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Abstract:

Angiosarcomas (AS) represent a heterogeneous group of tumors with variable clinical presentation. AS share an important morphologic and immunohistochemical overlap with other sarcomas, hence the differential diagnosis is challenging, especially in poorly-differentiated tumors. Although molecular studies provide significant clues, especially in the differential diagnosis with other vascular neoplasms, a thorough hematoxylin and eosin analysis remains an essential tool in AS diagnosis. In this review, we discuss pathological and molecular insights with emphasis on implications for differential diagnosis in cutaneous, breast, soft tissue and visceral AS.

Key words: angiosarcomas, immunohistochemistry, molecular biology, differential diagnosis.

Introduction

Angiosarcoma (AS) is a rare but highly aggressive vasoformative sarcoma characterized by high rates of recurrence and tumor-related death (Naka et al., 1995; Mark et al., 1996; Fury et al., 2005; Fayette et al., 2007; Abraham et al., 2007; Buehler et al., 2014; Wang et al., 2017; Zhang et al., 2019; Painter et al., 2020; Weidema et al., 2020). Survival for AS patients is generally poor, with reported five-year survival rates of around 40% and close to 15% in metastatic tumors (Buehler et al., 2014; Weidema et al., 2020). AS prognosis may be influenced by clinical and pathological factors and histological high grade is related with poor prognosis (Fury et al., 2005; Abraham et al., 2007; Buehler et al., 2014; Lee et al., 2019; Weidema et al., 2020). Depending on previous treatment (radiotherapy or systemic treatment), the mainstay of localized AS therapy consists of surgery with adjuvant radiotherapy and/or doxorubicin-based or taxane single-agent chemotherapy (Abraham et al., 2007; Hoang et al., 2018; Lodhi et al., 2018; Florou et al., 2019; Painter et al., 2020). For locally
advanced disease, without local treatment options or metastatic disease, the best choice is systemic treatment within clinical trials (Pasquier et al., 2016). Although recent advances in oncology, such as targeted therapy and immunotherapy may have benefited some AS patients, a more precise role for these new treatment options remains unclear (Fujii et al., 2014; Honda et al., 2016; Shimizu et al. 2016; Botti et al., 2017; Shinhu et al., 2017; D’Angelo et al., 2018; Wolina et al., 2018; Florou et al., 2019;).

In this review, we discuss pathological and molecular insights focussing on implications for differential diagnosis.

1. A wider spectrum of clinical presentation in Angiosarcomas

AS can occur in any organ or tissue, either as a primary AS or as a secondary AS linked to lymphedema or external damaging factors (radiation therapy or vinyl chloride exposure). In addition, AS have also been reported in association with other neoplasms (malignant peripheral nerve sheath tumor, germ cell tumor or schwannoma) (Hunt et al., 2004; Carpino et al., 2005; Fury et al., 2005; Antonescu et al., 2014; Matoso et al., 2015; Baker et al., 2017; Ginter et al., 2017; Leduc et al., 2017; Requena et al., 2017; Shustef et al., 2017; van IJzendoorn et al., 2027; Gourley et al., 2018; Ishida et al., 2018; Ginter et al., 2019; Abdou et al., 2019; Alves et al., 2019; Singh et al., 2019; Weiss et al., 2019; Wilson et al., 2019; Yasir et al., 2019; Pazhenkotill et al., 2020) or associated with foreign bodies (vascular grafts, prosthetic material) (Agaimy et al., 2016). The association between UV light exposure and AS is under debate (Requena et al., 2017; Shon et al., 2017; Shustef et al., 2017; Ishida et al., 2018).

The most frequent locations of AS include skin (especially the head and neck area), soft tissue and breast, whereas it is less common in liver, spleen, heart and bone (Ginter et al., 2019; Abdou et al., 2019; Alves et al., 2019; Singh et al., 2019; Weiss et al., 2019; Wilson et al., 2019; Yasir et al., 2019; Pazhenkotill et al., 2020). Therefore, depending on the primary site, AS can be divided into cutaneous, breast, soft tissue or visceral (Figure 1A-F). The majority of secondary AS are cutaneous, although rare cases have been reported in deeper-seated tissues (Seo et al., 2003; Weaber et al., 2009; Mentzel et al.,
Clinically, secondary AS related to radiation are frequently located in breast areas in female patients (Backer et al., 2017; Abdou et al., 2019; Corradini et al., 2020) while primary cutaneous AS of the head and neck region occurs mainly in elderly men (Pawlik et al., 2013; Ishida et al., 2018; Lee et al., 2019).

Cutaneous angiosarcoma may initially appear as a bruise, or a raised purplish-red papule, it is typically multifocal and can be mistaken for a simple benign lesion such an ecchymoses or cellulitis, leading to delayed diagnosis (Requena et al., 2017; Shon et al., 2017; Shustef et al., 2017; Ishida et al., 2018). As tumor size increases, tissue infiltration, oedema, tumor fungation (Figure 1), ulceration, and haemorrhage may develop (Requena et al., 2017). Deeper soft tissue and visceral lesions present as an expanding mass associated with pain or discomfort (Leduc et al., 2017; Ginter et al., 2019; Abdou et al., 2019; Alves et al., 2019; Singh et al., 2019; Weiss et al., 2019; Wilson et al., 2019; Yasir et al., 2019). Breast AS secondary to radiotherapy are usually superficial (dermis and subcutis), while primary AS of breast are usually intraparenchymal and appear in young women (Baker et al. 2017; Abdou et al., 2019; Beca et al., 2020; Corradini et al., 2020).

Hematogenous spread is frequent in AS, with the lungs presenting as the most common site for metastatic disease, where it may occur as pleural disease, haemorrhagic pleural effusion, or pneumothorax.

2. Histopathology in Angiosarcomas: from well-differentiated subtypes to undifferentiated and unrecognizable tumors.

Histologically, AS displays a wide range of appearances, ranging from well-formed vascular spaces with minimal cytologic atypia (Figure 2 A and B) which resemble hemangiomas, to poorly-differentiated tumors with solid sheets of spindled (Figure 2C), epithelioid (Figure 2D), round, or anaplastic cells (Figure 2F) that lack evident vascular structures (Hunt et al., 2004; Antonescu et al., 2014; Doyle et al., 2014; Baker et al., 2017; Shon et al., 2017; Alves et al., 2019;
Weis et al., 2019; Jung et al., 2020; Papke et al., 2020). This varied spectrum of morphological appearance complicates the differential diagnosis.

Secondary AS, especially those related to radiation, are frequently located in the dermis (Figure 2A). Several anastomosing and dissecting vascular channels are observed under hematoxylin and eosin (H&E) examination. In fact, the histopathology of cutaneous well-differentiated AS is not dissimilar to post-radiation atypical vascular proliferation (PRAVP) (Hunt et al., 2004; Guo et al., 2011; Mentzel et al., 2012; Ginter et al., 2014; Backer et al., 2017; Requena et al., 2017). PRAVP refers to a small, usually lymphatic-type vascular proliferation (Figure 2C) and although most atypical vascular lesions pursue a benign course, they may recur (Weaver et al., 2009; Mentzel et al., 2012; Wick et al., 2016; Requena et al., 2017). The option of classifying PRAVP as a precursor lesion of AS is still under debate. In both lesions (cutaneous well-differentiated AS and PRAVP) the neoplastic cells lining the vascular channel exhibit hyperchromatic and irregular nuclei with variable nuclear atypia, while mitotic figures or necrosis are infrequent (Hunt et al., 2004; Guo et al., 2011; Mentzel et al., 2012; Ginter et al., 2014; Backer et al., 2017; Requena et al., 2017). The differential diagnosis between PRAVP and AS is described in section 6. The non-well-differentiated AS may display poorly-differentiated areas (Figure 2D,E and F) leading to occasional misdiagnosis of high-grade AS, either when focal vascular differentiation cannot be clearly distinguished or where immunohistochemical results are inconclusive. In visceral location, for instance the liver, AS may present several histological patterns, including vasoformative, epithelioid or spindled cell morphology and sinusoidal or peliotic growth, with the last two being more difficult to recognize (Alves et al., 2019; Wilson et al., 2019; Yasir et al., 2019; Zeng et al., 2020).

3. Immunohistochemistry and electron microscopy may support the histological diagnosis of AS, while either neuroendocrine or epithelial differentiation are not exceptional in AS.

In several scenarios immunoreactivity for vascular markers will confirm the histological diagnosis of AS (Fernandez et al., 2012; Fisher et al., 2013; Ginter
et al., 2014; Wang et al., 2017; Corradini et al., 2020; Di Battista et al., 2020; Papke et al., 2020). A combination of CD31 (Figure 3A), CD34, D2-40 (Figure 3C), VE-cadherin, VEGFR (1, 2, and 3) are frequently used as an appropriate tool in AS diagnosis, however cytoplasmic immunoreactivity of these antibodies may occasionally demonstrate an inconsistent staining background (Fisher et al., 2013; Antonescu et al., 2014; Backer et al., 2017; Wang et al., 2017). Strong and nuclear immunopositivity for ERG or FLI1 (Figure 3B) can aid interpretation of the above-mentioned antibodies, which is sometimes problematic (Hunt et al., 2004; Ko et al., 2015; Machado et al., 2018; Kuhn et al., 2019). Vascular markers have the advantage that they stain the endothelial cells of any tissue, thus they can be used as positive internal control, indicative of tissue quality, especially in poorly-fixed tissue (Hunt et al., 2004; Antonescu et al., 2014; Marusic et al., 2017; Machado et al., 2018).

An important observation is that vascular markers, both for nuclear or cytoplasmic & membranous immunoreactivity are not completely specific for AS since many other benign and malignant tumors of diverse histogenesis (carcinomas, sarcomas or lymphomas) may sometimes disclose immunoreactivity for one or various of these markers (Backer et al., 2017; Alves et al., 2019; Di Battista et al., 2020). A list of source, dilution and staining pattern of vascular markers is summarized in Table 1.

Secondary AS shows consistent MYC expression by IHC (Figure 3D), but primary AS may sporadically reveal MYC immunoreactivity (Guo et al., 2011; Fernandez et al., 2012; Mentzel et al., 2012; Laé et al., 2015; Ginter et al., 2017; Requena et al., 2017; Requena et al., 2018; Papke et al., 2020). Although MYC immunoreactivity cannot discriminate between primary and secondary AS, this protein expression has not been observed in radiation-induced sarcomas other than AS (Ginter et al., 2017; Requena et al., 2017; Requena et al., 2018; Papke et al., 2020).

Unusual expression of either or both the epithelial (EMA and cytokeratins) and neuroendocrine markers (synaptophysin, chromogranin-A) with focal or diffuse staining patterns has been reported in vascular tumors, such as AS and composite hemangioendotheliomas. This aberrant expression of unusual markers in AS increases the list of differential diagnoses, especially in cases with poorly-differentiated histology (Antonescu et al., 2014; Tessier Cloutier et
Electron microscopy may provide important clues in the final diagnosis of AS (Seo et al., 2003; Carpino et al., 2005). The ultrastructural identification of Weibel-Palade bodies (Figure 3F) confirms the occurrence of endothelial differentiation in AS.


Graphic 1 depicts the main factors related to angiosarcoma biology (Mellberg et al., 2009; Kan et al., 2012; Young et al., 2014; Bagaria et al., 2018; Florou et al., 2018; Khan et al., 2018; Habeeeb et al., 2019; Weidema et al., 2019; Painter et al., 2020). All these factors are interrelated. The main genetic and epigenetic alterations are described in section 5. In this section we summarize the angiogenic factors, oncogenic pathways and tumor microenvironment factors.

4.1 Angiogenesis in AS

AS expresses multiple angiogenic growth factors, including VEGF, and both primary and secondary AS have increased expression of angiogenic tyrosine kinase receptor transcripts, including VEGFR1/2/3, implying an activated angiogenic program (Mellberg et al., 2009; Buehler et al., 2013; Young et al., 2014; Khan et al., 2018; Habeeeb et al., 2019; Weidema et al., 2019). In addition, many AS have one or more mutations in several angiogenesis-related genes, consequently several angiogenic pathways are upregulated or mutated in a large proportion of AS. The ANGPT-TIE system is essential to developmental angiogenesis and consists of two tyrosine kinase receptors, TIE1 and TEK (TIE2), and three corresponding ligands, angiopoietins-1, 2 and 4. Angiopoietin-1 and 2 play a key role in maintaining the integrity of existing vessels, vascular remodelling and angiogenesis (Mellberg et al., 2009; Buehler et al., 2013; Young et al., 2014; Khan et al., 2018). Buehler et al. reported a cohort of 56 AS where 62% of tumors expressed at least low levels of angiopoietin-2 (Buehler et al., 2013).
This finding is consistent with endothelial differentiation and is related to the upregulation of Angiopoietin-2 mRNA in AS when compared with other soft tissue sarcomas (Buehler et al., 2013). The study by Buehler et al. demonstrated that increased expression of ANGPT-TIE system components was associated with both a well-differentiated histological pattern and improved overall survival. In contrast, loss of expression was associated with poor histological differentiation and more aggressive disease (Buehler et al., 2013). Bevacizumab, Pazopanib, Sorafenib and Axitinib have been used as targeted therapy linked to angiogenesis in AS and a wide spectrum of tumor responses have been reported (Weidema et al., 2019).

4.2 Oncogenic pathways in angiosarcoma

Genetic alterations involving the RAS/RAF/MEK/Erk pathway have been reported in a variable proportion of AS ranging from no mutation on NRAS/BRAF to mutations in 53% of AS samples (Behjati et al., 2014; Murali et al., 2015; Weidema et al., 2019). An additional oncogenic pathway of interest in AS is the PI3K/AKT/mTOR-pathway, which is known to control cell survival, cell growth and cell cycle progression (Weidema et al., 2019). Mutations of the PIK3CA gene have been reported in less than 20% of AS (Behjati et al., 2014; Weidema et al., 2019). Finally, the p16(INK4A) pathway is also involved in AS, and genomic studies have revealed loss of CDKN2A in 26% of AS from different origins (Murali et al., 2015). Previous studies have demonstrated poor survival in patients with soft tissue sarcomas and loss of p16, but no significant difference in survival has been reported in AS patients (Weidema et al., 2019).

4.3 Microenvironment in Angiosarcoma

The response rate to chemo-radiotherapy in AS is usually low, therefore alternative therapeutic options are urgently needed, particularly in patients with metastatic disease (Honda et al., 2016; Shimizu et al., 2016; Sindhu et al., 2017; Botti et al., 2017; D’Angelo et al., 2018; Wollina et al., 2018; Florou et al., 2019; Weidema et al., 2019). The use of immune checkpoint inhibitors is a promising
treatment modality that has yielded long-term clinical benefits in historically therapeutically refractory cancers (D’Angelo et al., 2018). The immunologic tumor microenvironment in AS has not been systematically studied, with controversial results regarding prognosis reported in limited studies (Shimizu et al., 2016; Sindhu et al., 2017; Botti et al., 2017; D’Angelo et al., 2018; Wollina et al., 2018; Florou et al., 2019; Weidema et al., 2019). Shimizu et al. reported PD-L1 immunoreactivity related with poor prognosis in cutaneous AS (Shimizu et al., 2016), but Botti et al. in a cohort of primary AS did not confirm a prognostic significance of PD-L1 immunoexpression in AS (Botti et al., 2017). Despite the controversial results for PD-L1 expression and prognosis in AS, a complete response has been described in AS treated with CTLA-4 monotherapy (Sindhu et., al. 2017; Weidema et al., 2019). This finding may in part be related to an overall mutation burden in some AS that confer a relative clinical benefit from checkpoint inhibition (Weidema et al., 2019). At present, it is unclear as to what degree previous treatment altered the tumor microenvironment to subsequently sensitize them to checkpoint inhibition (Shimizu et al., 2016; Sindhu et al., 2017). Although PD-L1 expression appears to be present in a subset of AS, the relationship between PD-L1 IHC expression and susceptibility to anti-PD-1 treatment is so far unclear. These findings suggest a need for a thoughtful and targeted approach to the use of immunotherapy in AS (Weidema et al., 2019). The tumor microenvironment has been postulated to limit immune cell infiltration and impair their function in the tumors (D’Angelo et al., 2018; Wollina et al., 2018; Florou et al., 2019; Weidema et al., 2019). AS may have different tumor microenvironments depending on the location (soft tissue, breast, viscera or cutaneous). Indeed, the stromal compartment is highly heterogeneous in AS and may influence the interrelationship between stroma, neoplastic cells, and immune cells. Further studies are critical to better characterize the immune microenvironment of AS, especially the effects of location and implication of previous therapy.
5. Molecular Biology as an emerging tool in a differential diagnosis workflow of AS and other vascular neoplasms

AS includes a genetically heterogeneous group of tumors with various molecular alterations, including gene amplifications, point mutations, translocations or gene fusions as well as epigenetic alterations (Antonescu et al., 2009; Guo et al., 2011; Benhjati et al., 2014; Knosel et al., 2014; Huan et al., 2016, 2017; da Costa et al., 2017; Habeed et al., 2019; Beca et al., 2020; Painter et al., 2020). Table 2 summarizes the main genetic alterations and their clinical significance.

MYC plays a key oncogenic role in AS, and MYC gene amplification (Figure 3E) and MYC protein overexpression have been well documented in this type of sarcoma (Manner et al., 2010; Guo et al., 2011; Fernandez et al., 2012; Ginter et al., 2014; Huang et al., 2016; Weidema et al., 2019, Painter et al., 2020). MYC gene amplification (Figure 3E) is almost the exclusive genetic anomaly in many secondary AS, being present in around 55% of all secondary AS and up to 91% of secondary AS in breast (Guo et al., 2011; Fernandez et al., 2012; Ginter et al., 2014; Huang et al., 2016; Weidema et al., 2019, Painter et al., 2020). Accordingly, MYC amplification is a useful tool to differentiate between primary and secondary AS, even in morphologically indistinguishable tumors (Fernandez et al., 2012; Ginter et al., 2014; Knosel et al., 2014; Huan et al., 2016; Habeed et al., 2019; Beca et al., 2020; Painter et al., 2020). Moreover, MYC amplification has not been found in either PRAVP or radiation-induced sarcomas other than AS (Guo et al., 2011; Fernandez et al., 2012; Ginter et al., 2014). It is important to remark that MYC gene amplification is often, but not always, related to MYC protein overexpression (Fernandez et al., 2012; Ginter et al., 2014; Huang et al., 2016). Thus, MYC overexpression has been observed in 24% of primary AS without MYC amplification, suggesting an alternative potential regulatory pathway for MYC protein expression, such as epigenetic control in some primary AS (Fernandez et al., 2012; Ginter et al., 2014; Huang et al., 2016). Co-amplification of FLT4 (VEGFR3) with MYC has been identified in 25% of secondary AS (Guo et al., 2011, Weidema et al., 2019) and KDR mutation has been noted typically in breast AS and is apparently mutually
exclusive with PLCG1 mutation (Behjati et al., 2014; Huang et al., 2016; Weidema et al., 2019, Beca et al., 2020; Painter et al., 2020).

PLCG1 (Phospholipase C, gamma 1) and PTPRB (Protein Tyrosine Phosphatase, Receptor Type B) are two angiogenic genes related to AS carcinogenesis (Behjati et al., 2014; Huang et al., 2016). Around 26% of AS have inactivating PTPRB mutations, while 9% of AS have activating PLCG1 mutations (Huang et al., 2016; Weidema et al., 2019; Painter et al., 2020). PTPRB alterations have been reported in 45% of secondary AS or AS with unknown primary or secondary scenario, in addition secondary AS may exhibit concomitant MYC amplification and PTPRB or PLCG1 mutation (Behjati et al., 2014; Huang et al., 2016; Weidema et al., 2019; Painter et al., 2020). Although primary AS usually lack PTPRB mutations, either primary or secondary AS may harbour PLCG1 mutations (Behjati et al., 2014; Huang et al., 2016; Weidema et al., 2019; Painter et al., 2020). Overall, almost all AS with PLCG1 mutation will also harbour PTPRB mutations, however only half of AS with PTPRB mutations reveal PLCG1 mutation (Huang et al., 2016; Weidema et al., 2019).

CIC (capicua transcriptional repressor) is found in 9% of primary AS, genetic alterations include mutation, mutation and rearrangement or rearrangement without mutation. These AS subtypes with CIC alteration usually have round/epithelioid morphology, worse prognosis and specific clinical presentation (younger age at presentation) (Huang et al., 2016)

Corradini et al. recently reported a high mutation burden in TP53, EGFR, KRAS, HRAS, NRAS and hTERT genes in high grade (Grade 3) post-radiation AS. In addition, they detected H-TER mutation in both PRAVP and post-radiation AS, which opens up a new scenario in the association between PRAVP and secondary post-radiation AS (Corradini et al., 2020).

So far, it has been difficult to correlate clinical presentation with specific genetic alterations, such as mutations or gene amplifications, except for MYC amplification, which is present in the vast majority of secondary post-radiation AS (Fernandez et al., 2012; Ginter et al., 2014; Knosel et al., 2014; Huan et al., 2016; Habeed et al., 2019; Beca et al., 2020; Painter et al., 2020). However, various studies have suggested a relationship between mutational genetic profile and AS location (Weidema et al., 2019). For example, a recent study documented a higher frequency of KDR and PIK3CA mutations in primary
breast AS in comparison with other AS (Beca et al., 2020). Another study reported ATRX loss to be significantly associated with deep soft tissue and hepatic AS, while decreased NOTCH1 and NOTCH2 expression were more frequent in cutaneous and visceral AS, respectively (Panse et al., 2018). Likewise, Verbeke et al. also observed that TGF-β signaling and PTEN expression differ between bone and soft tissue AS (Verbeke et al., 2013). NUP160-SLC43A3 gene fusion has been discovered in one case of cutaneous AS and although the detection of fusion genes, including EWSR1–ATF1 or CEP85L–ROS1, has been reported in AS (Marks et al., 2019), these were seen in single cases, and were not specific to AS. The SLC43A3 gene is associated with microvascularization which may contribute to the pathogenesis of angiosarcoma (Marks et al., 2019).

DNA methylation in AS has been poorly investigated, although Weidema et al. recently performed a methylation profiling study, where for the first time they demonstrated different AS clusters in 36 angiosarcoma samples from different locations (Weidema et al., 2020). These clusters correlated well with clinical subtype, overall survival and chromosomal stability (Weidema et al., 2020). Notably, UV-induced AS and post-radiation AS fell in cluster A, while both visceral and soft tissue AS almost exclusively fell into cluster B (Weidema et al., 2020). This finding supports the idea that AS pathogenesis may be related to tumor location or previous external damage (radiation treatment).

Molecular methods have certainly enriched the reproducible assessment of vascular neoplasms. As a result, molecular approaches provide better and more precise diagnosis and classification, which assists in discovering potential targets for treatment.

6. Differential diagnosis in AS beyond tumor location and previous exposure to external carcinogenic factors.

Tumor location and previous exposure to external risk substances (radiotherapy treatment) influence the dynamic of differential diagnosis in AS. Here we review the most important differential diagnoses (Figure 4).
6.1 Cutaneous angiosarcomas

Although Kaposi sarcoma, pseudomyogenic hemangioendothelioma (PHE) and benign cutaneous vascular proliferation/malformation may enter in the differential diagnosis of cutaneous AS, the most challenging differential diagnosis is post-radiation atypical vascular proliferation (PRAVP) (Hunt et al., 2004; Weaver et al., 2009; Fisher et al., 2013; Ginter et al., 2014; Ginter et al., 2017; Baker et al., 2017; Shon et al., 2017; Shustef et al., 2017; Weiss et al., 2019; Papke et al., 2020). The differential diagnosis is even more challenging when dealing with a cutaneous lesion in a patient with a previous history of radiotherapy. In both tumors (well-differentiated AS and PRAVP) the histology shows dilated vessels with hyperchromatic endothelial cells with variable nuclear atypia and commonly lack of necrosis (Figure 2C). Of note, PRAVP is a relatively well-circumscribed and not infiltrative lesion with anastomosing growth pattern of irregular slit-like vascular spaces dissecting dermal collagen but not extending into the subcutis (Hunt et al. 2004; Weaver et al., 2009; Fisher et al., 2013; Ginter et al., 2014; Ginter et al., 2017; Baker et al., 2017; Shon et al., 2017; Weiss et al., 2019; Papke et al., 2020). In addition, PRAVP usually reveal less nuclear atypia and mitoses. In contrast, cutaneous AS display multilayering of endothelial cells, evident nuclear atypia, conspicuous nucleoli, mitoses and extension into deep dermis and subcutaneous tissues even in initial stages (Hunt et al. 2004; Weaver et al., 2009; Fisher et al., 2013; Ginter et al., 2017; Papke et al., 2020). While histological analysis may provide significant clues in the differential diagnosis when dealing with small biopsies, the IHC and molecular approach usually helps to reach an accurate diagnosis, especially in lesions with uncertain histological features. PRAVP has not been found to overexpress MYC protein or reveal MYC amplification so far, thus the knowledge of MYC protein and gene status can be a useful tool in differential diagnosis (Hunt et al. 2004; Weaver et al., 2009; Guo et al., 2011; Fisher et al., 2013; Ginter et al., 2014; Ginter et al., 2017; Baker et al., 2017; Shon et al., 2017; Shustef et al., 2017; Weiss et al., 2019; Papke et al., 2020). Of note, MYC overexpression may be observed in a small proportion of primary cutaneous AS; thus MYC protein status by IHC does not provide additional
information in the differential diagnosis between primary and secondary AS (Fernandez et al., 2012; Ginter et al., 2014; Corradini et al., 2020).

Kaposi sarcoma (KS) may resemble a well-differentiated AS but usually displays a characteristic clinical picture with histological spindle cell proliferation, hemosiderin deposits, vascular clefts and intracytoplasmic hyaline globules (Figure). This entire histological finding in addition to the nuclear HHV-8 immunoreactivity (Figure 4D) facilitates an accurate diagnosis (Schwartz et al., 2003).

PHE most often arises in the extremities of young adult males and many cases have cutaneous involvement (Antonescu et al., 2014; Ko et al., 2015; Papke et al., 2020). Under optical light microscope, the tumor is composed of plump spindle cell proliferation (Figure 4C), neutrophilic inflammation, and scattered cells with epithelioid morphology, while some spindle cells harbour distinctive brightly eosinophilic cytoplasm with rhabdomyoblast appearance (Antonescu et al., 2014; Ko et al., 2015; Sugita et al., 2016; Shon et al., 2017; Hung et al., 2017; Habeet et al., 2019; Papke et al., 2020; Ramos-Fuentes et al., 2020). Tumor cells express cytokeratin and FOSB, but are negative for S100 and desmin (Sugita et al., 2016; Shon et al., 2017; Hung et al., 2017; Habeet et al., 2019; Papke et al., 2020). FOSB immunoreactivity with strong and diffuse nuclear expression is highly specific for PHE and although FOSB overexpression is not limited to PHE, this positivity, together with the histology, offers very important clues in narrowing the final diagnosis. SERPINE1-FOSB gene fusion is exclusive so far for PHE (Papke et al., 2020).

Atypical fibroxanthoma (AFX) is a dermal spindle-histiocytoid cell tumor that typically occurs on the sun-damaged skin of head and neck in elderly people. In AFX, either prominent vascularization or extensive hemosiderin deposits may mimic AS (Mentzel et al., 2017). Adding a vascular marker to the IHC panel, such as CD31 and ERG (usually negative in AFX), helps to rule out AS (Mentzel et al., 2017). In addition, CD10 immunoreactivity favours AFX since AS does not present CD10 expression (Kaddu et al., 2002; Soleymani et al., 2019).

The differential diagnosis of cutaneous AS with benign vascular lesions (hemangiomas, lymphangiomas etc) and vascular malformation is relatively straightforward and usually does not require additional IHC or molecular
analysis (Baker et al., 2017). Detailed differential diagnosis with specific benign vascular lesions with predominant cutaneous clinical presentation is beyond the scope of the present review.

Regarding malignant cutaneous lesions, poorly-differentiated squamous cell carcinoma, Merkel cell carcinoma or melanoma may occasionally present a pseudoangiomatous pattern resembling AS, hence in this setting, clinical correlation and IHC analysis is very important to define the accurate histogenesis of the tumor (Hunt et al., 2004; Fisher et al., 2013; Baker et al., 2017; Machado et al., 2018). In addition, cutaneous leiomyosarcoma (LMS) may also resemble a cutaneous AS with spindle cell morphology, positivity for at least two smooth muscle immunohistochemical markers (SMA, Desmin, H-Caldesmon) favours the diagnosis of LMS.

6.2 Breast angiosarcomas

AS represent the most common sarcoma of the breast and occur most frequently secondary to radiation therapy for breast carcinoma or secondary to longstanding lymphedema, frequently in older female patients (Baker et al., 2017; Abdou et al., 2019; Beca et al., 2020; Corradini et al., 2020). Breast AS may also arise as primary sarcoma of the breast, more commonly in younger patients (Abdou et al., 2019). Secondary AS often presents with skin changes, and primary AS presents as a palpable mass (Abdou et al., 2019). The histopathological features range from morphologically low grade tumors demonstrating well-formed vessels with mild cytologic atypia, to histologically high-grade tumors showing pleomorphism, mitoses and a solid growth pattern resembling an undifferentiated sarcoma (Baker et al., 2017; Abdou et al., 2019; Beca et al., 2020; Corradini et al., 2020). Furthermore, cutaneous secondary AS arising in the breast area demonstrate the same morphological features as any extramammary cutaneous secondary AS (see section of cutaneous AS). The most deep-seated tumors have a morphological spectrum similar to those AS located in soft tissues (see section on soft tissue AS). PRAVP, pseudoangiomatous stromal hyperplasia (PASH), benign vascular lesions (hemangioma, angiolipoma), metaplastic carcinoma and other sarcomas with
pseudoangiomatous growth patterns or extensive hemosiderin deposits are within the differential diagnostic spectrum (Ginter et al., 2014; Ginter et al., 2017; Baker et al. 2017; Abdou et al., 2019; Ginter et al., 2019; Beca et al., 2020; Corradini et al., 2020). The morphological differential diagnostic consideration with PRAVP is described in the cutaneous AS section. MYC amplification is seen in secondary breast AS and not seen in PRAVP, in addition, FLT4 co-amplification is observed in a subset of secondary AS, but not in PRAVP or other radiation-associated sarcomas (Guo et al., 2011; Fernandez et al., 2012; Mentzel et al., 2012; Beca et al., 2020). PASH represents a benign proliferation of stromal cells with an anastomosing pattern of slit-like clefts lined by a single layer of flat spindle cells simulating vascular spaces that may resemble a low-grade AS, especially in limited biopsy material (Mantilla et al., 2016; Baker et al., 2017; Ginter et al., 2017). The presence of vascular channels containing red blood cells with invasion into breast parenchyma, papillary endothelial growth and endothelial cells with hyperchromatic nuclei and mitoses in addition to vascular marker immunoreactivity favour low-grade AS. In contrast, in PASH the spindle cells display hormonal receptor positivity (oestrogen and progesterone) (Mantilla et al., 2016; Baker et al., 2017; Ginter et al., 2017). The presence of a convincing infiltrative growth pattern in AS is a major feature that will distinguish a well-differentiated AS from benign vascular lesions (hemangioma etc.) (Mantilla et al., 2016; Baker et al., 2017; Ginter et al., 2017). An angiosarcomatous component in a metaplastic breast carcinoma is a rare event, but a spindle cell component in breast metaplastic carcinoma may resemble a spindle cell AS, therefore the identification of any histological epithelial component or epithelial differentiation is important to reach a definite diagnosis (Baker et al. 2017; Abdou et al., 2019; Beca et al., 2020; Corradini et al., 2020). The differential diagnosis with other sarcomas with a pseudoangiomatous growth pattern is detailed in the soft tissue tumor section. It is important to remark that a strong clinical correlation is mandatory in breast AS diagnosis, and so during the diagnostic workflow of breast tumor in patients with a previous history of radiation therapy, the possibility of post-radiation AS should be promptly excluded.
6.3 Soft tissue angiosarcomas

Differential diagnosis in soft tissue AS is related predominantly to morphological findings, either the histological pattern or cytological appearance (epithelioid, spindle, round or anaplastic tumor).

6.3.1 Epithelioid AS

Differential diagnosis of epithelioid AS is extensive, and includes benign and malignant lesions with mesenchymal, epithelial or melanocytic differentiation. Epithelioid hemangioma (EH) (Figure 4A) and epithelioid hemangioendothelioma (EHE) (Figure 4B) are vascular neoplasms that may occasionally resemble a well-differentiated epithelioid AS, although AS with epithelioid morphology usually exhibit more significant nuclear atypia and mitoses, hence further molecular analysis is not usually required (Hunt et al., 2004; Fisher et al., 2013; Antonescu et al., 2014; Ko et al., 2015; Matoso et al., 2015; Shon et al., 2017; van IJzendoorn et al., 2017; Alves et al., 2019; Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020). In cases with an unconvincing histological picture, intravascular growth, prominent stromal inflammation and FOS rearrangement or FOSB overexpression favour EH (Fisher et al., 2013; Antonescu et al., 2014; Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020). Of note, FOS rearrangement or FOSB overexpression are not specific for EH since other tumors may display these anomalies, for instance pseudomyogenic hemangioendothelioma and osteoblastoma (Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020). Histologically, EHE is composed of predominantly epithelioid cells embedded in myxochondroid or sclerotic hyalinized stroma and the presence of evident intracytoplasmic vacuolation (Figure 4B) is a useful diagnostic clue (Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020). Nuclear pleomorphism, necrosis and increased mitotic activity are not exceptional in EHE and in such cases the differential diagnosis with epithelioid AS is more challenging.

CAMTA1 immunoreactivity or CAMTA1 (Calmodulin binding transcription activator 1) rearrangement favour a diagnosis of EHE (Habeeb et al., 2019;
Rosenbaum et al., 2019; Pepke et al., 2020), although a subcategory of EHE may reveal TFE3 immunoreactivity instead of CAMTA1 (Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020). Gene fusions WWTR1-CAMTA1, and less frequently YAP1-TFE3, have both been described in EHE, but not in other epithelioid mesenchymal soft tissue tumors or in a wide range of other vascular tumors/proliferations (Habeeb et al., 2019; Pepke et al., 2020).

Carcinomas, melanomas and epithelioid malignant mesenchymal tumors, such as sclerosing epithelioid fibrosarcoma and epithelioid sarcoma are a potential differential diagnosis of epithelioid AS and vascular markers (ERG, CD31, D2-40, VE-cadherin), MUC4, S100, SOX10 and INI1 are often necessary to resolve this differential (Hunt et al., 2004; Fisher et al., 2013; Antonescu et al., 2014; Ko et al., 2015; Matoso et al., 2015; Shon et al., 2017; van IJzendoorn et al., 2017; Alves et al., 2019; Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020). Epithelioid AS can express CK or EMA immunoreactivity and may be confused with metastatic carcinoma, especially in limited biopsy material. Nevertheless, unlike carcinomas, epithelioid AS almost always present intense diffuse staining for endothelial immunomarkers (Fisher et al., 2013; Antonescu et al., 2014; Habeeb et al., 2019; Rosenbaum et al., 2019; Pepke et al., 2020).

6.3.2 Spindle cell AS

Spindle cell hemangioma (SCH) and composite hemangioendothelioma (CHE) are potential vascular candidates for differential diagnosis when dealing with spindle cell AS (Marusic et al., 2017; Habeeb et al., 2019; Pepke et al., 2020). SCH is considered a benign neoplasm, the histology of which resembles the combination of cavernous hemangioma and KS, hence the KS area may resemble spindle cell AS. IDH1 (isocitrate dehydrogenase) or IDH2 mutations represent diagnostically significant findings in support of SCH (Habeeb et al., 2019; Pepke et al., 2020). CHE may harbour focal areas with a low-grade angiosarcoma-like histological pattern that may be confused with spindle cell AS, but which usually have other intermixed or combined patterns including retiform hemangioendothelioma-like, spindle cell hemangioma-like or EHE-like. Endothelial marker immunoreactivity is the rule; however, neuroendocrine differentiation, possibly related to poor prognosis, has been reported (Habeeb et
Molecular studies do not provide additional diagnostic information. Other spindle cell sarcomas (synovial sarcoma, MPNST, fibrosarcoma, leiomyosarcoma), metastatic spindle cell/desmoplastic melanoma or sarcomatoid carcinoma are potential differential diagnoses of spindle cell AS, although the integration of clinical findings, specific IHC profile and the complement of specific molecular studies usually provide an accurate final diagnosis (Fisher et al., 2013; Antonescu et al., 2014; Habeeb et al., 2019; Pepke et al., 2020). It is essential to emphasize that IHC findings are not completely specific in AS and pathologists should be aware that many vascular markers may be expressed in a wide variety of tumor types, many of which are included in the histological differential diagnosis of AS.

6.3.3 Round cells or anaplastic AS

Round cell or anaplastic AS are the less frequent variants, and the differential diagnosis should be established especially with the Ewing family of tumors (EFT), Ewing-like tumors (ELT), rhabdomyosarcomas and pleomorphic undifferentiated sarcomas, metastatic carcinomas with anaplastic morphology (predominantly lung or pancreatic carcinoma), malignant melanoma or less frequently CD30 anaplastic lymphoma (Antonescu et al., 2014; Machado et al., 2018; Habeeb et al., 2019; Pepke et al., 2020). It should be noted that neuroendocrine differentiation in some AS, especially round cell AS with a solid pattern (lack of vascular formation) can complicate the differential diagnosis with neuroendocrine tumors and EFT (Machado et al., 2018). In addition, EFT may reveal a hemangioendothelial pattern (Figure 4 E) and vascular marker immunoreactivity such as FLI1 and ERG positivity (Machado et al., 2018). In this setting, additional IHC and molecular studies may help to differentiate between Ewing tumors and AS. CD31 and D2-40 expression is very rare in EFT. Furthermore, neither nuclear NKX2.2 positivity, PAX7 positivity, strong membranous CD99 immunoreactivity, nor the EWSR1 rearrangement have been documented in AS to date (Machado et al., 2018). Conversely, the
differential diagnosis between ELS with CIC-rearrangement and round cell AS may still be challenging considering that D2-40 and CD31 immunoreactivity has been reported in CIC-rearranged sarcomas, and CIC rearrangement has been reported in a subset of AS (Yoshida et al., 2016; Machado et al., 2018). Undifferentiated pleomorphic soft tissue sarcoma (UPS) and poorly-differentiated AS (figure 4F) are difficult to distinguish, especially in AS with poor vascular marker immunoreactivity. Furthermore, AS may result as a dedifferentiation process in other sarcomas such as MPNST, leiomyosarcoma, liposarcoma (da Cunha et al., 2005) or malignant solitary fibrous tumor, hence MDM2 or STAT6 immunoreactivity may help in this setting. Differential diagnosis with carcinoma, melanoma and anaplastic lymphoma is discussed in the visceral AS section.

6.4 Visceral angiosarcomas
Poorly-differentiated AS, especially those with solid morphology can closely mimic poorly-differentiated carcinoma, melanoma or anaplastic lymphoma (Seo et al., 2003; Ko et al., 2015; Baker et al., 2017; Ginter et al., 2017; Habeeb et al., 2019; Jung et al., 2019; Alves et al., 2019, Papke et al., 2020). In addition, AS (especially epithelioid subtype) and poorly-differentiated carcinomas may demonstrate IHC similarities (Seo et al., 2003; Ko et al., 2015; Ginter et al., 2017; Habeeb et al., 2019; Jung et al., 2019; Alves et al., 2019, Papke et al., 2020). In visceral tumors (liver, heart etc.) with clear, well-defined vasoformation, AS diagnosis is relatively straightforward, but challenging when epithelioid or spindle morphology predominates (Alves et al., 2019). Notably, the fact that epithelioid AS are positive for cytokeratin markers prompts us to consider epithelial histogenesis. Given the overlapping histological and IHC features in both, a panel of IHC stains is often needed to distinguish between carcinomas and poorly-differentiated AS arising in visceral organs (Alves et al., 2019, Machado et al., 2019). ERG and CD31 are rarely expressed in carcinomas or melanomas and melanocytic marker expression (S100, SOX10, Melan A and HMB45) has not so far been found in AS (Alves et al., 2019, Machado et al., 2019).
CD30 IHC expression occurs in a noteworthy subset of AS and creates a problem of differential diagnosis with other CD30-positive malignancies, especially anaplastic large cell lymphomas (ALCL), diffuse large B-cell lymphomas and germ cell tumors, some of which may be morphologically very similar to epithelioid AS (Alimchandani et al., 2014). In addition, AS may co-occur with germ cell tumors. Expression of various endothelial markers is rare in anaplastic lymphoma or germ cell tumors and lymphoid markers or germ cell immunomarkers are not expressed in AS to our knowledge (Alimchandani et al., 2014).

In conclusion, the integration of clinical, morphological, immunohistochemical and molecular findings are relevant in AS diagnosis. There is no doubt that molecular studies provide significant clues, especially in the differential diagnosis with other vascular neoplasms; nevertheless, a thorough hematoxylin and eosin analysis remains an essential tool in AS diagnosis.

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**Figures**

**Figure 1 (Clinical and grossly detail in AS)**


**Figure 2 (AS histological spectrum)**

A. Secondary cutaneous AS with dermal and subcutaneous infiltration, hematoxylin and eosin (H&E), 10x B. Vasoformative, well-differentiated

Figure 3 (Immunohistochemistry, FISH and electron microscope in AS)

A. CD31 positivity soft tissue epithelioid AS, 20x B. Nuclear ERG expression in well-differentiated cutaneous AS, 20x C. D2-40 immunoreactivity in round cell AS 20x, D. MYC nuclear immunoexpression in breast secondary AS 40x, E. MYC amplification (FISH) in secondary AS, F. Electron microscopy showing Weibel-Palade bodies in AS.

Figure 4 (Differential diagnosis in AS)

A. Epitheliod hemangioma, H&E 40x, B. Epithelioid hemangioendothelioma with mitoses, H&E 40x, C. Pseudomyogenic hemangioendothelioma H&E 20x, D. Kaposi sarcoma with hemosiderin deposits, H&E 40x, E. Ewing sarcoma with pseudoangiomatous (hemangioendothelial) growth pattern, H&E 20x, F. Pleomorphic undifferentiated sarcoma with pseudovascular pattern mimic a pleomorphic or anaplastic AS, H&E 40x.

Graphic 1. Interaction effects of several factors in Angiosarcoma biology

References


molecular features of 74 cases with long term follow up and literature review. Histopathology. 11. doi: 10.1111/his.14090.


tumors is highly associated with loss of ATRX expression and is frequently observed in hepatic angiosarcomas. Hum. Pathol. 46, 1360-6.


separates angiosarcoma of bone from its soft tissue counterpart. Mod. Pathol. 26, 1211-1221.


Table 1. Antibodies, source, dilution and conditions of vascular markers in angiosarcoma

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Source</th>
<th>Clone</th>
<th>Dilution</th>
<th>Pretreatment condition</th>
<th>Staining pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD31</td>
<td>DAKO M0823</td>
<td>JC70A</td>
<td>1/50</td>
<td>Autoclave, Low Ph</td>
<td>M, C</td>
</tr>
<tr>
<td>CD34</td>
<td>DAKO M7165</td>
<td>QBEnd-10</td>
<td>1/50</td>
<td>Autoclave, Low Ph</td>
<td>M</td>
</tr>
<tr>
<td>D2-40</td>
<td>DAKO IR072</td>
<td>D2-40</td>
<td>Prediluted</td>
<td>PTLINK, High Ph</td>
<td>M, C</td>
</tr>
<tr>
<td>ERG</td>
<td>DAKO IR659</td>
<td>EP-111</td>
<td>Prediluted</td>
<td>Autoclave, High Ph</td>
<td>N</td>
</tr>
<tr>
<td>Fli1</td>
<td>MASTER DIAGNOSTIC MAD-210407-Q</td>
<td>MRQ1</td>
<td>1/40</td>
<td>Autoclave, High Ph</td>
<td>N</td>
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<tr>
<td>VE-Cadherin</td>
<td>SANTA CRUZ BIOTECHNOLOGY SC-6458</td>
<td>POLYCLONAL</td>
<td>1/50</td>
<td>Autoclave, Low Ph</td>
<td>C</td>
</tr>
<tr>
<td>VEGF</td>
<td>NEOMARKERS</td>
<td>Mab MS-</td>
<td>1/50</td>
<td>Autoclave, Low Ph</td>
<td>M, C</td>
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<td>Antibody</td>
<td>Manufacturer</td>
<td>Dilution</td>
<td>Staining Conditions</td>
<td>Localization</td>
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<tr>
<td>VEGFR1 (FLT-1)</td>
<td>SANTA CRUZ BIOTECHNOLOGY SC-316</td>
<td>POLYCLONAL 1/400</td>
<td>Autoclave, Low Ph</td>
<td>M,C</td>
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<tr>
<td>VEGFR2 (FLK-1)</td>
<td>SANTA CRUZ BIOTECHNOLOGY SC-315</td>
<td>POLYCLONAL 1/400</td>
<td>Autoclave, Low Ph</td>
<td>M,C</td>
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<tr>
<td>VEGFR3 (FLT-4)</td>
<td>SANTA CRUZ BIOTECHNOLOGY SC-321</td>
<td>POLYCLONAL 1/400</td>
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<tr>
<td>MYC</td>
<td>(ROCHE) VENTANA Nº CAT 790-4628</td>
<td>RABBIT MONOCLONAL Y69</td>
<td>Prediluted</td>
<td>Cell Conditioning Solution (CC1)</td>
<td>N</td>
</tr>
</tbody>
</table>

N: nuclear, C: cytoplasmic, M: membranous
Table 2. Main genetic alterations in Angiosarcomas (AS) with their clinical significance

<table>
<thead>
<tr>
<th>Type of genetic alteration</th>
<th>Genes involved</th>
<th>Clinical significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene amplification</td>
<td>MYC, FLT4 amplification, MYC and FLT4 (VEGFR-3) coamplification</td>
<td>Secondary AS (post-radiation), Primary or secondary AS 25% of secondary AS</td>
<td>Behjati et al., 2014, Huang et al., 2016, Weidema et al., 2019</td>
</tr>
<tr>
<td>Mutation</td>
<td>KDR (VEGFR-2), PTPRB, PLCG1, HTER, TP53, EGFR, KRAS, HRAS, NRAS (high mutation burden), PIK3CA</td>
<td>Typically in primary breast AS, Secondary AS &gt; primary AS, Primary or secondary AS Post-radiation AS and PRAVP, High-grade secondary post-radiation AS, High frequency in primary breast AS</td>
<td>Huang et al., 2016, Behjati et al., 2014, Huang et al., 2016, Corradini et al., 2020, Corradini et al., 2020, Behjati et al., 2014</td>
</tr>
<tr>
<td>Translocation or gene fusion</td>
<td>CIC, EWSR1–ATF1, CEP85L–ROS1, NUP160–SLC43A3</td>
<td>Worse prognosis and younger age at presentation, Sporadically described in primary AS, but not specific, Cutaneous AS</td>
<td>Huang et al., 2016, Marks et al., 2019, Marks et al., 2019</td>
</tr>
<tr>
<td>Epigenetic alteration</td>
<td>Cluster A, Cluster B</td>
<td>Post-radiation secondary AS</td>
<td>Weidema et al., 2020</td>
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<tr>
<td>Visceral and soft tissue AS</td>
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AS: angiosarcoma, PRAVP: post-radiation atypical vascular proliferation