-67 antibodies. Twenty-seven patients were treated with standard doses of conventional adjuvant radiation therapy for the prior breast carcinoma. Three patients were treated with intraoperative radiotherapy with electrons. The post-radiation latency interval from breast carcinoma to AVL was 48.5 months (ranged from 1 to 146 months). Most of the lesions were classified as lymphatic type (78.6 %) based on D2-40 positivity. No extension into subcutaneous tissue or significant atypia was noted in all cases. Despite the fact that the AVL of our series have shown benign behavior in 93.3 %, one patient developed local recurrence of AVL, and two cases progressed to angiosarcoma at the previous AVL site. Further studies should be conducted to better understand the clinical behavior and to propose additional histopathologic diagnostic criteria to distinguish AVL from low grade angiosarcoma and those AVL with increased risk for malignant progression. Concerning current treatments of AVL, we recommend complete excision with free surgical margins and close follow up.

Keywords Atypical vascular lesion · Radiation therapy · Vascular lesions · Breast cancer · Skin · Angiosarcoma

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Introduction

Post-radiation vascular proliferations are well-known complications of radiation exposure, and show heterogeneous clinical presentations and histopathologic features [1–6]. A direct link has been made between adjuvant radiotherapy and subsequent development of cutaneous angiosarcoma (AS) and atypical vascular lesions (AVL) in the radiation field [4, 7]. In 1994, Fineberg and Rosen [8]

reported for the first time cases of an unusual group of vascular lesions occurring in mammary skin after radiation therapy for breast carcinoma which they called "atypical vascular lesion". AVL are believed to arise as a result of lymphatic obstruction following surgery and/or radiotherapy, causing acquired dilation of superficial vascular channels [9]. The skin of the breast or chest wall is by far the most frequently affected site following radiation treatment for breast carcinoma [4, 10].

AVL are usually classified as one of two immunohistochemical patterns, based on D2-40 positivity, which are designated as lymphatic type (LT-AVL, D2-40 positive) or vascular type (VT-AVL, D2-40 negative) [11]. Most AVL reported in the literature are of the LT-AVL, and the diagnosis of LT-AVL and VT-AVL seems to have different implications. In contrast to LV-AVL, VT-AVL seems to have worse prognosis [11].

After the first description, most data on outcomes favored a benign process, but some studies have questioned this concept, suggesting that AVL and post-radiation angiosarcoma represent a morphologic continuum, implying AVL as a precursor to angiosarcoma [9–12]. However, the risk of malignancy is still undefined due to small number of reported cases with follow-up information.

Aiming to further characterize these lesions clinically and histopathologically, and to study their behavior, we report our experience in a series of 30 patients with AVL of the breast occurring after radiation exposure in the setting of conservative therapy for breast carcinoma.

Materials and methods

A computerized search of the database from the Department of Anatomic Pathology of the European Institute of Oncology (IEO) disclosed all female patients that were diagnosed as AVL of the breast from 1999 to 2012. Post-radiation AVL arising at sites other than the breast and AVL not associated with radiation were not included in this study.

Clinical information was obtained from the hospital records and the following data were collected: age, gender, diagnosis of primary breast cancer and type of treatment, time latency interval elapsed between radiation therapy and diagnosis of the vascular lesion, clinical presentation, location, and number of lesions. Follow-up information was available for all patients. Local recurrence was defined as any tumor recurrence at the primary disease site and the loco-regional areas of the same histological subtype.

Hematoxylin and eosin stained sections from all cases were reviewed by two authors (H.G and C.F.G), using criteria proposed by Fineberg and Rosen (Table 1) to define AVL [8]. Immunohistochemical study was performed in all cases, using the following antibodies: CD31 (clone JC70A, 1:100 dilution, Dako/USA), D2-40, podoplanin (clone D2-40, 1:100 dilution, Signet/USA), CD105, endoglin, (clone SN6h, 1:100 dilution, Dako/USA), and Ki-67 (clone MIB-1, 1:100 dilution, Dako/USA). All immunostainings were performed automatically using a Ventana Benchmark XT staining system (Ventana Medical Systems, Tucson, Arizona, USA) at A.C.Camargo Cancer Center. The proportion of positive vessels for CD31, D2-40, Ki-67, and CD105 was estimated as <25 % (+), 25–50 % (++), and >50 % (+++), regardless the staining intensity. A semi-quantitative analysis of Ki-67 was performed, considering the percentage of endothelial cells staining for this marker. Expression of Ki-67 was classified as follows: negative (no staining); low (<10 %), intermediate (10–25 %), and high (>25 %) expression.

The Ethical Committees from the IEO and Federal University of Minas Gerais approved the study, and informed consent was obtained from all patients and/or guardians.

Table 1 Histopathological features used to distinguish between atypical vascular lesions from angiosarcoma (adapted from Fineberg and Rosen [8])

Histopathological feature	Atypical vascular lesion	Angiosarcoma
Papillary endothelial hyperplasia	−	+++
Infiltration into subcutis	−	+++
Mitotic figures	−	+++
Prominent nucleoli	−	+++
Significant cytological atypia	−	+++
"Blood lakes"	−	++
Dissection of dermal collagen	+/−	+++
Anastomotic vessels	++	+++
Hypercromatic endothelial cells	+++	++
Chronic inflammation	+++	+
Relative circumscription	+++	−
Projections of stroma into lumen	+++	−

Results

From 1999 to 2012, 30 patients treated at the IEO were diagnosed with atypical vascular lesions of the breast (Table 2). All patients were women diagnosed, treated and followed at the IEO. The median age at diagnosis of AVL was 58.5 years old (ranging from 36 to 81 years old). All

Table 2 Clinical features of the 30 atypical vascular lesions of the breast developed in the field of prior radiation therapy

#	Age	Primary disease	Surgery for breast cancer	Latency interval[a] (months)	Clinical presentation	Treatment of AVL	Follow-up (months)
1	67	Ipsilateral BC	Conservative	117	Two erythematous papules	PB	Alive NED (16)
2	47	Ipsilateral BC	Conservative	57	Single erythematous nodule	EB	Dead of metastatic CRC(25)
3	67	Ipsilateral BC	Radical	1	Axillary erythematous papules	EB	Alive NED (25)
4	51	Ipsilateral BC	Conservative	50	Two erythematous papules	EB	Dead of metastatic BC (63)
5	43	Ipsilateral BC	Conservative	19	Nodular lesion	EB	Alive NED (93)
6	54	Ipsilateral BC	Radical	31	Erythematous plaque	PB	Dead of metastatic BC (24)
7	60	Ipsilateral BC	Conservative	26	Erythematous plaque	PB	Alive NED (31)
8	59	Ipsilateral BC	Conservative	42	Three erythematous papules	PB	Alive NED (90)
9	57	Ipsilateral BC	Conservative	49	Single erythematous nodule	EB	Alive NED (93)
10	37	Ipsilateral BC	Conservative	28	Single erythematous nodule	PB	Alive, with recurrent AVL (26)
11	47	Ipsilateral BC	Conservative	124	Single erythematous papule	EB	Alive NED (27)
12	81	Ipsilateral BC	Conservative	124	Single erythematous nodule	EB	Alive NED (36)
13	67	Ipsilateral BC	Conservative	48	Not available	PB	Dead of cardiomyopathy (54)
14	65	Ipsilateral BC	Conservative	33	Single erythematous nodule	EB	Alive NED (61)
15	62	Ipsilateral BC	Conservative	80	Single erythematous nodule	EB	Alive NED (109)
16	67	Ipsilateral BC	Conservative	83	Nodular lesion	PB	Alive NED (72)
17	68	Ipsilateral BC	Conservative	93	Three erythematous papules	EB	Alive NED (38)
18	55	Ipsilateral BC	Conservative	77	Single erythematous nodule	PB	Alive NED (60)
19	54	Ipsilateral BC	Conservative	37	Single erythematous nodule	EB	Alive NED (8)
20	75	Ipsilateral BC	Conservative	17	Erythematous plaque	PB	Alive NED (41)
21	43	Ipsilateral BC	Conservative	56	Two erythematous nodules	EB	Lost to follow-up
22	51	Ipsilateral BC	Conservative	79	Erythematous plaque	S	Dead of metastatic BC (18)
23	62	Ipsilateral BC	Conservative	54	Single erythematous nodule	PB	Alive, progression to AS (97)
24	65	Bilateral BC	Conservative	40	Nodular lesion	PB	Alive, progression to AS (107)
25	75	Ipsilateral BC	Conservative	34	Nodular lesion	PB	Alive NED (5)
26	73	Ipsilateral BC	Conservative	146	Axillary nodular lesion	EB	Alive NED (6)
27	44	Ipsilateral BC	Nipple Sparing	72	Erythematous plaque	PB	Alive NED (12)
28	50	Ipsilateral BC	Conservative	25	Erythematous plaque	PB	Alive NED (2)
29	68	Ipsilateral BC	Conservative	41	Single erythematous nodule	EB	Alive NED (3)
30	50	Ipsilateral BC	Conservative	10	Single erythematous nodule	PB	Alive NED (2)

BC breast carcinoma, *NA* not available, *PB* punch biopsy, *EB* excisional biopsy, *S* surgery, *NED* no evidence of disease (AVL), *CRC* colorectal cancer, *AS* angiosarcoma

[a] Latency interval between radiotherapy and development of AVL

patients had a history of previous infiltrating breast carcinoma (ductal, 26; lobular, 1; medullary, 1; unknown, 2), and were surgically treated by excision (quadrantectomy, 27; modified radical mastectomy, 2; nipple sparing mastectomy, 1) with adjuvant radiation therapy. Twenty-seven patients were treated with standard doses of conventional adjuvant radiation therapy, fractionated over the course of 4–6 weeks. Three patients were treated with intraoperative radiotherapy with electrons (ELIOT): two patients underwent intraoperative radiotherapy boost (12 Gy) followed by conventional radiation therapy (37 Gy), and one patient received nipple–areola intraoperative radiotherapy (16 Gy), in the context of nipple sparing mastectomy. Therefore, all patients had received a total dose ranging from 12 to 60 Gy (median 50 Gy). The post-radiation latency interval from breast carcinoma to AVL was 48.5 months (ranged from 1 to 146 months).

Axillary dissection was previously performed in 20/30 patients (66.6 % of the cases), and sentinel node biopsy in 9/30 patients (30 % of the cases). In one case, axillary treatment was ignored.

Clinical presentation

Clinically, AVL presented as well-circumscribed and small reddish papules in the irradiation field, including the skin

of the breast (20 patients) or the thoracic region (2 patients), and axillary skin (1 patient). Six patients presented with a small, erythematous plaque. The lesion size ranged from 0.3 cm to 1.1 cm (median 0.5 cm). Sixteen patients had a solitary lesion and five patients presented with multiple lesions synchronously (two or more lesions). Clinical appearance was not given for one patient.

Imaging

No imaging studies performed were informative. Among 11 patients who underwent mammography, 90.9 % had a negative report, and in only one patient a cutaneous nodule was observed. Five patients underwent ultrasonography, which was negative in 40 % of the cases. In three patients, skin lesions were observed upon examination.

Diagnostic methods

The main method used for the pathological diagnosis of AVL was punch biopsy (50 % of cases). Excisional biopsy was performed in 14 patients (46.6 % of cases). One patient with inflammatory breast carcinoma underwent a mastectomy which showed the presence of one AVL in the breast skin.

Histopathological features

The AVL were relatively well-circumscribed wedge-shaped lesions confined to the upper dermis, composed of anastomosing vascular channels infiltrating the dermal collagen and around pilar muscle bundles when present (Figs. 1a, b, 2a, b). The endothelial cell nuclei were usually mild hyperchromatic with some hobnailing (Figs. 1c, 2c) but without pleomorphism, mitotic figures, endothelial multilayering or stroma bleeding. Intraluminal papillary projections were present in 16 cases (Fig. 1c). The most frequent finding was a patchy chronic inflammatory infiltrate that accompanied all AVL to varying degree (Figs. 1b, 2b). No extension into subcutaneous tissue was noted. Intraluminal red blood cells were seen focally in two lesions and projections of endothelium-covered stroma protruding into lumina were a constant finding (Fig. 1c). Microscopically, the lesion size ranged from 0.3 to 1.1 cm,

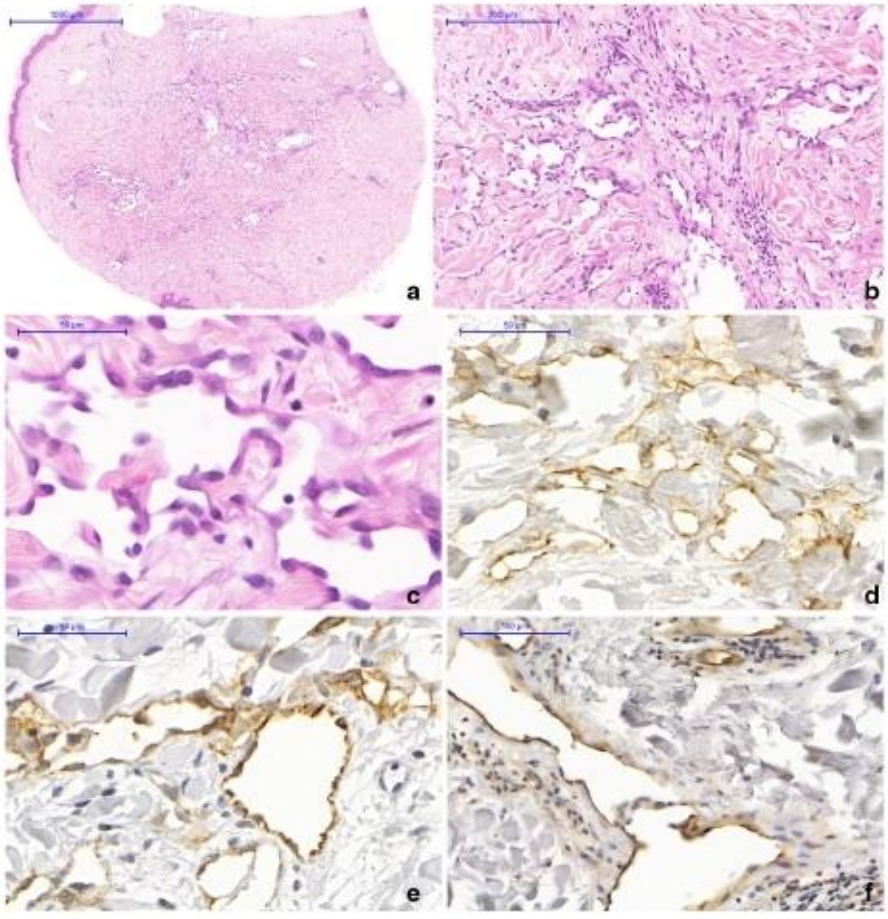

Fig. 1 Atypical vascular lesion of mammary skin (case 24). **a** Atypical vascular proliferation in the upper dermis, dissecting collagen fibers. Hematoxylin and eosin (×20). **b** Proliferating anastomosing vessels with adjacent lymphocytic infiltration. Hematoxylin and eosin ×100. **c** Proliferated vessels showing papillary projections within the vascular lumen and mild nuclear atypia. Hematoxylin and eosin (×400). **d** Endothelial cells positive for CD-31 (×400). **e** Vessels with endothelial cells positive for D2-40 (×400). **f** Proliferated vessels with endothelial cells positive for CD 105 (×200)

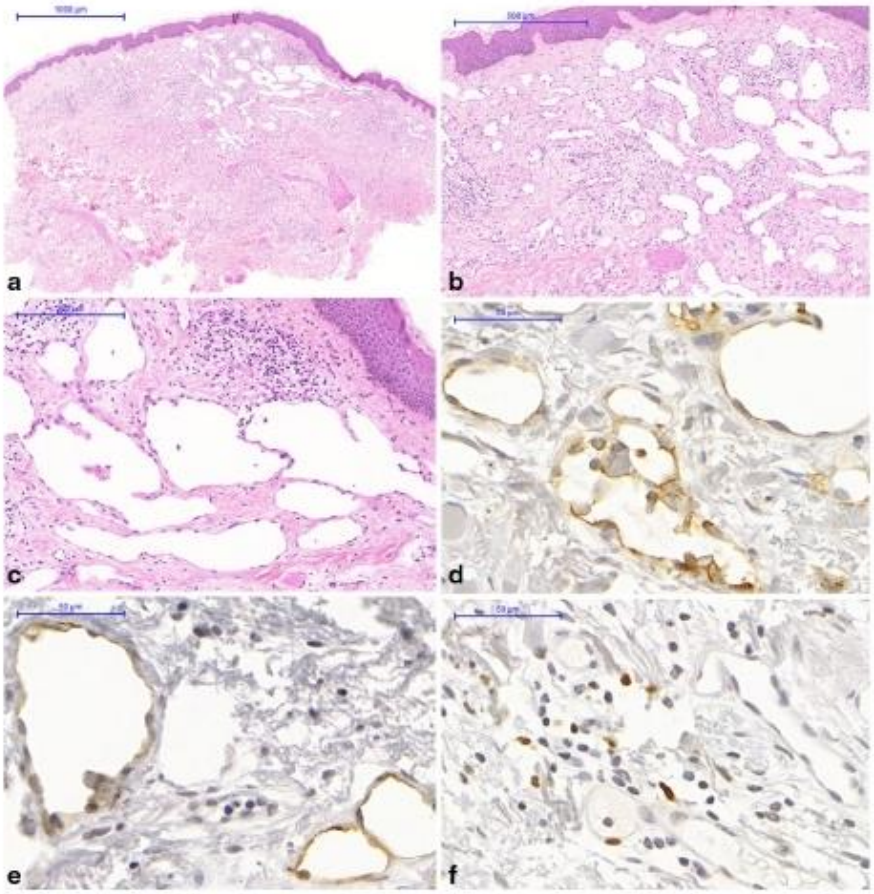

Fig. 2 Atypical vascular lesion of mammary skin (case 23). **a** Well circumscribed atypical vascular proliferation within the upper dermis (Hematoxylin and eosin, ×20). **b** Small vessels proliferating in the upper dermis with adjacent lymphocytic infiltration (Hematoxylin and eosin, ×50). **c** Vascular channels covered by monolayer of endothelial cells with very mild hyperchromatic nuclei (Hematoxylin and eosin, ×100). **d** Endothelial cells positive for CD-31 (×400). **e** Endothelial cells weakly positive for D2-40 (×400). **f** Endothelial cells focally positive for MIB-1 (×400)

with a median of 0.5 cm. Twenty-one cases achieved tumor-free surgical margins.

Immunohistochemistry

Immunohistochemistry was performed in all AVL. Endothelial cells stained positive for CD31 in 18 of 30 cases (Figs. 1d, 2d). AVLs appeared as one of two immunohistochemical patterns according to Patton et al.'s classification: lymphatic type, LT, (D2-40 positive: Figs. 1e, 2e) or vascular type, VT (D2-40 negative). Twenty-two AVL were classified as LT, 6 cases were classified as VT, and D2-40 was not tested in 2 cases. The proportion of positive vessels for D2-40 varied from <25 % ($n = 5$ lesions), to 25–50 % ($n = 5$ lesions). Twelve tumors showed diffuse and strong immunoreactivity for D2-40 in almost 100 % of the endothelial cells (Fig. 1e and 2e). Twenty-five AVL (83 %) stained positive for CD105 (<25 % = 5 cases; 25–50 % = 7 cases; >50 % = 13 cases) (Fig. 1f). Positive cells for Ki-67 (Fig. 2f) were noted in 11/30 cases (low = 6 cases; intermediate = 3 cases, and high expression: 2 cases).

Treatment

Because AVLs were considered as benign lesions, twenty-nine patients were initially treated conservatively, either with punch biopsy alone (15 patients) or with simple excision (14 patients). One patient was elected to undergo mastectomy because of synchronous recurrent breast carcinoma.

Free surgical margins were found in 25 patients, and in four patients, who underwent only a punch biopsy, the margins were compromised. One patient who underwent excisional biopsy had the description of positive margins.

Clinical outcomes

Follow-up information was available for 29 out of 30 patients with a median follow-up of 31 months (range 3–109 months). One patient was lost to follow-up and 9 patients had a follow-up >60 months.

One patient developed local recurrence of AVL 32 months after the diagnosis of AVL in a punch biopsy for AVL, with compromised margins. This patient

presented with 2 red papules in the previous biopsy site by the moment of recurrence and died of metastatic breast cancer 2 years later. Of 29 patients with clinical follow-up, 26 are alive with no progression of disease.

Two patients developed angiosarcoma in the previous biopsy site. One patient was submitted to punch biopsy with positive margins for a LT-AVL 19 months earlier, and developed a high grade angiosarcoma of the breast. She is still alive, with no evidence of disease during the 78-months follow-up period. The other patient was initially submitted to conservative therapy for synchronous bilateral breast cancer. She underwent punch biopsy with compromised margins reference in the context of AVL's diagnosis (VT-AVL), and developed a bilateral high grade epithelioid angiosarcoma 89 months later. This latter VT-AVL lesion stained positive for Ki-67 on immunohistochemistry. She is still alive after 39 months of follow-up, but she has progressive disease with bone and lung metastasis from angiosarcoma, histologically confirmed.

To date, four patients died (13.3 %), three of them from metastatic breast cancer and one from unspecified cardiomyopathy. None developed any new vascular lesions prior to death.

Discussion

In this study, we evaluated a series of AVLs diagnosed, treated and followed in the same institution. All breast AVLs of our series were well circumscribed vascular proliferations, with mild endothelial atypia and were placed within the differential diagnosis with other vascular lesions [10]. AVLs diagnosed post-radiotherapy have been categorized as having benign behavior, but there is disagreement in the literature if they would not, in fact, be premalignant or at risk for the development of angiosarcoma [4, 10, 11]. Some authors believe that these lesions are underdiagnosed angiosarcomas [2, 8].

When isolated, in limited samples, these lesions enter in the context of differential diagnosis with low-grade angiosarcoma because of histological similarity and previous history of RT [5]. To date, there are no specific criteria to distinguish them completely [5].

In our series, the median age at diagnosis of AVL was 58.5 years, and the latency period between the RT for breast cancer and the detection of AVL of 4 years, similar to data reported in other studies [2, 4, 11, 13]. All AVL of our series presented clinically as small solitary or multiple vesicles or papules, with a wedge-shaped and circumscribed appearance. We did not find extension into subcutis or significant atypia that was described by some authors [2, 11]. Chronic inflammatory cell infiltrate was a constant finding in all cases of our series, as described in previous reports [2, 7, 9–11, 14].

Thus far, the consensus which exists in the literature is that these lesions have a benign behavior [2], but the development of angiosarcoma in patients with a pre-existing atypical vascular lesion has also been documented [4, 11]. In our series, only a punch or an excisional biopsy was performed, and it was observed that, in 93.3 % of patients, this approach was sufficient. In our series, margin involvement was also included in the analysis to establish its importance as well as the prognosis. In three cases when margin was compromised, the patients presented with unfavorable outcomes: one patient had local recurrence of AVL and the other two cases progressed to high-grade angiosarcoma.

Among the AVL cases reported in the literature, there are only two reports of progression to angiosarcoma [4, 11]. In our series, we observed two cases of angiosarcoma that developed at the previous AVL site. The two initial biopsies showed wedge-shaped lesions within the superficial to mid dermis, accompanied by a chronic inflammatory infiltrate, but no cytologic atypia or multilayering was observed. However, in both cases, subsequent angiosarcomas presented as high grade tumors. We cannot say whether this was a progression from a benign lesion towards malignancy in these cases or the sample available for the initial diagnosis was not representative of the entire lesion. Angiosarcomas are very aggressive tumors, and even low-grade tumors tend to have rapid growth and more prominent clinical signs. It would be unlikely that a tumor with such behavior would remain underdiagnosed for prolonged periods, as observed in these two patients from our series (19 and 89 months, respectively). Nevertheless, we cannot rule out the possibility of an angiosarcoma which was not presented in the initial diagnostic biopsy.

Patton et al. proposed two histologic patterns of AVLs based on immunohistochemical endothelial markers expression, which they designated as lymphatic type (LT) or vascular type (VL). They suggested that the VT type represents a premalignant lesion or "incipient angiosarcoma" [11]. In our series, most of the lesions were classified as LT (78.6 %). However, among the patients who developed angiosarcoma at the previous AVL site, one had LT-AVL, and the other had VT-AVL. This latter case of VT-AVL lesion also stained strongly positive for Ki-67 on immunohistochemistry. The lesion of the patient who developed local recurrence of AVL was LT, strongly immunoreactive for D2-40. Therefore, based on our immunohistochemical findings, we cannot confirm Patton's suggestion that VL-AVL are at higher risk for malignant progression.

Endoglin (CD105) has been suggested as an appropriate marker for tumor-related angiogenesis and neovascularization [15]. To our knowledge, there are no previous reported studies of endoglin expression in atypical vascular

lesion of the breast. Hara H reported strong expression of endoglin in endothelial cells of cutaneous angiosarcoma and weak or negative expression in vascular endothelium of normal tissues [16]. In our series, endothelial cells of 25 AVL stained positive for CD105, ranging from low to high expression. However, we also noted low to high expression for CD105 in the endothelium of normal vessels of the adjacent stroma, suggesting nonspecific staining. It is possible that the high sensitivity of the antibody used (clone SN6h) may have overestimated the expression of this marker.

Ki-67 expression has been used as a tool to distinguish between benign and malignant lesions exhibiting histologic overlap and to assess prognosis. Rare studies investigated Ki-67 in vascular lesions of the breast [17] and, to our knowledge, only one has been performed in AVL [3]. Theses authors reported complete negative staining for Ki-67 in their cases, different from our findings. In our series, 20 % of the AVL cases showed low Ki-67 expression, using a semi-quantitative analysis of this proliferation marker. The two cases of our study that developed angiosarcoma showed high or intermediate expression for Ki-67. However, the remaining cases that expressed significant staining had a benign clinical behavior. Our results, therefore, cannot support the Ki-67 immunohistochemical stain for assessing prognosis.

There remains considerable overlap features between AVL and angiosarcoma, and distinction may not always be possible based only on clinical and morphologic and immunohistochemical findings. Santi et al. [18] investigated the presence of p53 alterations in a series of 12 cases of AVL in comparison with 8 cases of postradiation cutaneous angiosarcoma. They reported TP53 variations in the majority (83 %) of AVL and in a similar percentage (87.5 %) of postradiation cutaneous AS, suggesting a molecular link between AVL and AS. Their results support the hypothesis that AVL and AS are biologically related entities, the extremes of a morphological continuum [18].

Recently, few studies have explored the use of interphase FISH and immunohistochemistry for the detection of *Myc* amplification in both post-radiation angiosarcoma and AVL [19–21]. A consistent *Myc* amplification has been reported in post-radiation angiosarcoma, but not in AVL after radiotherapy for breast cancer [19, 20, 22]. The lack of *Myc* amplification in post-radiation AVL questions its role as a true precursor of AS, and it could be speculated that AVL has a distinct pathogenesis. This finding could be a useful diagnostic tool in the assessment of secondary angiosarcoma and AVL of the breast, however, *Myc* amplification is not demonstrated in all cases of secondary AS. Manner et al. [23] found *Myc* amplification in only 55 % of post-radiation AS. These different results suggest that the pathogenesis of AS and AVL and their relationship are still unclear.

The specific type, technique, and dosage of prior radiation were not available for all cases and were not specifically analyzed in our study, however all AVLs were observed within the radiation field. Twenty-seven patients of our series have previously undergone conventional radiation therapy, and three patients developed AVL after intraoperative radiation therapy with electrons (ELIOT). To our knowledge, these three patients are the first reported cases of AVL following ELIOT reported in the literature. One patient underwent ELIOT in the nipple–areola complex in the context of nipple sparing mastectomy (16 Gy) and developed AVL in the nipple 72 months later. The other two patients underwent ELIOT boost (12 Gy) plus conventional radiotherapy (37 Gy), and developed AVL in the radiation field 8 and 10 months later, respectively. We did not retrieve any case of AVL after standard ELIOT single dose (21 Gy), a skin sparing technique.

Skin changes in the breast after radiation therapy should alert the clinician. Our data indicates the need to sample a significant amount of tissue for a definitive diagnosis, since, to date, the proposed treatment of AVLs, considering them benign lesions, is completely different from the treatment of angiosarcomas.

Longer follow-up is necessary to determine the rate of progression from AVL to cutaneous angiosarcoma. Concerning current treatments of AVL for patients with a history of surgery and/or radiation therapy, we strongly recommend complete excision with free surgical margins and close follow up, accompanied by clinical exams rather than imaging until the natural history of the AVLs is better explained.

In conclusion, data from our study and the controversies found in the literature indicate that further studies should be conducted to better understand the clinical behavior and to propose additional histopathologic and immunohistochemical diagnostic criteria to distinguish AVL from low grade angiosarcoma and those AVL with increased risk for malignant progression.

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References

1. Fraga-Guedes C, Gobbi H, Mastropasqua MG, Botteri E, Luini A, Viale G (2012) Primary and secondary angiosarcomas of the

breast: a single institution experience. Breast Cancer Res Treat 132(3):1081–1088. doi:10.1007/s10549-011-1931-2
2. Gengler C, Coindre JM, Leroux A, Trassard M, Ranchere-Vince D, Valo I, Michels JJ, Guillou L (2007) Vascular proliferations of the skin after radiation therapy for breast cancer: clinicopathologic analysis of a series in favor of a benign process: a study from the French Sarcoma Group. Cancer 109(8):1584–1598. doi:10.1002/cncr.22586
3. Requena L, Kutzner H, Mentzel T, Duran R, Rodriguez-Peralto JL (2002) Benign vascular proliferations in irradiated skin. Am J Surg Pathol 26(3):328–337
4. Brenn T, Fletcher CD (2005) Radiation-associated cutaneous atypical vascular lesions and angiosarcoma: clinicopathologic analysis of 42 cases. Am J Surg Pathol 29(8):983–996
5. Mattoch IW, Robbins JB, Kempson RL, Kohler S (2007) Post-radiotherapy vascular proliferations in mammary skin: a clinicopathologic study of 11 cases. J Am Acad Dermatol 57(1):126–133. doi:10.1016/j.jaad.2006.10.025
6. Lucas DR (2009) Angiosarcoma, radiation-associated angiosarcoma, and atypical vascular lesion. Arch Pathol Lab Med 133(11):1804–1809. doi:10.1043/1543-2165-133.11.1804
7. Sener SF, Milos S, Feldman JL, Martz CH, Winchester DJ, Dieterich M, Locker GY, Khandekar JD, Brockstein B, Haid M, Michel A (2001) The spectrum of vascular lesions in the mammary skin, including angiosarcoma, after breast conservation treatment for breast cancer. J Am Coll Surg 193(1):22–28
8. Fineberg S, Rosen PP (1994) Cutaneous angiosarcoma and atypical vascular lesions of the skin and breast after radiation therapy for breast carcinoma. Am J Clin Pathol 102(6):757–763
9. Brodie C, Provenzano E (2008) Vascular proliferations of the breast. Histopathology 52(1):30–44. doi:10.1111/j.1365-2559.2007.02892.x
10. Brenn T, Fletcher CD (2006) Postradiation vascular proliferations: an increasing problem. Histopathology 48(1):106–114. doi:10.1111/j.1365-2559.2005.02293.x
11. Patton KT, Deyrup AT, Weiss SW (2008) Atypical vascular lesions after surgery and radiation of the breast: a clinicopathologic study of 32 cases analyzing histologic heterogeneity and association with angiosarcoma. Am J Surg Pathol 32(6):943–950
12. Di Tommaso L, Fabbri A (2003) Cutaneous angiosarcoma arising after radiotherapy treatment of a breast carcinoma. Description of a case and review of the literature. Pathologica 95(4):196–202
13. Mandrell J, Mehta S, McClure S (2010) Atypical vascular lesion of the breast. J Am Acad Dermatol 63(2):337–340. doi:10.1016/j.jaad.2009.08.017
14. Diaz-Cascajo C, Borghi S, Weyers W, Retzlaff H, Requena L, Metze D (1999) Benign lymphangiomatous papules of the skin following radiotherapy: a report of five new cases and review of the literature. Histopathology 35(4):319–327
15. Nassiri F, Cusimano MD, Scheithauer BW, Rotondo F, Fazio A, Yousef GM, Syro LV, Kovacs K, Lloyd RV (2011) Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy. Anticancer Res 31(6):2283–2290
16. Hara H (2012) Endoglin (CD105) and claudin-5 expression in cutaneous angiosarcoma. Am J Dermatopathol 34(7):779–782. doi:10.1097/DAD.0b013e318252fc32
17. Shin SJ, Lesser M, Rosen PP (2007) Hemangiomas and angiosarcomas of the breast: diagnostic utility of cell cycle markers with emphasis on Ki-67. Arch Pathol Lab Med 131(4):538–544. doi:10.1043/1543-2165(2007)131[538:HAAOTB]2.0.CO;2
18. Santi R, Cetica V, Franchi A, Pepi M, Cesinaro AM, Miracco C, Paglierani M, De Giorgi V, Delfino C, Difonzo EM, Pimpinelli N, Bianchi S, Sardi I, Santucci M, Massi D (2011) Tumour suppressor gene TP53 mutations in atypical vascular lesions of breast skin following radiotherapy. Histopathology 58(3):455–466. doi:10.1111/j.1365-2559.2011.03770.x
19. Fernandez AP, Sun Y, Tubbs RR, Goldblum JR, Billings SD (2012) FISH for MYC amplification and anti-MYC immunohistochemistry: useful diagnostic tools in the assessment of secondary angiosarcoma and atypical vascular proliferations. J Cutan Pathol 39(2):234–242. doi:10.1111/j.1600-0560.2011.01843.x
20. Mentzel T, Schildhaus HU, Palmedo G, Buttner R, Kutzner H (2012) Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. Mod Pathol 25(1):75–85. doi:10.1038/modpathol.2011.134
21. Guo T, Zhang L, Chang NE, Singer S, Maki RG, Antonescu CR (2011) Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. Genes Chromosomes Cancer 50(1):25–33. doi:10.1002/gcc.20827
22. Jayalakshmy PS, Sivaram AP, Augustine J, Bindu P (2012) Postmastectomy-postirradiation atypical vascular lesion of the skin: report of 2 cases. Case Rep Pathol 2012:710318. doi:10.1155/2012/710318
23. Manner J, Radlwimmer B, Hohenberger P, Mossinger K, Kuffer S, Sauer C, Belharazem D, Zettl A, Coindre JM, Hallermann C, Hartmann JT, Katenkamp D, Katenkamp K, Schoffski P, Sciot R, Wozniak A, Lichter P, Marx A, Strobel P (2010) MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. Am J Pathol 176(1):34–39. doi:10.2353/ajpath.2010.090637

4- ARTIGO 2 SUBMETIDO A REVISTA "*CANCER*"

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Keywords:	angiosarcoma, post-irradiation, MYC, atypical vascular lesion, FISH, cutaneuous

Original Article

Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of *MYC* gene amplification and protein expression

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Precis for use in the Table of Contents

MYC amplification is a highly specific but poorly sensitive marker for SAS and, therefore, a negative result does not exclude the diagnosis of angiosarcoma. *MYC* amplification was associated with adverse prognosis, suggesting a prognostic role of *MYC* amplification status on SAS of the breast.

ABSTRACT

Background: *MYC* amplification has been reported as a prominent feature of secondary angiosarcomas (SAS). The differential diagnosis between atypical vascular lesion (AVL) and low-grade angiosarcoma (AS) can be occasionally very difficult or even impossible, and *MYC* amplification status has been pointed as an important diagnostic tool to distinguish cutaneous vascular lesions of the breast. **Methods:** We assessed *MYC* amplification and protein expression status by fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC), respectively, in 49 patients diagnosed with breast AS, and 30 patients diagnosed with post-radiation AVL of the breast. Clinical and pathological features, and follow-up data were collected, and survival analyses were performed. **Results:** Among 37 patients with SAS, twenty patients had tumors with

high-level *MYC* amplification and protein overexpression (54%). None of primary angiosarcomas (PAS) or AVL cases showed *MYC* amplification or protein expression. Concordance between *MYC* amplification (FISH) and protein expression (IHC) was 100% in AVL, PAS and SAS. Survival analysis of the SAS patients demonstrates that those with *MYC* amplification had a significantly worse overall survival compared to cases without MYC amplification (P=0.035). There was a non-significant trend toward a poor disease-free survival between cases with and without *MYC* amplification (P=0,155). **Conclusions:** Our findings show that *MYC* amplification is a highly specific but poorly sensitive marker for SAS and, therefore, a negative result does not exclude the diagnosis of angiosarcoma. *MYC* amplification was associated with adverse prognosis, suggesting a prognostic role of *MYC* amplification status on SAS of the breast.

Keywords

angiosarcoma; post-irradiation; MYC; cutaneous; FISH; atypical vascular lesion

Introduction

Secondary angiosarcoma (SAS) is an aggressive tumor that arises in the setting of previous irradiation field or chronic lymphedema (Stewart-Treves syndrome) and is associated with poor prognosis [1-3]. Atypical vascular lesions (AVL) of the breast, however, are a benign radiation-associated vascular proliferation that shows morphological similarities with low-grade secondary angiosarcomas [4-9]. The differential diagnosis between AVL and low-grade angiosarcoma (AS) can be occasionally very difficult or even impossible, mostly in small sized skin biopsies, due to the histologic overlap characteristics between low-grade AS and AVL [5, 10]. Some studies reported patients with AVL who later developed AS, suggesting that AVL may be a precursor to or an incipient angiosarcoma [6, 7, 11].

Recently, some studies have shown that *c-myc* amplification is a recurrent genetic alteration in secondary angiosarcomas, but not in primary angiosarcomas and AVL, suggesting distinct pathogenetic mechanisms between them [12-15]. *MYC* is a proto-oncogene that codes for a transcription factor involved in the regulation of cellular proliferation, cell growth, and apoptosis [4, 12]. The most common mechanisms by which *MYC* activation occurs in tumors are gene amplification and gene rearrangement [4, 10]. The deregulation of *c-myc* has been associated with human cancers and has also been implicated in angiogenesis [16]. MYC protein expression promotes cell proliferation through inappropriate entry to S-phase from G1 phase following ionizing radiation, resulting in its function as an oncogene [17].

As accurate morphological diagnosis between low-grade AS and AVL can be difficult and the utility of immunohistochemical (IHC) stains for this particular diagnostic

dilemma has not been established [11]. New methods to distinguish these two biologic entities are a welcome tool in the clinical setting.

In this study, we aim to evaluate the diagnostic utility of *MYC* amplification and overexpression in the scenario of AVL and AS to further assess whether *MYC* amplification is implicated in prognosis.

MATERIAL AND METHODS

We searched the database from the Department of Anatomic Pathology of the European Institute of Oncology (IEO) Milan, Italy, and Portuguese Institute of Oncology (IPO), Lisbon, Portugal, and retrieved all female patients with the diagnosis of either angiosarcoma or AVL of the breast from 1999 to 2012. Post-radiation AVL and angiosarcoma arising at sites other than the breast and cases without representative tumor blocks were not included in this study. The medical records were reviewed and hematoxylin and eosin-stained slides were re-examined by three of the authors (CFG, SA and HG) to confirm the diagnosis. Histopathological diagnostic criteria proposed by Fineberg and Rosen [18] were used in the pathological review. Suitable blocks were chosen to obtain additional sections for *MYC* immunostainings and interphase FISH analysis.

For IHC analysis, standard whole sections were immunostained for *MYC* with a rabbit monoclonal anti-*c-MYC* antibody (Y69, 1:50, Epitomics [Cat no. 1472-1], Burlingame, CA) using heat-induced epitope retrieval and an automated immunostainer (Ventana, Oro Valley, AZ, USA). Appropriate positive and negative controls were used. Only nuclear reactivity was considered positive. Immunostained sections were then examined

by routine light microscopy. The cases were scored as 'negative' (≤5% positive cells), '1+`' (5-25% positive cells), '2+' (26-50% positive cells), or '3+' (≥ 51% positive cells), as previously proposed by Shon et al [12].

Interphase FISH was performed using commercially available FISH probe for *MYC* (8q24), and a probe designed to detect CEP8 (Abbot Molecular, Des Plines, IL, USA). All tissue sections were pretreated, digested and washed as recommended by the probes supplier. A minimum of 50 non-overlapping intact interphase nuclei was assessed for the presence of amplification and were analyzed by two observers blinded to the original diagnosis. The hybridized slides were reviewed and the ratio of *MYC* (red) and CEP8 (green) signals was calculated. MYC/CEP8 ratio of 2.0 or higher defined *MYC* amplification.

When comparing characteristics between primary, secondary angiosarcomas with *MYC* amplification and secondary angiosarcomas without *MYC* amplification, for categorical variables we used the Fisher's exact test or the Chi-square test. For continuous variables, the non-parametric median two-sample test was used. Disease-free survival (DFS) was calculated from the date of diagnosis of angiosarcoma to any local, regional, distant relapse or death from any cause, whichever occurred first, or to last visit date in case of no events. Overall survival (OS) was defined as the time interval from date of diagnosis of angiosarcoma to death from any cause or to last date of follow-up. DFS and OS were calculated with the Kaplan–Meier method and compared across different subgroups by means of the Log-rank test or Log-rank test for trend, as appropriate. Multivariable Cox regression models were used to adjust the effect of the different types of angiosarcoma on survival. Variables that were significant in the univariate analysis were tested in the multivariable models and only significant or borderline significant

(P<0.10) variables in the multivariable models were included in the final model. Hazard ratios (HR) and 95% confidence intervals (CI) were reported. All analyses were carried out with the Statistical Analysis System (SAS) software (SAS Institute, Cary, NC) and the R (http://cran.r-project.org/) software. All the reported P values were two sided.

Informed consent was obtained from all patients and/or guardians and institutional review board approvals were obtained for all parts of the study.

RESULTS

Clinicopathological features

Forty-nine patients were diagnosed with breast angiosarcoma. Of these 49 patients diagnosed with breast angiosarcoma, thirty-seven patients had a previous history of breast carcinoma treated with radiation therapy (Figure 1d), and twelve patients had a diagnosis of primary (sporadic) angiosarcoma. Clinicopathological features from 28 cases of AS included in the present series were previously described [3]. Thirty patients were diagnosed with post-radiation AVL of the breast (Figure 1a), but one patient was excluded from this study because there was no more representative tumor block available for *MYC* analysis. Clinicopathological details of these patients with AVL were published previously [11]. The clinicopathological characteristics of the three study groups are summarized in Tables 1, 2, and 3.

Immunohistochemical data

Immunohistochemical stains for MYC protein were performed on all cases of AVL and AS. None of the 29 cases of AVL displayed nuclear immunoreactivity for *MYC* (Table

1, Figure 1b). Twenty specimens of secondary angiosarcoma (54%) stained positive for *c-myc* (20/37 cases= 54%), with strong positive staining ('3+' or >51% positive) observed in 10 cases (10/20 cases=50%) (Table 3, Figure 1e). None of the PAS cases showed MYC protein expression (Table 2).

Fluorescence in situ hybridization (FISH)

None of the 29 cases of AVL (Figure 1c) or PAS showed *MYC* amplification (Tables 1 and 2). Twenty out of 37 cases (54%) of SAS showed high-level *MYC* amplification using interphase FISH analysis (Table 3, Figure 1f). Of 3 patients with secondary angiosarcoma with epithelioid features, 2 cases showed *MYC* amplification and MYC protein overexpression.

Concordance between FISH and IHC

Concordance between protein expression (IHC) and *MYC* amplification was 100% in AVL, PAS and SAS. The two cases of AVL that progressed to AS (Cases 22 and 23, Table 1) showed no *MYC* amplification or protein overexpression both at diagnosis of AVL, as at the time of diagnosis of AS (Cases 4 and 11, Table 3).

***MYC* amplification/protein overexpression as a prognostic factor**

Clinical follow-up data of patients with diagnosis of breast angiosarcoma were available for 48 of the 49 patients (median 32 months, range from 1 to 163 months).

There was no correlation between histological tumor grade and *MYC* amplification (P=0.365). No significant association of tumor size and *MYC* amplification was found (P=0.289).

When comparing DFS between PAS, SAS without *MYC* amplification and SAS with *MYC* amplification, there were statistically significant differences at both univariate and multivariable analyses (P=0.033, Figure 2 and Table 5; P=0.016; Table 6a). When comparing OS between PAS, SAS without *MYC* amplification and SAS positive for *MYC* amplification, there were statistically significant differences only at multivariable analyses (P=0.084, Figure 3 and Table 5; P=0.012; Table 6a). When limiting the survival analysis to the SAS patients, we observed that cases with *MYC* amplification had a significantly worse OS compared to cases without *MYC* amplification (size and grade-adjusted HR: 3.47 (1.09-11.1)). There was a non-significant trend toward a poor DFS between cases with and without *MYC* amplification (size and grade =adjusted HR: 1.89 (0.78-4.55), Table 6b).

Other well established disease parameters (tumor size and tumor grade) were also independently predictive of worse outcome in both primary and secondary angiosarcomas. Patients that presented tumor size > 5cm had a short-term DFS (*P*=0.054) and poor OS (*P*=0.020) when compared with cases showing tumor size ≤ 5cm in multivariable analysis (Table 6a). High-grade tumors were associated with worse DFS and OS (*P*=0.056 and *P*=0.018, respectively; Table 6a) when compared with low-grade tumors.

MYC amplification, however, had no significant association with a shorter latency period (time from radiation therapy to the diagnosis of secondary angiosarcoma) in cases with versus in cases without *MYC* amplification (*P*=0.161).

DISCUSSION

MYC amplification has been described in different solid tumors. *MYC* high-level amplification has been reported as a prominent feature of radiation-induced angiosarcomas and is also prevalent in other radiation-induced sarcomas, suggesting a strong association between irradiation and *MYC* gene amplification [19]. The perspective of *MYC* as an important anticancer target determines the importance to understand the specific role of *MYC* in different subsets of sarcomas [20, 21].

Our study evaluated the largest series of *MYC* amplification in the largest series of primary and secondary angiosarcomas and AVL of the breast, and it is the unique study that explored exclusively vascular lesions occurring within the breast. In our study, *MYC* amplification was detected in 54% of secondary breast angiosarcomas. Two other studies found 55% and 67% of *MYC* amplification in secondary angiosarcomas, respectively [14, 22]. We did not find *MYC* amplification or protein overexpression in any case of AVL or primary angiosarcoma of the breast, and our results confirm previous findings of other series [5, 10, 13-15].

Some studies however, have found that a high level of amplification of *MYC* on chromosome 8q24-21 is present in 100% of the patients with post-radiation angiosarcoma and lymphedema-associated angiosarcoma, but not in AVL and primary angiosarcoma [5, 10, 13, 15], suggesting *MYC* analysis as a crucial diagnostic tool in the setting of vascular lesions. All these studies, however, involved small case series. They also studied cases of angiosarcoma from nonmammary sites and lymphedema-associated angiosarcoma all together, and this fact may represent a selection bias. Kacker et al. found 58% frequency of *MYC* high-level amplifications in their series of radiation-induced angiosarcomas, including sarcomas of the breast but they also

included AS of other organs. When only AS of the breast were counted, the frequency of *MYC* high-level amplifications was 86% [19].

Based on our findings, the diagnostic usefulness of interphase and *MYC* immunohistochemistry in distinguishing low-grade secondary angiosarcoma from AVL is limited due to the low sensitivity of these assays. Indeed, a negative result does not exclude the diagnosis of angiosarcoma.

In our series, none of the 12 primary angiosarcoma of the breast showed *MYC* amplification. Recent studies, however, have shown a small subset of primary angiosarcoma that also presents *MYC* amplification, suggesting that genomic amplification of *MYC* is not restricted to secondary AS, as previously recognized [10, 12, 22]. Shon et al. detected *MYC* abnormalities in a small number of primary cutaneous angiosarcomas, but they included male and female patients with angiosarcomas from nonmammary skin [12]. Italiano et al. found *MYC* amplification in three out of the six primary cases of angiosarcoma (2 out of the 3 cases occurring within the breast) [22]. It seems that the absence of high-level gene amplifications does not exclude a possible role of *MYC* in the pathogenesis of primary angiosarcomas. We can also hypothesize that *MYC* amplification is not a specific genomic aberration induced by ionizing radiation in secondary angiosarcomas, as *MYC* amplification has also been shown even in lymphedema-associated angiosarcomas [10, 13-15].

Two patients of our series with initial diagnosis of AVL showed progression to high-grade cutaneous angiosarcoma (Cases 22 and 23, Table 1). Both at diagnosis of AVL, as at the time of diagnosis of AS, no *MYC* amplification and protein overexpression were observed. Santi et al. found a common mutational pathway (mutational inactivation of *TP53* gene) among AVL and AS, suggesting that they are biologically related entities

and could represent the extremes of a morphological continuum [23]. Most studies that explored *MYC* amplification status cast doubt on this hypothesis, since, to date, no case of *MYC* amplification in AVL has been identified [13, 15]. However, based on our findings, the hypothesis that AVL may represent a precursor lesion should not be discarded based only on *MYC* amplification status, since not all cases of post-radiation angiosarcoma shows *MYC* amplification.

We also found excellent FISH and IHC concordance in primary and secondary angiosarcomas, and AVL of the breast, confirming previous studies [5, 13]. Different from our results, Ginter et al. found a poor concordance (65%) between IHC and FISH for *MYC* in AS of nonmammary sites [10]. Shon et al. reported MYC protein overexpression in cases lacking gene amplification in primary cutaneous angiosarcomas, suggesting other mechanisms of *MYC* activation, but they also included cases of AS from other organs [12]. Therefore, we can conclude that *MYC* amplification and protein overexpression in angiosarcomas is a highly specific but low sensitive marker for the diagnosis of angiosarcomas of the breast and other nonmammary sites.

Our findings confirm the fact that secondary tumors have a worse prognosis when compared with primary disease, as we previously reported in a series addressing a smaller number of cases [3, 24]. We also found a significant association between *MYC* amplification and poor prognosis in secondary angiosarcomas of the breast. Previous studies have shown an association between gene amplification and/or protein overexpression of *MYC* and advanced stage in a variety of non-angiosarcoma human malignancies [12, 25], but none of the available studies that explored the prognostic role of *MYC* gene in angiosarcomas was able to find any association between *MYC* amplification and clinical prognosis or tumor grade [12, 14, 19, 22].

Our study is the first one to demonstrate a consistent association between *MYC* amplification status and clinical outcome on secondary angiosarcoma. We did not find any association between *MYC* amplification and tumor size, tumor grade or shorter latency period from radiation therapy and diagnosis of AS. To date, only the study of Kacker et al. found a non-significant statistical trend toward a shorter latency between primary tumor and sarcoma in cases with *MYC* amplification (p=0,2).

Data from current literature and our results demonstrate that the genetic and molecular aberrations involved in AS tumorigenesis remain poorly understood. The genetic heterogeneity of these tumors has been observed by other authors, and new potential genomic events have been investigated. Guo et al. identified *FLT4* gene coamplification with *MYC* in 25% of secondary angiosarcomas, but none of AVL and primary AS showed this abnormality [15]. Italiano et al. observed that the NOTCH pathway effector gene *MAML1* (5q35.3) is amplified and overexpressed in 18% of secondary angiosarcomas, in all these cases, coamplified with *FLT4*. They did not find any difference in clinical or pathologic characteristics between AS with and without 5q35 amplification [22].

Radiation-induced AS seems to be genetically different from primary angiosarcomas and AVL, but there is a clear evidence of genetic heterogeneity even among secondary cases. Therefore, further studies are necessary to identify the oncogenic trigger events of this subset of tumors.

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References

1. Billings SD, McKenney JK, Folpe AL, Hardacre MC, Weiss SW. Cutaneous angiosarcoma following breast-conserving surgery and radiation: an analysis of 27 cases. *Am J Surg Pathol* 2004;28(6):781-788.

2. Kirova YM, Vilcoq JR, Asselain B, Sastre-Garau X, Fourquet A. Radiation-induced sarcomas after radiotherapy for breast carcinoma: a large-scale single-institution review. *Cancer* 2005;104(4):856-863.

3. Fraga-Guedes C, Gobbi H, Mastropasqua MG, Botteri E, Luini A, Viale G. Primary and secondary angiosarcomas of the breast: a single institution experience. *Breast Cancer Res Treat* 2012;132(3):1081-1088.

4. Feller JK, Mahalingam M. c-myc and cutaneous vascular neoplasms. *Am J Dermatopathol* 2013;35(3):364-369.

5. Fernandez AP, Sun Y, Tubbs RR, Goldblum JR, Billings SD. FISH for MYC amplification and anti-MYC immunohistochemistry: useful diagnostic tools in the assessment of secondary angiosarcoma and atypical vascular proliferations. *J Cutan Pathol* 2011;39(2):234-242.

6. Patton KT, Deyrup AT, Weiss SW. Atypical vascular lesions after surgery and radiation of the breast: a clinicopathologic study of 32 cases analyzing histologic heterogeneity and association with angiosarcoma. *Am J Surg Pathol* 2008;32(6):943-950.

7. Brenn T, Fletcher CD. Radiation-associated cutaneous atypical vascular lesions and angiosarcoma: clinicopathologic analysis of 42 cases. *Am J Surg Pathol* 2005;29(8):983-996.

8. Mattoch IW, Robbins JB, Kempson RL, Kohler S. Post-radiotherapy vascular proliferations in mammary skin: a clinicopathologic study of 11 cases. *J Am Acad Dermatol* 2007;57(1):126-133.

9. Lucas DR. Angiosarcoma, radiation-associated angiosarcoma, and atypical vascular lesion. *Arch Pathol Lab Med* 2009;133(11):1804-1809.

10. Ginter PS, Mosquera JM, MacDonald TY, D'Alfonso TM, Rubin MA, Shin SJ. Diagnostic utility of MYC amplification and anti-MYC immunohistochemistry in atypical vascular lesions, primary or radiation-induced mammary angiosarcomas, and primary angiosarcomas of other sites. *Hum Pathol* 2013;45(4):709-716.

11. Fraga-Guedes C, Gobbi H, Mastropasqua MG, et al. Clinicopathological and immunohistochemical study of 30 cases of post-radiation atypical vascular lesion of the breast. *Breast Cancer Res Treat* 2014;146(2):347-354.

12. Shon W, Sukov WR, Jenkins SM, Folpe AL. MYC amplification and overexpression in primary cutaneous angiosarcoma: a fluorescence in-situ hybridization and immunohistochemical study. *Mod Pathol* 2014;27(4):509-515.

13. Mentzel T, Schildhaus HU, Palmedo G, Buttner R, Kutzner H. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. *Mod Pathol* 2012;25(1):75-85.

14. Manner J, Radlwimmer B, Hohenberger P, et al. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. *Am J Pathol* 2010;176(1):34-39.

15. Guo T, Zhang L, Chang NE, Singer S, Maki RG, Antonescu CR. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. *Genes Chromosomes Cancer* 2011;50(1):25-33.

16. Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. *Nat Rev Cancer* 2002;2(10):764-776.

17. Sheen JH, Dickson RB. Overexpression of c-Myc alters G(1)/S arrest following ionizing radiation. *Mol Cell Biol* 2002;22(6):1819-1833.

18. Fineberg S, Rosen PP. Cutaneous angiosarcoma and atypical vascular lesions of the skin and breast after radiation therapy for breast carcinoma. *Am J Clin Pathol* 1994;102(6):757-763.

19. Kacker C, Marx A, Mossinger K, et al. High frequency of MYC gene amplification is a common feature of radiation-induced sarcomas. Further results from EORTC STBSG TL 01/01. *Genes Chromosomes Cancer* 2013;52(1):93-98.

20. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 2011;146(6):904-917.

21. Soucek L, Whitfield J, Martins CP, et al. Modelling Myc inhibition as a cancer therapy. *Nature* 2008;455(7213):679-683.

22. Italiano A, Thomas R, Breen M, et al. The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification. *Genes Chromosomes Cancer* 2012;51(6):569-578.

23. Santi R, Cetica V, Franchi A, et al. Tumour suppressor gene TP53 mutations in atypical vascular lesions of breast skin following radiotherapy. *Histopathology* 2011;58(3):455-466.

24. Toesca A, Spitaleri G, De Pas T, et al. Sarcoma of the breast: outcome and reconstructive options. *Clin Breast Cancer* 2012;12(6):438-444.

25. Nesbit CE, Tersak JM, Prochownik EV. MYC oncogenes and human neoplastic disease. *Oncogene* 1999;18(19):3004-3016.

FIGURE LEGENDS

Fig. 1a AVL of the breast skin showing circumscribed proliferation of vessels in the upper dermis, mild endothelial atypia and associated lymphocytic infiltration. Hematoxylin and eosin, x200.

Fig. 1b In a MYC-immunostained section of the same AVL, there was no nuclear staining in endothelial cells.

Fig. 1c FISH for *MYC* amplification showing no MYC amplification in AVL.

Fig. 1d Secondary AS with epithelioid morphology showing proliferation of atypical vessels. Hematoxylin and eosin, x200.

Fig. 1e Secondary AS showing nuclear MYC protein expression in proliferative tumor cells.

Fig. 1f FISH analysis showed high-level *MYC* gene amplification in the secondary AS.

Fig. 2 Disease-free survival according to type of angiosarcoma and MYC amplification

Fig. 3 Overall survival according to type of angiosarcoma and MYC amplification

Table 1: Clinicopathological, FISH and IHC data for atypical vascular lesion

#	Age	Latency interval *(months)	Location	Follow-up (months)	FISH	IHC
1	67	117	Breast skin	Alive NED (16)	NAMP	-
2	47	57	Breast skin	Dead of metastatic CRC(25)	NAMP	-
3	67	1	Axillary skin	Alive NED (25)	NAMP	-
4	43	19	Breast skin	Alive NED (93)	NAMP	-
5	54	31	Breast skin	Dead of metastatic BC (24)	NAMP	-
6	60	26	Breast skin	Alive NED (31)	NAMP	-
7	59	42	Breast skin	Alive NED (90)	NAMP	-
8	57	49	Breast skin	Alive NED (93)	NAMP	-
9	37	28	Breast skin	Alive, with recurrent AVL (26)	NAMP	-
10	47	124	Breast skin	Alive NED (27)	NAMP	-
11	81	124	Breast skin	Alive NED (36)	NAMP	-
12	67	48	Not available	Dead of cardiomyopathy (54)	NAMP	-
13	65	33	Breast skin	Alive NED (61)	NAMP	-
14	62	80	Breast skin	Alive NED (109)	NAMP	-
15	67	83	Breast skin	Alive NED (72)	NAMP	-
16	68	93	Breast skin	Alive NED (38)	NAMP	-
17	55	77	Breast skin	Alive NED (60)	NAMP	-
18	54	37	Breast skin	Alive NED (8)	NAMP	-
19	75	17	Breast skin	Alive NED (41)	NAMP	-
20	43	56	Breast skin	Lost to follow-up	NAMP	-
21	51	79	Breast skin	Dead of metastatic BC (18)	NAMP	-
22	**62**	**54**	**Breast skin**	**Alive, progression to AS (97)**	**NAMP**	**-**
23	**65**	**40**	**Breast skin**	**Dead, progression to AS (107)**	**NAMP**	**-**
24	75	34	Breast skin	Alive NED (5)	NAMP	-
25	73	146	Axillary skin	Alive NED (6)	NAMP	-
26	44	72	Breast skin	Alive NED (12)	NAMP	-
27	50	25	Breast skin	Alive NED (2)	NAMP	-
28	68	41	Breast skin	Alive NED (3)	NAMP	-
29	50	10	Breast skin	Alive NED (2)	NAMP	-

NED, no evidence of disease (AVL); BC,breast cancer; CRC, colorectal cancer; AS, angiosarcoma. NAMP, no amplification

*Latency interval between radiotherapy and development of AVL.

Table 2: Clinicopathological, FISH an IHC data for primary angiosarcomas (AS)

#	Age	Location	Tumor size	Tumor grade	FISH	IHC	Follow-up (months)
1	61	Breast	1.0cm	Intermediate	NAMP	-	Lost to follow-up
2	55	Breast	2.1cm	Low	NAMP	-	Alive NED (133)
3	38	Breast	2.2cm	Intermediate	NAMP	-	Alive NED (164)
4	34	Breast	3.0cm	Intermediate	NAMP	-	Alive NED (149)
5	69	Breast	3.0cm	High	NAMP	-	Dead (53)
6	42	Breast	3.0cm	Low	NAMP	-	Alive LR (104)
7	42	Breast	10.0cm	High	NAMP	-	Alive NED (58)
8	36	Breast	8.0cm	High	NAMP	-	Dead MT (12)
9	30	Breast	15.0cm	Intermediate	NAMP	-	Dead MT (30)
10	45	Breast	14.0cm	Intermediate	NAMP	-	Dead (42)
11	77	Breast	10.0cm	High	NAMP	-	Dead LR (14)
12	59	Breast	10.0cm	High	NAMP	-	Alive NED (28)

NAMP, no amplification; NED, no evidence of disease (AS); LR, local recurrence; MT, metastasis

Table 3: Clinicopathological, FISH and immunohistochemical (IHC) data for secondary angiosarcomas (SAS)

#	Age	Location	Latency interval *(months)	Tumor size	Tumor grade	FISH	IHC	Follow-up (months)
1	69	Breast	18	1.4cm	Low	NAMP	-	Alive NED (159)
2	66	Breast	70	1.2cm	High	NAMP	-	Dead LR(48)
3	78	Breast	192	7.0cm	High	NAMP	-	Dead MT(9)
4	**64**	**Breast**	**79**	**5.5cm**	**High**	**NAMP**	-	**Alive NED (122)**
5	50	Breast	118	6.0cm	High	AMP	+	Dead (20)
6	48	Breast	105	0.7cm	Low	NAMP	-	Dead LR (31)
7	63	Breast	82	2.0cm	High	AMP	++	Dead LR (24)
8	81	Breast	66	2.0cm	High	AMP	+++	Dead LR (63)
9	43	Breast	283	9.5cm	Intermediate	NAMP	-	Alive LR (86)
10	80	Breast	48	6.0cm	Low	NAMP	-	Alive LR (81)
11	**73**	**Breast**	**134**	**8.0cm**	**High/Epithelioid**	**NAMP**	-	**Dead CR (32)**
12	46	Breast	111	2.5cm	Low	NAMP	-	Alive NED (71)
13	62	Breast	41	11.0cm	High/Epithelioid	AMP	+++	Dead MT(12)
14	66	Breast	104	NA	High	AMP	+	Dead MT (2)
15	77	Breast	120	4.2cm	High	AMP	+	Dead MT (19)
16	70	Breast	60	3.0cm	High	AMP	+++	Dead LR (13)
17	69	Breast	97	2.0cm	Low	AMP	+++	Alive NED (52)
18	37	Breast	51	5.5cm	High	NAMP	-	Alive LR (65)
19	88	Breast	113	2.0cm	High	AMP	+	Dead LR (25)
20	73	Breast	118	8.9cm	High/Epithelioid	AMP	+	Dead LR (8)
21	60	Breast	90	3.5cm	Low	AMP	+	Alive LR (34)
22	40	Breast	82	3.0cm	High	AMP	+++	Alive LR (22)
23	66	Breast	71	4.0cm	High	AMP	+++	Dead MT (25)
24	71	Breast	53	5.5cm	Low	AMP	+	Alive LR (40)
25	70	Breast	146	2.5cm	High	AMP	+	Alive MT (32)
26	81	Breast	60	14.0cm	Low	AMP	+++	Alive NED (66)
27	56	Breast	118	20.0cm	Intermediate	NAMP	-	Dead of disease (35)
28	77	Breast	71	10.0cm	High	NAMP	-	Dead of disease (37)
29	77	Breast	135	6.0cm	Intermediate	AMP	++	Dead of disease (8)
30	67	Breast	138	17.0cm	High	NAMP	-	Dead of disease (1)
31	64	Breast	331	7.0cm	Intermediate	NAMP	-	Dead of disease (4)

#	Age	Location	Latency interval *(months)	Tumor size	Tumor grade	FISH	IHC	Follow-up (months)
32	79	Breast	93	4.5cm	Low	NAMP	-	Alive NED (21)
33	66	Breast	75	12.0cm	High	AMP	+++	Dead of disease (6)
34	84	Breast	106	5.0cm	Low	AMP	+++	Alive NED (12)
35	72	Breast	93	7.0cm	Intermediate	AMP	+++	Dead of disease (17)
36	79	Breast	87	3.0cm	Intermediate	NAMP	-	Dead of disease (133)
37	69	Breast	261	14.0cm	Low	NAMP	-	Alive NED (2)

NA, not available; NAMP, no amplification; AMP, amplification; NED, no evidence of disease (AS); LR, local recurrence; MT, metastasis; CR, contralateral recurrence

* Latency interval between radiotherapy and development of AS. **Cases 4 and 11 (highlighted) had previous diagnosis of AVL that progressed to AS.**

Table 4. Population characteristics

Variable	*Classification*	***PAS***	***SAS*** **MYC AMP negative**	***SAS*** **MYC AMP positive**	**P-*value***	**P-*value*[a]**
		No. (col %)	***No. (col %)***	***No. (col %)***		
All patients		*12 (100.0)*	*17 (100.0)*	*20 (100.0)*		
Age (years)	***Median (range)***	*44 (30-77)*	*67 (37-80)*	*70 (40-88)*	*0.027*	*0.278*
	≤ 60	*9 (75.0)*	*5 (29.4)*	*3 (15.0)*	*0.020*	*0.637*
	61-70	*2 (16.7)*	*6 (35.3)*	*8 (40.0)*		
	> 70	*1 (8.3)*	*6 (35.3)*	*9 (45.0)*		
Diameter (cm)	***Median (range)***	*5.5 (1.0-15.0)*	*6.0 (0.7-20.0)*	*4.2 (2.0-14.0)*	*0.460*	*0.210*
	≤ 2	*1 (8.3)*	*3 (17.7)*	*3 (15.8)*	*0.524*	*0.289*
	2.1-5	*5 (41.7)*	*3 (17.7)*	*8 (42.1)*		
	>5	*6 (50.0)*	*11 (64.7)*	*8 (42.1)*		
	Missing	*0 (-)*	*0 (-)*	*1 (-)*		
Grade	***1***	*2 (16.7)*	*6 (35.3)*	*5 (25.0)*	*0.254*	*0.365*
	2	*5 (41.7)*	*4 (23.5)*	*2 (10.0)*		
	3	*5 (41.7)*	*7 (41.2)*	*13 (65.0)*		
Time from primary radiotherapy (years)	***Median (range)***	*-*	*9.3 (1.5-27.7)*	*7.2 (3.4-12.2)*	*-*	*0.260*
	< 7	*-*	*5 (29.4)*	*8 (40.0)*	*-*	*0.161*
	7 - 10	*-*	*5 (29.4)*	*9 (45.0)*		
	> 10	*-*	*7 (41.2)*	*3 (15.0)*		

PAS, primary angiosarcoma; SAS, secondary angiosarcoma; *MYC AMP*, *MYC* amplification. [a]Among secondary angiosarcomas only. Percentage calculations did not include missing values. Age and time from radiotherapy were categorized in tertiles, diameter according to standard categorization.

Table 5. Univariate survival analysis

Variable	*Classification*	*At risk No.*	*DFS: Events (5-year survival)*	**P-value**	*OS: Events (5-year survival)*	**P-value**
Age (years)	***≤ 60***	*17*	*11 (33.6)*	*0.158*	*6 (60.1)*	*0.035*
	61-70	*15*	*10 (24.1)*		*10 (23.0)*	
	> 70	*16*	*13 (17.1)*		*11 (38.4)*	
Diameter (cm)	***≤ 5***	*22*	*13 (40.5)*	*0.044*	*10 (56.0)*	*0.045*
	>5	*25*	*20 (14.7)*		*16 (30.7)*	
Grade	***1***	*13*	*5 (54.0)*	*0.006*	*1 (90.0)*	*<0.001*
	2	*10*	*8 (30.0)*		*7 (40.0)*	
	3	*25*	*21 (8.8)*		*19 (20.5)*	
Time from primary radiotherapy (years)	***< 7***	*13*	*10 (23.1)*	*0.445*	*7 (52.8)*	*0.092*
	7 - 10	*14*	*10 (30.6)*		*8 (36.5)*	
	> 10	*10*	*8 (11.3)*		*7 (22.5)*	
Angiosarcoma group	***PAS***	*11*	*6 (39.8)*	*0.033*	*5 (49.1)*	*0.084*
	***SAS* MYC AMP neg**	*17*	*12 (29.3)*		*9 (47.9)*	
	***SAS* MYC AMP pos**	*20*	*16 (15.6)*		*13 (35.9)*	

PAS, primary angiosarcoma; SAS, secondary angiosarcoma; *MYC AMP*, *MYC* amplification; neg, negative for MYC amplification; pos, positive for MYC amplification.

Table 6a. Multivariable analysis in all patients

Variable	***Comparison***	***DFS*** ***HR (95% CI)***	**P-** ***value***	***OS*** ***HR (95% CI)***	**P-** ***value***
Diameter	***> 5 vs ≤ 5***	*2.15 (0.99-4.69)*	*0.054*	*3.15 (1.20-8.26)*	*0.020*
Grade	***2 vs 1***	*2.46 (0.76-7.97)*	*0.056*	*15.9 (1.79-141)*	*0.018*
	3 vs 1	*3.42 (1.25-9.35)*		*19.6 (2.50-154)*	
Angiosarcoma group	***SAS* MYC AMP neg *vs* PAS**	*2.31 (0.86-6.24)*	*0.016*	*1.44 (0.47-4.43)*	*0.012*
	***SAS* MYC AMP pos *vs* PAS**	*4.56 (1.62-12.8)*		*5.69 (1.67-19.4)*	

SAS, secondary angiosarcoma; *MYC AMP neg*, negative for *MYC* amplification; *MYC AMP pos*, positive for *MYC* amplification; PAS, primary angiosarcoma.

Table 6b. Multivariable analysis in secondary angiosarcoma patients (SAS)

Variable	***Comparison***	***DFS*** ***HR (95% CI)***	**P-*value***	***OS*** ***HR (95% CI)***	**P-*value***
Diameter	***> 5 vs ≤ 5***	*2.14 (0.89-5.21)*	*0.090*	*2.92 (0.99-8.68)*	*0.053*
Grade	***2 vs 1***	*2.67 (0.72-9.91)*	*0.055*	*13.6 (1.46-125)*	*0.030*
	3 vs 1	*3.90 (1.29-11.8)*		*16.7 (2.09-133)*	
MYC AMP	**Pos *vs* Neg**	*1.89 (0.78-4.55)*	*0.155*	*3.47 (1.09-11.1)*	*0.035*

MYC AMP Pos, positive for *MYC* amplification; MYC AMP *Neg*, negative for MYC amplification.

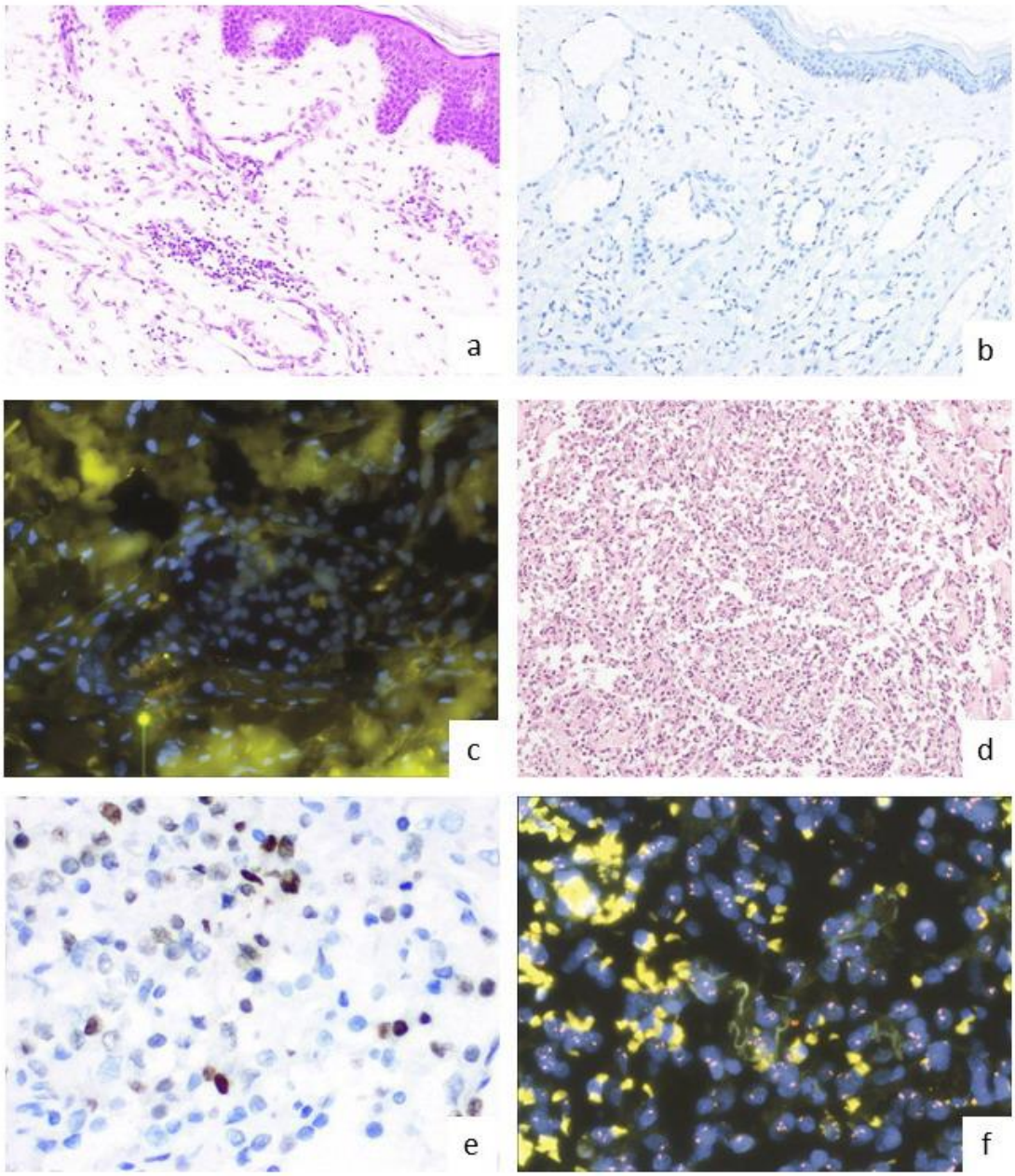


Figure 1: a) AVL of the breast skin showing circumscribed proliferation of vessels in the upper dermis, mild endothelial atypia and associated lymphocytic infiltration. HE, x200; b) in a MYC-immunostained section of the same AVL, there was no nuclear staining in endothelial cells; c) FISH for *MYC* amplification showing no MYC amplification in AVL; d) Secondary AS with epithelioid morphology showing proliferation of atypical vessels. HE, x200; e) secondary AS showing nuclear MYC protein expression in proliferative tumor cells; f) FISH analysis showed high-level *MYC* gene amplification in the secondary AS.

Figure 2. Disease-free survival according to type of angiosarcoma and *MYC* amplification

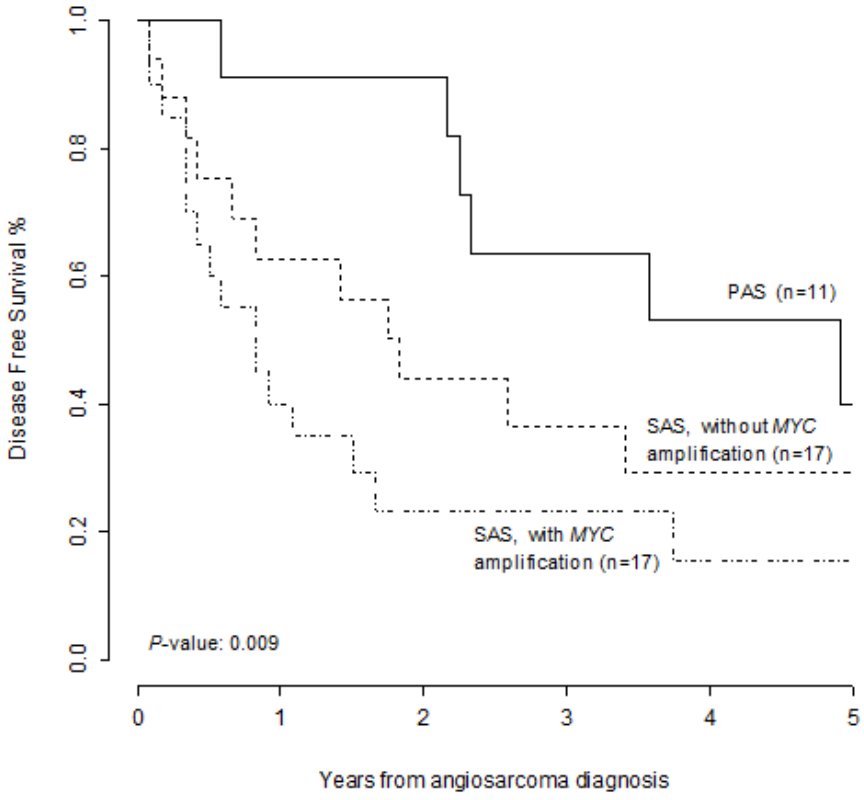
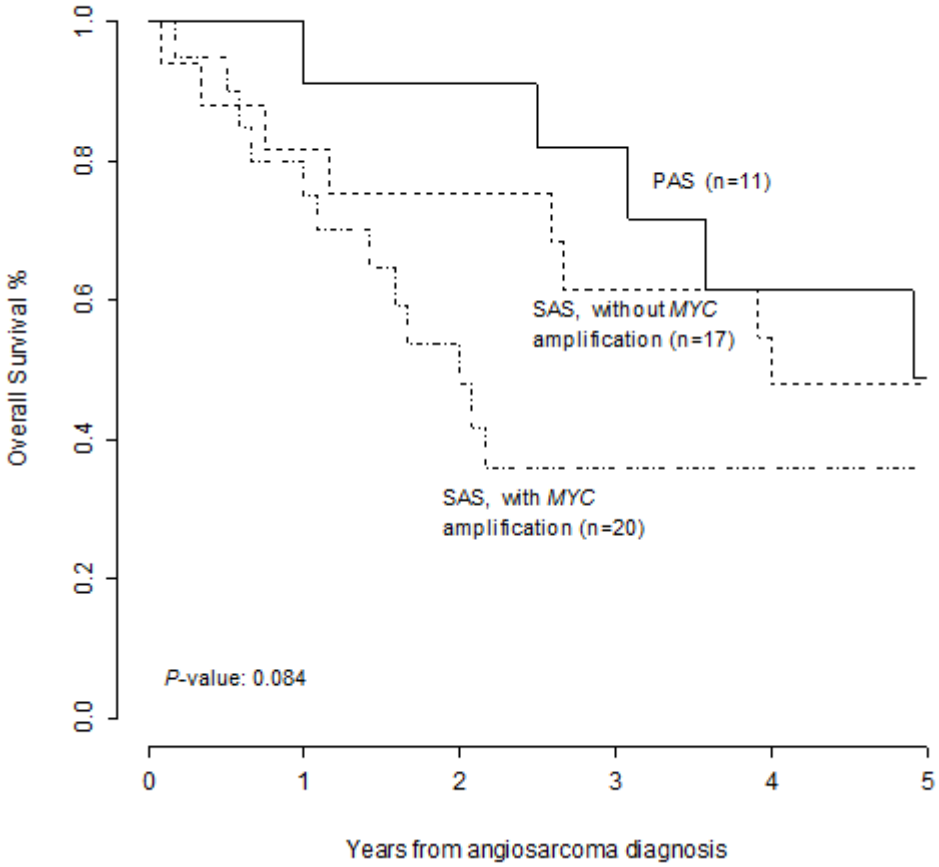


Figure 3. Overall survival according to type of angiosarcoma and MYC amplification



5. CONSIDERAÇÕES FINAIS

Nossos resultados mostraram que LVA são lesões que se apresentam em pacientes com mediana de idade de 58,5 anos, após um intervalo de latência entre a radioterapia e diagnóstico de cerca de 4 anos. Clinicamente, as LVA se apresentam como diminutas pápulas solitárias ou múltiplas no sítio de irradiação prévia da mama, e costumam ser bem circunscritas, sem extensão para o tecido subcutâneo e sem atipia significativa. A presença de infiltrado inflamatório é achado frequente, mas lagos de sangue, polimorfismo nuclear ou múltiplas camadas endoteliais não foram observados em nenhum dos nossos casos.

Identificamos três casos de LVA que surgiram na mama após o emprego de radioterapia intraoperatória, e estes foram os únicos casos descritos na literatura até a presente data. É válido, entretanto, ressaltar que, nestes casos, a pele da mama não foi poupada, uma vez que duas destas pacientes receberam dose de ataque de 12 Gy (*boost*) no intraoperatório, porém, realizou-se RT convencional complementar de 37 Gy na sequência do tratamento. Estas pacientes desenvolveram LVA após 8 e 10 meses da conclusão da RT, respectivamente. A terceira paciente recebeu a dose total de 16 Gy no complexo aréolo-papilar no contexto de adenomastectomia preservadora do CAP, tendo detectado LVA 72 meses após a realização da RT. O fato destas três pacientes terem desenvolvido LVA na mama pode, portanto, ser explicado pela radiação que atingiu a pele e em dose ainda mais concentrada de uma única vez. Há dois trabalhos na literatura que relataram a ocorrência de angiossarcoma após braquiterapia para câncer de mama com MammoSite®, técnica em que também há exposição da pele à radiação (ANDREWS et al., 2010; MANSFIELD; ZYNGER; AGNESE, 2014). Contudo, não há, até a presente data, nenhum registro na literatura de LVA ou angiossarcoma desenvolvido na mama no contexto de radioterapia intraoperatória de única dose (21Gy), técnica que classicamente poupa a pele da radiação. Este fato ratifica a importância da participação da RT da pele da mama no desenvolvimento destas lesões secundárias.

Dado relevante deste trabalho foi a progressão para AS de dois casos com diagnóstico prévio de LVA. Apesar deste achado já ter sido relatado por Brenn e Fletcher e Patton et al.(BRENN; FLETCHER, 2005; PATTON; DEYRUP; WEISS, 2008) nós tivemos a oportunidade de estudar estes dois casos também do ponto de vista molecular, que é inédito até o momento. Observamos que estes dois casos apresentaram características comuns, como a presença de margens comprometidas quando da realização da biópsia

cutânea (*punch biopsy)* da LVA, a expressão do marcador Ki-67 (>10%), assim como ausência de amplificação do *MYC*, tanto no momento do diagnóstico de LVA, quanto no momento do diagnóstico do AS. O Ki-67 é um marcador de proliferação celular já bem estudado em carcinoma de mama e que está relacionado à maior agressividade tumoral (INIC et al., 2014; NISHIMURA et al., 2014). Um questionamento que poderia ser levantado seria a possibilidade destes casos se tratarem, na verdade, de angiossarcomas, subamostrados e subdiagnosticados no momento da biópsia cutânea que identificou a LVA. Porém, ambos os casos evoluíram para AS de alto grau histológico 19 e 89 meses após o diagnóstico de LVA, respectivamente. Seria pouco provável que um AS de alto grau fosse confundido com uma LVA, ainda que em uma biópsia de pequeno fragmento tecidual. Além disso, angiossarcomas são tumores muito agressivos, e mesmo os de baixo grau histológico tendem a ter um crescimento rápido e sinais clínicos expressivos. A ausência de amplificação do *MYC* no momento do diagnóstico de LVA é outro fato que corrobora com seu diagnóstico, ainda que não afaste por completo a possibilidade de AS.

A importância das margens cirúrgicas livres também pôde ser verificada no único caso de recidiva de LVA que observamos neste estudo. A paciente foi submetida à biópsia cutânea quando teve diagnóstico de LVA, e este foi o único tratamento oferecido. Trinta e dois meses após o diagnóstico de LVA, a paciente apresentou outras duas lesões no sítio da biópsia prévia. Tendo em vista os três casos de LVA com margens comprometidas que tiveram prognóstico desfavorável, a despeito da biópsia cutânea ter sido um tratamento suficiente em 93,3% dos nossos casos, sugerimos ressecção completa da LVA, com margens livres de doença.

Outra contribuição de nosso trabalho foi a caracterização imuno-histoquímica das LVA. Mostramos que o uso de marcador D2-40 com a finalidade de subdividir estas lesões em LVA tipo-linfático e LVA tipo-vascular segundo a proposta de Patton et al. (PATTON; DEYRUP; WEISS, 2008) não é de utilidade na prática clínica, uma vez que identificamos evolução desfavorável em ambos os tipos de LVA (um caso de progressão para AS era do tipo linfático, e o outro era do tipo vascular). Todavia, conseguimos identificar que 20% dos nossos casos apresentavam baixa expressão de Ki-67 e 17% apresentavam intermediária ou alta expressão deste marcador. O único estudo disponível na literatura sobre a expressão do Ki-67 em LVA foi o de Requena et al., que mostraram negatividade para este marcador em todos os casos de LVA que analisaram (REQUENA et al., 2002). De acordo com nossos resultados, o estudo do Ki-67 em LVA não pode ser usado para

fins de prognóstico ou diagnóstico diferencial com angiossarcomas, uma vez que outros casos que apresentaram maior expressão do Ki-67 tiveram um comportamento clínico benigno.

Durante a revisão das lâminas, pudemos constatar a dificuldade em diferenciar alguns casos de LVA de angiossarcomas de baixo-grau histológico, sobretudo em fragmentos de biópsia muito diminutos. Mesmo seguindo os critérios de Fineberg e Rosen (FINEBERG; ROSEN, 1994), em alguns casos, o diagnóstico diferencial baseado nas características histopatológicas foi difícil. Estas lesões guardam semelhanças entre si, daí a necessidade de se encontrar marcadores moleculares e/ou imuno-histoquímicos que possam auxiliar o patologista nesta diferenciação.

Não conseguimos identificar nenhum marcador que pudesse oferecer um diagnóstico inequívoco de LVA. Estudamos a expressão da endoglina (CD105), avaliado pela primeira vez na literatura nestes casos. Hara H relatou forte expressão deste marcador nas células endoteliais de angiossarcomas cutâneos (HARA, 2012). Contudo, obtivemos positividade para o CD105 em quase todos os casos de LVA (83%), porém com marcação, além de células endoteliais das lesões, também das células endoteliais de vasos normais do estroma adjacente. Realizamos a IHQ com dois clones diferentes para CD105 (clones 8A1 e SN6) e o desfecho se repetiu. É possível que a alta sensibilidade destes clones tenha interferido e superestimado o resultado encontrado, porém, devido à inespecificidade da marcação, em nosso trabalho, o CD105 não se mostrou um marcador útil.

Nos últimos cinco anos, surgiram alguns trabalhos que pareciam, finalmente, ter encontrado importante ferramenta diagnóstica para diferenciação das proliferações vasculares pós-radioterapia: o estudo da expressão protéica do produto do gene *MYC* por IHQ ou a amplificação do *MYC* pelo FISH. Alguns autores apontaram a amplificação do *MYC* como condição exclusiva dos angiossarcomas secundários, afirmando que, quando presente, descartaria o diagnóstico de LVA e angiossarcomas primários, uma vez que encontraram amplificação em 100% dos tumores secundários e não identificaram nenhum caso de amplificação do *MYC* em LVA nem em angiossarcomas primários (FERNANDEZ et al., 2011; GUO et al., 2011; MENTZEL et al., 2012). Esta descrição motivou estudos posteriores que investigarem a importância do *MYC* em LVA e AS, mas resultados conflitantes vêm sendo descritos.

Nosso trabalho avaliou a maior casuística de LVA e angiossarcomas da mama que foram submetidos ao estudo do *MYC*. Ao contrário de outros trabalhos que apontaram 100% dos ASS com *MYC* amplificado, detectamos amplificação do *MYC* em 54% dos nossos casos de ASS. Dois outros trabalhos demonstraram 55% e 67% de amplificação do *MYC* em ASS, respectivamente (MANNER et al., 2010; ITALIANO et al., 2012). Não identificamos amplificação do *MYC* em nenhum dos casos de LVA ou ASP da mama, confirmando os achados prévios de outras séries de casos (MANNER et al., 2010; FERNANDEZ et al., 2011; GUO et al., 2011; MENTZEL et al., 2012; GINTER et al., 2013). Diante destas observações, questionamos a utilidade do estudo do *MYC* por IHQ ou pelo FISH para distinção entre LVA e AS de baixo grau histológico, uma vez que, além de implicar em aumento de custos, um resultado negativo para amplificação do *MYC* não exclui o diagnóstico de AS.

Ainda que não haja nenhum caso de LVA mostrando amplificação do *MYC* descrito na literatura até a presente data, trabalhos recentes apontaram casos de amplificação do *MYC* em ASP (ITALIANO et al., 2012; GINTER et al., 2013; SHON et al., 2014). Este dado sugere que a amplificação do *MYC* não é achado restrito aos ASS como se pensava, e pode-se inferir um possível papel do *MYC* também na patogênese dos ASP. Ainda mais relevante, podemos levantar a hipótese de que a alteração genômica do *MYC* não seja um fenômeno específico induzido pela radiação ionizante, uma vez que casos de angiossarcomas secundários a linfedema crônico (Síndrome de Stewart-Treves) apresentando amplificação do *MYC* também já foram descritos na literatura (MANNER et al., 2010; GUO et al., 2011; MENTZEL et al., 2012; GINTER et al., 2013).

Nosso estudo foi o único a conseguir demonstrar o papel do *MYC* no prognóstico dos casos de AS que apresentaram amplificação. Os poucos trabalhos disponíveis na literatura que avaliaram sobrevida *versus* amplificação do *MYC* em AS não encontraram nenhuma associação com a evolução clínica dos pacientes estudados (MANNER et al., 2010; ITALIANO et al., 2012; KACKER et al., 2013; SHON et al., 2014). Nós observamos piora significativa da sobrevida global entre os casos de ASS com *MYC* amplificado *versus* os que não apresentaram amplificação, além de uma tendência não-significativa a pior sobrevida livre de doença nos casos com amplificação do *MYC*. Os ASS apresentam pior prognóstico que os ASP confirmando achados prévios de nosso grupo e agora com maior número de casos (FRAGA-GUEDES et al., 2012). Demonstramos ainda que o grau histológico e o diâmetro tumoral são também fatores prognósticos em angiossarcomas.

A despeito do conceito vigente de que as LVA são lesões benignas, de bom prognóstico, o fato de dois de nossos casos de LVA terem progredido para AS é um achado relevante do nosso estudo, e levanta discussão sobre a patogênese destas lesões e seu potencial como possível lesão precursora de AS. Estudando a amplificação do *MYC* em ambos os casos, não identificamos amplificação nas LVA nem nos AS que surgiram posteriormente. Portanto, não podemos afirmar nem descartar a possibilidade de LVA serem lesões precursoras de AS baseados no estudo do gene *MYC*.

Nossos resultados acrescentam informações importantes à literatura, uma vez que utilizamos critérios de seleção de pacientes mais rígidos, que diminuem a possibilidade de vieses de seleção. Todas as pacientes foram do sexo feminino, todos os tumores foram restritos às mamas, e tanto LVA quanto ASS se desenvolveram no contexto de radioterapia prévia para carcinoma mamário. Não incluímos casos de Síndrome de Stewart- Treves, assim como tumores de outros sítios além das mamas como todos os demais trabalhos já publicados que avaliaram a amplificação do *MYC* nestas proliferações vasculares. Da mesma forma, nossos achados também devem ser avaliados com cautela na tentativa de reproduzi-los quando no contexto de proliferações vasculares de outros sítios além das mamas e em demais tipos de sarcomas que não foram o foco do nosso trabalho.

Por fim, concluímos que as alterações genéticas e moleculares envolvidas na patogênese dos AS e LVA ainda não estão bem esclarecidas. Nossos achados, assim como os achados da literatura, sugerem a possibilidade de LVA e AS se tratarem de lesões geneticamente distintas, mas também alertam para a importante heterogeneidade que existe mesmo entre os angiossarcomas secundários e primários da mama. Novos estudos são necessários para identificar outros possíveis eventos genômicos que consigam explicar a patogênese deste grupo de lesões vasculares da mama.

6- CONCLUSÕES

a) A mediana de idade das pacientes com LVA foi 58,5 anos, após intervalo de latência entre a RT e diagnóstico de 4 anos. LVA se apresentaram como pápulas circunscritas, solitárias ou múltiplas no sítio de irradiação prévia da mama. A maioria é positiva para CD31, D2-40 e CD105, com baixa expressão de Ki-67 e com evolução benigna, entretanto, dois casos progrediram para AS.

b) Amplificação do *MYC* foi encontrada apenas em ASS (54% dos casos), sendo marcador de alta especificidade e baixa sensibilidade para ASS, porém um resultado negativo não descarta a possibilidade de ASS.

c) Houve 100% de concordância entre as técnicas de FISH e IHQ para o *MYC* em LVA, ASP e ASS, porém, devido à baixa sensibilidade dos dois métodos, sua utilização no diagnóstico diferencial entre LVA e AS de baixo-grau não é recomendada.

d) A amplificação do MYC nos ASS correlacionou-se com pior sobrevida global e tendência não-significativa a pior sobrevida livre de doença.

7- BIBLIOGRAFIA

ANDREWS, S.et al. Angiosarcoma following MammoSite partial breast irradiation. **Breast Cancer Res Treat**, v.124, n.1, p.279-82, Nov. 2010.

BAUDINO, T. A.et al. c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression. **Genes Dev**, v.16, n.19, p.2530-43, Oct 1. 2002.

BERNS, E. M.et al. Prognostic factors in human primary breast cancer: comparison of c-myc and HER2/neu amplification. **J Steroid Biochem Mol Biol**, v.43, n.1-3, p.13-9, Sep. 1992.

BISWAS, T.et al. Angiosarcoma of the breast: a rare clinicopathological entity. **Am J Clin Oncol**, v.32, n.6, p.582-6, Dec. 2009.

BRENN, T.; FLETCHER, C. D. Radiation-associated cutaneous atypical vascular lesions and angiosarcoma: clinicopathologic analysis of 42 cases. **Am J Surg Pathol**, v.29, n.8, p.983-96, Aug. 2005.

BRENN, T.; FLETCHER, C. D. Postradiation vascular proliferations: an increasing problem. **Histopathology**, v.48, n.1, p.106-14, Jan. 2006.

BRODIE, C.; PROVENZANO, E. Vascular proliferations of the breast. **Histopathology**, v.52, n.1, p.30-44, Jan. 2008.

CHEN, K. T.;KIRKEGAARD, D. D.; BOCIAN, J. J. Angiosarcoma of the breast. **Cancer**, v.46, n.2, p.368-71, Jul 15. 1980.

CLARKE, M.et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. **Lancet**, v.366, n.9503, p.2087-106, Dec 17. 2005.

COZEN, W.et al. The risk of angiosarcoma following primary breast cancer. **Br J Cancer**, v.81, n.3, p.532-6, Oct. 1999.

DALLA-FAVERA, R.et al. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. **Proc Natl Acad Sci U S A**, v.79, n.24, p.7824-7, Dec. 1982.

DARBY, S.et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. **Lancet**, v.378, n.9804, p.1707-16, Nov 12. 2011.

DELMORE, J. E.et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. **Cell**, v.146, n.6, p.904-17, Sep 16. 2011.

DI TOMMASO, L.; FABBRI, A. [Cutaneous angiosarcoma arising after radiotherapy treatment of a breast carcinoma. Description of a case and review of the literature]. **Pathologica**, v.95, n.4, p.196-202, Aug. 2003.

DIAZ-CASCAJO, C.et al. Benign lymphangiomatous papules of the skin following radiotherapy: a report of five new cases and review of the literature. **Histopathology**, v.35, n.4, p.319-27, Oct. 1999.

FELLER, J. K.; MAHALINGAM, M. c-myc and cutaneous vascular neoplasms. **Am J Dermatopathol**, v.35, n.3, p.364-9, May. 2013.

FERNANDEZ, A. P.et al. FISH for MYC amplification and anti-MYC immunohistochemistry: useful diagnostic tools in the assessment of secondary angiosarcoma and atypical vascular proliferations. **J Cutan Pathol**, v.39, n.2, p.234-42, Feb. 2011.

FINEBERG, S.; ROSEN, P. P. Cutaneous angiosarcoma and atypical vascular lesions of the skin and breast after radiation therapy for breast carcinoma. **Am J Clin Pathol**, v.102, n.6, p.757-63, Dec. 1994.

FRAGA-GUEDES, C.et al. Primary and secondary angiosarcomas of the breast: a single institution experience. **Breast Cancer Res Treat**, v.132, n.3, p.1081-8, Apr. 2012.

FRAGA-GUEDES, C.et al. Clinicopathological and immunohistochemical study of 30 cases of post-radiation atypical vascular lesion of the breast. **Breast Cancer Res Treat**, v.146, n.2, p.347-54, Jul. 2014.

FURY, M. G.et al. A 14-year retrospective review of angiosarcoma: clinical characteristics, prognostic factors, and treatment outcomes with surgery and chemotherapy. **Cancer J**, v.11, n.3, p.241-7, May-Jun. 2005.

GENGLER, C.et al. Vascular proliferations of the skin after radiation therapy for breast cancer: clinicopathologic analysis of a series in favor of a benign process: a study from the French Sarcoma Group. **Cancer**, v.109, n.8, p.1584-98, Apr 15. 2007.

GINTER, P. S.et al. Diagnostic utility of MYC amplification and anti-MYC immunohistochemistry in atypical vascular lesions, primary or radiation-induced mammary angiosarcomas, and primary angiosarcomas of other sites. **Hum Pathol**, v.45, n.4, p.709-16, Apr. 2013.

GLAZEBROOK, K. N.;MAGUT, M. J.; REYNOLDS, C. Angiosarcoma of the breast. **AJR Am J Roentgenol**, v.190, n.2, p.533-8, Feb. 2008.

GUO, T.et al. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. **Genes Chromosomes Cancer**, v.50, n.1, p.25-33, Jan. 2011.

HARA, H. Endoglin (CD105) and claudin-5 expression in cutaneous angiosarcoma. **Am J Dermatopathol**, v.34, n.7, p.779-82, Oct. 2012.

HELFRICH, Y. R.;SACHS, D. L.; VOORHEES, J. J. Overview of skin aging and photoaging. **Dermatol Nurs**, v.20, n.3, p.177-83; quiz 84, Jun. 2008.

HUNG, J.et al. Sporadic versus Radiation-Associated Angiosarcoma: A Comparative Clinicopathologic and Molecular Analysis of 48 Cases. **Sarcoma**, v.2013, p.798403. 2013.

INIC, Z.et al. Difference between Luminal A and Luminal B Subtypes According to Ki-67, Tumor Size, and Progesterone Receptor Negativity Providing Prognostic Information. **Clin Med Insights Oncol**, v.8, p.107-11. 2014.

ITALIANO, A.et al. The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification. **Genes Chromosomes Cancer**, v.51, n.6, p.569-78, Jun. 2012.

KACKER, C.et al. High frequency of MYC gene amplification is a common feature of radiation-induced sarcomas. Further results from EORTC STBSG TL 01/01. **Genes Chromosomes Cancer**, v.52, n.1, p.93-8, Jan. 2013.

KAHN, H. J.;BAILEY, D.; MARKS, A. Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcomas. **Mod Pathol**, v.15, n.4, p.434-40, Apr. 2002.

KAUFMANN, T.;CHU, F.; KAUFMAN, R. Post-mastectomy lymphangiosarcoma (Stewart-Treves syndrome): report of two long-term survivals. **Br J Radiol**, v.64, n.765, p.857-60, Sep. 1991.

KO, J. S.et al. Fully automated dual-color dual-hapten silver in situ hybridization staining for MYC amplification: a diagnostic tool for discriminating secondary angiosarcoma. **J Cutan Pathol**, v.41, n.3, p.286-92, Mar. 2014.

LUCAS, D. R. Angiosarcoma, radiation-associated angiosarcoma, and atypical vascular lesion. **Arch Pathol Lab Med**, v.133, n.11, p.1804-9, Nov. 2009.

LUINI, A.et al. Angiosarcoma of the breast: the experience of the European Institute of Oncology and a review of the literature. **Breast Cancer Res Treat**, v.105, n.1, p.81-5, Sep. 2007.

MANDRELL, J.;MEHTA, S.; MCCLURE, S. Atypical vascular lesion of the breast. **J Am Acad Dermatol**, v.63, n.2, p.337-40, Aug. 2008.

MANNER, J.et al. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. **Am J Pathol**, v.176, n.1, p.34-9, Jan. 2010.

MANSFIELD, S. A.;ZYNGER, D. L.; AGNESE, D. M. Angiosarcoma and breast cancer recurrence eight years following mammosite therapy. **Breast J**, v.20, n.6, p.658-60, Nov. 2014.

MATTOCH, I. W.et al. Post-radiotherapy vascular proliferations in mammary skin: a clinicopathologic study of 11 cases. **J Am Acad Dermatol**, v.57, n.1, p.126-33, Jul. 2007.

MEIS-KINDBLOM, J. M.; KINDBLOM, L. G. Angiosarcoma of soft tissue: a study of 80 cases. **Am J Surg Pathol**, v.22, n.6, p.683-97, Jun. 1998.

MENTZEL, T.et al. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. **Mod Pathol**, v.25, n.1, p.75-85, Jan.

MENTZEL, T.et al. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. **Mod Pathol**, v.25, n.1, p.75-85, Jan. 2012.

NESBIT, C. E.;TERSAK, J. M.; PROCHOWNIK, E. V. MYC oncogenes and human neoplastic disease. **Oncogene**, v.18, n.19, p.3004-16, May 13. 1999.

NISHIMURA, R.et al. Prognostic significance of Ki-67 index value at the primary breast tumor in recurrent breast cancer. **Mol Clin Oncol**, v.2, n.6, p.1062-68, Nov. 2014.

PANDEY, M.et al. Primary sarcoma of the breast. **J Surg Oncol**, v.87, n.3, p.121-5, Sep 1. 2004.

PATTON, K. T.;DEYRUP, A. T.; WEISS, S. W. Atypical vascular lesions after surgery and radiation of the breast: a clinicopathologic study of 32 cases analyzing histologic heterogeneity and association with angiosarcoma. **Am J Surg Pathol**, v.32, n.6, p.943-50, Jun. 2008.

PELENGARIS, S.;KHAN, M.; EVAN, G. c-MYC: more than just a matter of life and death. **Nat Rev Cancer**, v.2, n.10, p.764-76, Oct. 2002.

PENEL, N.et al. Angiosarcoma: state of the art and perspectives. **Crit Rev Oncol Hematol**, v.80, n.2, p.257-63, Nov. 2011.

PERTSCHUK, L. P.et al. Steroid hormone receptor immunohistochemistry and amplification of c-myc protooncogene. Relationship to disease-free survival in breast cancer. **Cancer**, v.71, n.1, p.162-71, Jan 1. 1993.

RAO, J.et al. Cutaneous angiosarcoma as a delayed complication of radiation therapy for carcinoma of the breast. **J Am Acad Dermatol**, v.49, n.3, p.532-8, Sep. 2003.

REQUENA, L.et al. Benign vascular proliferations in irradiated skin. **Am J Surg Pathol**, v.26, n.3, p.328-37, Mar. 2002.

SANTI, R.et al. Tumour suppressor gene TP53 mutations in atypical vascular lesions of breast skin following radiotherapy. **Histopathology**, v.58, n.3, p.455-66, Feb. 2011.

SENGUPTA, S.;BIARNES, M. C.; JORDAN, V. C. Cyclin dependent kinase-9 mediated transcriptional de-regulation of cMYC as a critical determinant of endocrine-therapy resistance in breast cancers. **Breast Cancer Res Treat**, v.143, n.1, p.113-24, Jan. 2014.

SHEEN, J. H.; DICKSON, R. B. Overexpression of c-Myc alters G(1)/S arrest following ionizing radiation. **Mol Cell Biol**, v.22, n.6, p.1819-33, Mar. 2002.

SHET, T.et al. Primary angiosarcoma of the breast: observations in Asian Indian women. **J Surg Oncol**, v.94, n.5, p.368-74, Oct 1. 2006.

SHON, W.et al. MYC amplification and overexpression in primary cutaneous angiosarcoma: a fluorescence in-situ hybridization and immunohistochemical study. **Mod Pathol**, v.27, n.4, p.509-15, Apr. 2014.

STEWART, F. W.; TREVES, N. Lymphangiosarcoma in postmastectomy lymphedema; a report of six cases in elephantiasis chirurgica. **Cancer**, v.1, n.1, p.64-81, May. 1948.

TAHIR, M.et al. Radiation induced angiosarcoma a sequela of radiotherapy for breast cancer following conservative surgery. **Int Semin Surg Oncol**, v.3, p.26. 2006.

TAUB, R.et al. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. **Proc Natl Acad Sci U S A**, v.79, n.24, p.7837-41, Dec. 1982.

THARIAT, J.et al. Not all sarcomas developed in irradiated tissue are necessarily radiation-induced--spectrum of disease and treatment characteristics. **Crit Rev Oncol Hematol**, v.83, n.3, p.393-406, Sep. 2012.

VINH-HUNG, V.; VERSCHRAEGEN, C. Breast-conserving surgery with or without radiotherapy: pooled-analysis for risks of ipsilateral breast tumor recurrence and mortality. **J Natl Cancer Inst**, v.96, n.2, p.115-21, Jan 21. 2004.

VORBURGER, S. A.et al. Angiosarcoma of the breast. **Cancer**, v.104, n.12, p.2682-8, Dec 15. 2005.

WEST, J. G.et al. Risk of angiosarcoma following breast conservation: a clinical alert. **Breast J**, v.11, n.2, p.115-23, Mar-Apr. 2005.

8- PRODUÇÃO CIENTÍFICA RELACIONADA À TESE

8.1 ARTIGO RELACIONADO À TESE

Fraga-Guedes C, Gobbi H, Mastropasqua MG, Rocha RM, Botteri E, Toesca A, Viale G. Clinicopathological and immunohistochemical study of 30 cases of post-radiation atypical vascular lesion of the breast. Breast Cancer Res Treat. 2014 Jul;146(2):347-54. doi: 10.1007/s10549-014-3020-9. Epub 2014 Jun 19.

8.2 OUTRAS PUBLICAÇÕES

Botteri E, Gentilini O, Rotmensz N, Veronesi P, Ratini S, **Fraga-Guedes C**, Toesca A, Sangalli C, Del Castillo A, Rietjens M, Viale G, Orecchia R, Goldhirsch A, Veronesi U. Mastectomy without radiotherapy: outcome analysis after 10 years of follow-up in a single institution. Breast Cancer Res Treat. 2012 Aug;134(3):1221-8. doi: 10.1007/s10549-012-2044-2. Epub 2012 Apr 26.

8.3 TRABALHOS APRESENTADOS EM EVENTOS, COM RESUMOS PUBLICADOS EM ANAIS OU PERIÓDICOS DE CONGRESSOS

1. **Fraga-Guedes C,** Gobbi H, Mastropasqua M., Botteri E, Luini A, Kneubil, MC, Viale, G. Aspectos clínico-patológicos e evolutivos de uma série de casos de Lesões Vasculares Atípicas da mama. In: XVI Congresso Brasileiro de Mastologia, 2011, Goiânia.

Revista Brasileira de Mastologia, Rio de Janeiro: Zeppelin Editorial, 2011. v.21. p.55 – 55

2. Gobbi H, **Fraga-Guedes C**, Mastropasqua M., Kneubil MC, Botteri E, Viale G. Atypical vascular lesion after radiation therapy for breast cancer: a clinicopathologic study and outcomes of 24 cases In: 24th European Congress of Pathology, 2012, Praga.

Virchows Archives. , 2012. v.461. p.S7 - S7

3. **Fraga-Guedes C**, Gobbi H, Mastropasqua MG, Botteri E, Kneubil MC, Viale G. Atypical Vascular Lesion of the Breast: clinopathological and immunohistochemical study of 24 cases from a single institution. Apresentação oral In: 17 World Congress on Breast Deseases from the Senology International Society, 2012, Salvador.-BA.

4. **Fraga-Guedes C**, Gobbi H, Mastropasqua MG, Malagoli R, Viale G. Post-radiation epithelioid angiosarcoma of the breast: an agressive and diagnostically challenging neoplasm. Apresentação de pôster In: 9 Jornada Paulista de Mastologia, Sociedade Brasileira de Mastologia Regional São Paulo, 2013, São Paulo.

8.4 PREMIAÇÕES

Prêmio de Melhor Tema Livre Pôster

Fraga-Guedes C, Gobbi H, Mastropasqua M., Botteri E, Luini A, Kneubil, MC, Viale, G. Aspectos clínico-patológicos e evolutivos de uma série de casos de Lesões Vasculares Atípicas da mama. In: XVI Congresso Brasileiro de Mastologia, 2011, Goiânia.

Entidade promotora: Sociedade Brasileira de Mastologia, no XVI Congresso Brasileiro de Mastologia

9- ANEXOS

9.1 ANEXO I: APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DA UFMG (COEP)

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 0216.0.203.000-10

Interessado(a): Profa. Helenice Gobbi
Depto. de Anatomia Patológica e Medicina Legal
Faculdade de Medicina - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 13 de julho de 2010, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Angiossarcoma e lesões vasculares atípicas da mama: estudo clínico, patológico e imunofenotípico comparativo"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2° andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

9.2 ANEXO II: APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DO IEO

IEO
Istituto Europeo di Oncologia

Istituto di Ricovero e Cura a Carattere Scientifico (DM 18/1/96)
Via Ripamonti 435, 20141 Milano - Italia

Comitato Etico
Tel +39 02 57 489 848 Fax +39 02 57 489 781
E-mail: comitato.etico@ieo.it

Milano, 10 novembre 2009
AN/dt

Dssa Conceicao Maria Fraga Guedes
IEO Sede

Oggetto: Angiosarcoma della mammella e lesioni vascolari atipiche: studio clinico, patologico e immuno-fenotipico comparativo.

Con la presente si prende atto, per quanto di competenza, dello studio in oggetto.

Questo studio retrospettivo dovrà essere condotto nel rispetto di quanto previsto dal Codice di protezione dei dati personali, utilizzando i dati clinici ed i campioni biologici di pazienti per i quali è disponibile il consenso informato, ottenuto attraverso l'apposito modulo IEO per il Consenso delle prestazioni sanitarie.

Si richiede che al termine dello studio venga resa disponibile una relazione riassuntiva dei risultati.

Distinti saluti.

Dott. Atanasio Nonis
Responsabile Segreteria Tecnico-Scientifica

9.3 ANEXO III: APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA DO IPO

INSTITUTO PORTUGUÊS DE ONCOLOGIA DE LISBOA
FRANCISCO GENTIL, E.P.E.

Unidade de Investigação Clínica

NOTA DE SERVIÇO

De: Unidade de Investigação Clínica

Data: 18/11/2014

Para: Dr. João Oliveira
Vogal do Conselho de Administração

N.º: 107/2014

ASSUNTO: Projecto de investigação intitulado **"Estudo clínico-patológico, imunohistoquímico e molecular (amplificação do gene cMYC) em angiossarcomas e lesões vasculares atípicas da mama em pacientes tratadas para carcinomas mamários com radioterapia"– UIC/926.**

Obtidos os pareceres favoráveis do Conselho de Investigação e da Comissão de Ética, junto envio o processo do estudo mencionado em epígrafe para autorização final.

Com os melhores cumprimentos,

P/ Unidade de Investigação Clínica
Conceição Costa

Aprovado.
20 NOV 2014

JOÃO OLIVEIRA
Vogal do Conselho de Administração

1299/14

DIRECÇÃO CLÍNICA

9.4 ANEXO IV: CÓPIA DA ATA DE DEFESA DE TESE

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

UFMG

ATA DA DEFESA DE TESE DA ALUNA

CONCEIÇÃO MARIA FRAGA GUEDES

Realizou-se, no dia 28 de novembro de 2014, às 14:00 horas, Sala 526 ou 340, Faculdade de Medicina da UFMG, da Universidade Federal de Minas Gerais, a defesa de tese, intitulada *ESTUDO CLÍNICO-PATOLÓGICO, IMUNO-HISTOQUÍMICO E MOLECULAR (AMPLIFICAÇÃO DO GENE MYC) EM ANGIOSSARCOMAS E LESÕES VASCULARES ATÍPICAS DA MAMA.*, apresentada por CONCEIÇÃO MARIA FRAGA GUEDES, número de registro 2010758433, graduada no curso de MEDICINA, como requisito parcial para a obtenção do grau de Doutor em PATOLOGIA, à seguinte Comissão Examinadora: Prof(a). Helenice Gobbi - Orientador (Universidade Federal de Minas Gerais, UFMG), Prof(a). Geovanni Dantas Cassali (Universidade Federal de Minas Gerais, UFMG), Prof(a). Henrique Lima Couto (Fundação Hospitalar de Minas Gerais), Prof(a). Isabela Werneck da Cunha (AC Camargo Câncer Center), Prof(a). Marina De Brot Andrade (Universidade Federal de Minas Gerais, UFMG), Prof(a). Rafael Malagoli Rocha (Fundação Antônio Prudente).

A Comissão considerou a tese:

(X) Aprovada

( ) Reprovada

Finalizados os trabalhos, lavrei a presente ata que, lida e aprovada, vai assinada por mim e pelos membros da Comissão.
Belo Horizonte, 28 de novembro de 2014.

Prof(a). Helenice Gobbi (Doutor)

Prof(a). Rafael Malagoli Rocha (Doutor)

Prof(a). Henrique Lima Couto (Doutor)

Prof(a). Isabela Werneck da Cunha (Doutora)

Prof(a). Marina De Brot Andrade (Doutora)

9.5 ANEXO V: CÓPIA DA FOLHA DE APROVAÇÃO

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

UFMG

FOLHA DE APROVAÇÃO

ESTUDO CLÍNICO-PATOLÓGICO, IMUNO-HISTOQUÍMICO E MOLECULAR (AMPLIFICAÇÃO DO GENE MYC) EM ANGIOSSARCOMAS E LESÕES VASCULARES ATÍPICAS DA MAMA.

CONCEIÇÃO MARIA FRAGA GUEDES

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em PATOLOGIA, como requisito para obtenção do grau de Doutor em PATOLOGIA, área de concentração PATOLOGIA MÉDICA.

Aprovada em 28 de novembro de 2014, pela banca constituída pelos membros:

Prof(a). Helenice Gobbi - Orientadora
Universidade Federal de Minas Gerais, UFMG

Prof(a). Henrique Lima Couto
Fundação Hospitalar de Minas Gerais

Prof(a). Isabela Werneck da Cunha
AC Camargo Cancer Center

Prof(a). Marina De Brot Andrade
Universidade Federal de Minas Gerais, UFMG

Prof(a). Rafael Malagoli Rocha
Fundação Antônio Prudente

Belo Horizonte, 28 de novembro de 2014.