

Solid Form of Angiomatoid Fibrous Histiocytoma: A Diagnostically Challenging Entity

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Abstract

Aim: Angiomatoid fibrous histiocytoma (AFH) is a rare tumor mainly affecting children and young adults. The classic morphology is described as being well encapsulated with a solid cellular proliferation containing histiocyte-like cells, cystic/hemorrhagic spaces and a surrounding dense lymphocytic infiltrate. Unfortunately, the morphology is not always typical and immunostains are often equivocal. Cytogenetic studies show that many instances of AFH have an *EWSR1* gene rearrangement. We describe 8 cases of AFH and review them for lack of any of the characteristic histologic features.

Methods and Results: Routine immunohistochemical (IHC) stains as well as FISH studies for *EWSR1* gene rearrangement were performed on available material. Seven of eight cases lacked at least one major histologic feature and three cases showed a uniformly solid architecture. In addition, three of the seven cases were positive for the *EWSR1* gene rearrangement.

Conclusion: It is important to recognize the solid form of AFH and not to confuse it with malignancy. FISH studies, when positive, are useful and practical in confirming the diagnosis of AFH in cases with unusual histology.

Keywords: Angiomatoid Fibrous Histiocytoma, *EWSR1*, Angiomatoid Malignant Fibrous Histiocytoma, Fluorescence *In Situ* Hybridization

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Introduction

Angiomatoid fibrous histiocytoma was first described by Enzinger in 1979, and is a rare tumor mainly affecting children and young adults [1]. It was initially called angiomatoid malignant fibrous histiocytoma, however as patients with this tumor were followed the malignant modifier was dropped, since the majority of these tumors generally behave in a benign fashion [2]. Nevertheless, metastatic rates have been reported as high as 5% [3,4]. It most commonly presents on the extremities as a painful or tender mass [5]. Occasionally systemic symptoms, such as anemia, weight loss and fever, accompany the lesion [6].

Most cases of AFH show some form of cystic/hemorrhagic spaces, hence the name "angiomatoid". However, these tumors are histologically heterogeneous and may lack any of the three characteristic features: lymphoid tissue, cystic spaces or a capsule, which can lead to diagnostic difficulty. Furthermore, Immunohistochemistry can be equivocal. We have encountered several cases of AFH which did not show the typical histologic features and subsequently reviewed our files for similar cases. Herein, we review the characteristic features of AFH, highlight

the solid form of the tumor, and study the utility of fluorescent *in situ* hybridization (FISH) for the *EWSR1* gene in confirming the diagnosis.

Methods

Eleven total cases of "Angiomatoid Fibrous Histiocytoma" or "Angiomatoid Malignant Fibrous Histiocytoma" were identified in the files at our institution. Three cases were consults and the original slides were not available for evaluation. Hematoxylin and eosin stained slides were available for eight cases and paraffin-embedded tissue was available for seven of them. Hematoxylin and eosin stained slides were reviewed and evaluated for encapsulation, hemorrhagic/cystic spaces and lymphoid aggregates. Atypia was determined on a scale of 0-3 corresponding to absent, mild, moderate or severe atypia. The number of mitotic figures was counted per 10 high power fields, and the presence of necrosis and hemosiderin were noted.

Immunohistochemical (IHC) stains for cytokeratin (AE1/3), S100 protein, smooth muscle actin, desmin, CD99 and CD68 were performed. FISH studies for *EWSR1* gene rearrangement were performed using a break-apart probe. Additional information

including patient's demographics, symptoms at presentation, duration, location of the tumor and the clinical impression were obtained via electronic medical records.

Immunohistochemistry procedure

Immunohistochemistry was performed on the automated Leica Microsystem Bond III platform using their Polymer Refine DAB kit. The pretreatments Enzyme 1 and ER2 (epitope retrieval solution-2) were supplied by Leica (Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, United Kingdom) (**Table 1**).

FISH procedure

Sections were cut to 4-6 μ m thickness then deparaffinized. The slides were baked overnight at 56°C, then immersed in Hemo-D for 3 times at 10 minutes each, dehydrated in 100% ethanol twice for 5 minutes and air dried on 45-50°C slide warmer for 2-5 minutes. Slides were pretreated by immersing them in 0.2 N Hydrochloric acid (HCl) for 21 minutes, followed by purified water for 3 minutes with a pretreatment wash buffer (2 X SSC) for 3 minutes. Slides were then immersed in a second pretreatment solution (Sodium Thiocyanate/NaSCN) at 80°C for 30 minutes, rinsed in purified water for 1 minute and placed in a pretreatment Wash Buffer (2 XSSC) for 5 minutes. Slides were then immersed in a protease solution (25 mg. Pepsin/50 mLs. 0.1 M HCl) at 37°C for >20 minutes until adequately digested, then in a Wash Buffer (2X SSC) for 5 minutes and dried.

The probe, Vysis LSI EWSR1 [22q12] (Abbott Molecular, Abbott Park, IL) was pipetted (10 μ l) onto the slide, coverslipped and placed in a VYSIS Hybrite at 73°C for overnight. Slides were post washed in 2X SSC/0.3% NP-40 to remove the coverslip then immersed in 2.0 X SSC/0.3%NP-40 at 73°C for 2 minutes. Slides were then air dried and DAPI was applied as a counterstain. FISH results were scored independently by two technologists and the laboratory cytogenetic director.

Results

Clinical

Table 2 summarizes the clinical features. The patients included 3 females and 4 males with a female to male ratio of 0.75:1. Ages ranged from 9 months to 32 years, with an average age of 13.2

Table 1 Antibody sources, dilution and retrieval method.

	Company	Dilution	Clone	Antigen retrieval
S100	Dako	1:500	Polyclonal	Enzyme 1/5 minutes
Cytokeratin	Dako	1:400	Ae1,Ae3	Enzyme 1/10 minutes
CD99	BioCare Medical	1:50	H036-1.1	ER2/20 minutes
CD68	Leica Microsystems	1:80	514H12	ER2/20 minutes
Smooth muscle actin	Dako	1:200	1A4	Enzyme 1/10 minutes
Desmin	Dako	1:100	D33	No pretreatment

years. The duration ranged from 1 week to 4 years, and 5 out of seven cases were increasing in size up to the time of resection. Two of the patients noted the lesion to be painful; however none reported systemic symptoms that are known to accompany AFH. Sites of involvement included upper extremity (3), back (3), neck (1) and abdomen (1). The clinical impression prior to operation ranged from benign entities such as lipoma and sebaceous cyst to malignant neoplasms such as sarcoma and lymphoma. Follow up was available on 7 cases and ranged from 1 to 96 months (average 16.57 months) with no evidence of recurrence or metastasis in any of the patients.

Histology

Grossly the tumors ranged from 2.2 cm to 4.0 cm with an average size of 2.94 cm. One case showed classic histology and all the major criteria for AFH. Seven cases lacked one major histologic feature (88%). Six cases were at least partially encapsulated (75%) and four exhibited lymphoid aggregates (50%). The presence of hemorrhagic/cystic spaces was identified in 5 cases (63%) which often corresponded to the presence of hemosiderin. Three cases (38%) were architecturally solid. Four cases exhibited mild cytologic atypia and four were moderately atypical. Mitotic figures were present in three cases and ranged from 1- 3 per 10 high power fields. None of the tumors showed necrosis (**Figure 1**).

IHC and FISH

Of the IHC, CD99 and CD68 showed the highest rates of positivity within the tumors at 86% and 100%, respectively. Three cases (43%) were reactive for desmin (**Figure 2**). All of the cases were negative for smooth muscle actin, S100 protein and cytokeratin (AE1/3). FISH studies revealed that three out of 7 cases (43%) had rearrangement of the *EWSR1* gene (**Table 3**).

Discussion

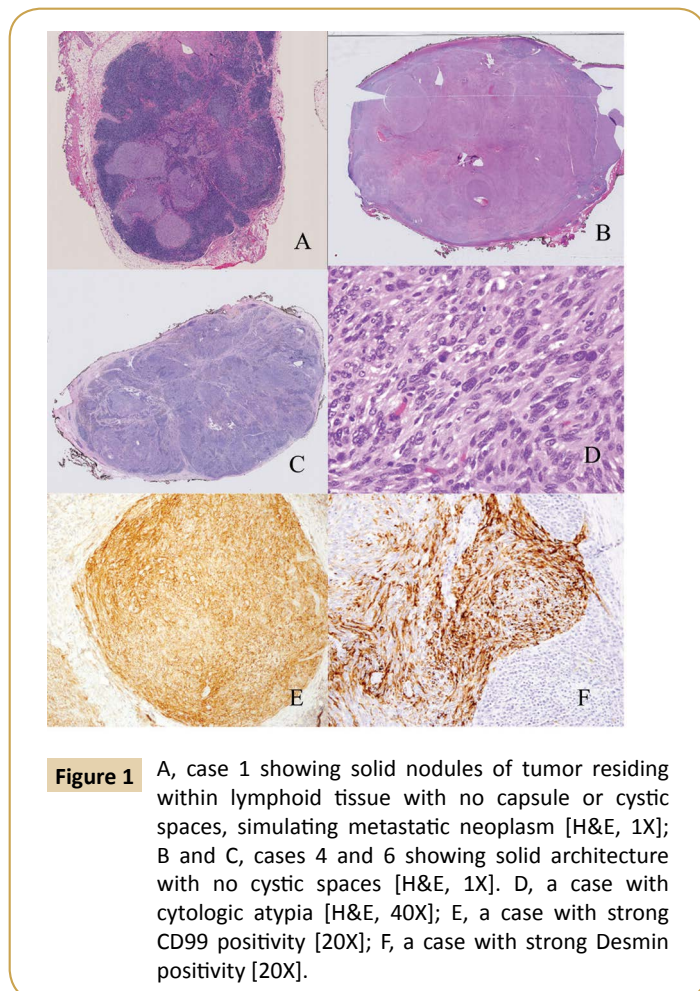
The classic morphology of AFH has been described as being well encapsulated and composed of a solid cellular proliferation of histiocyte-like cells with vesicular nuclei and eosinophilic cytoplasm [5]. Fanburg-Smith described four common histologic features: fibrous pseudocapsule, round or spindled fibrohistiocytic proliferation, pseudo-angiomatous pattern and a plasma-lymphocytic response [2]. The differential diagnosis for AFH includes vascular and myofibroblastic tumors such as nodular Kaposi sarcoma, aneurysmal benign fibrous histiocytoma, palisaded myofibroblastoma, inflammatory pseudotumor and metastatic tumors to lymph nodes [2,7].

Not infrequently, however, these tumors do not show the classic histology and/or present in unusual locations. Several authors described pleomorphic features and/or round cell morphology [8-10]. Chen et al., described cases with unusual morphologic patterns including clear cells, rhabdomyoblasts-like, pulmonary edema-like and cell cords. Unusual locations reported include brain, mediastinum, lung, bone, omentum, retroperitoneum, ovary, vulva and most recently endobronchial [11-13].

In our series, the majorities of the cases lacked one key histologic feature but were recognizable as AFH. On the other hand, three

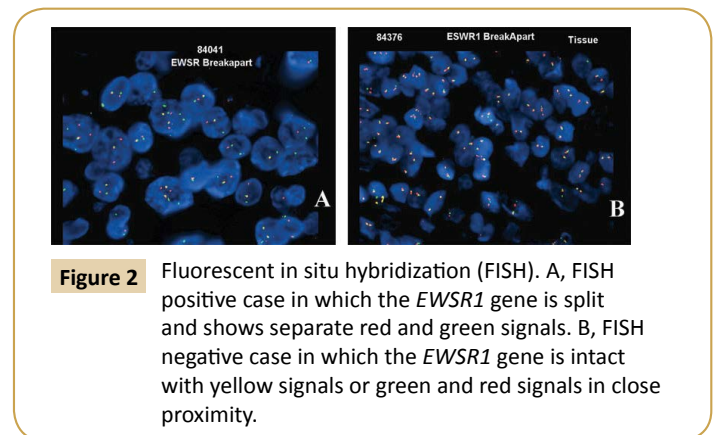
Table 2 Clinical features.

Case	Location	Increasing size	Duration	Pain	Systemic symptoms*	Sex	Age (years)	Clinical impression
1	Antecubital fossa	Yes	2 years	Yes	No	F	14	Perivascular tumor
2	Middle Back	Yes	2 years	Yes	No	F	14	Soft tissue mass
3	Right neck	Yes	1 week	No	No	M	32	Lymphoma
4	Left forearm	Yes	Several months	No	No	M	16	Sebaceous cyst vs. lipoma
5	Right lower quadrant	Yes	3 weeks	No	No	M	3	Sarcoma
6	Right forearm	Unknown	3-4 years	Unknown	No	F	10	Unknown
7	Right upper back	Unknown	Unknown	Unknown	No	M	15	Unknown
8	Middle Back	Unknown	Unknown	Unknown	Unknown	F	4	Unknown



cases had solid architecture and two cases in particular (cases 1 and 4) lacked 2 key histologic features (both capsule and cystic hemorrhagic spaces), and proved to be diagnostically challenging. We will refer to these cases as the solid form of AFH in this paper. This form can be mistaken for metastatic tumor to a lymph node (e.g. sarcoma, spindle cell melanoma or spindle cell carcinoma) and care should be taken not to inadvertently call it malignant.

By immunohistochemistry these tumors show variable positivity



for EMA, desmin, CD68 and CD99, whereas S100 protein and cytokeratin are negative [2,8,11,14,15]. Our cases showed high percentage of positivity for CD99 and CD68. Desmin was positive in less than half of the cases. Although none of these stains is specific for AFH, they are helpful in excluding other entities in the differential. One of the solid forms of AFH in our series was positive for both desmin and CD99, whereas the two other cases were only positive for CD99. Although CD68 is positive in the majority of cases, it is a much less specific marker.

Cytogenetic studies show that many instances of AFH have a Ewing sarcoma breakpoint region 1 (*EWSR1*) gene rearrangement. The rearrangement usually involves the N-terminal of the *EWSR1* gene being fused to a C-terminal DNA binding domain of another gene, this encoding a transcription factor. In AFH the most common fusions with *EWSR1* involve the *CREB1* or the *ATF1* genes [15,16]. Other gene rearrangements such as *FUS/ATF1* gene fusion have also been reported, but are far less common [6].

Some studies have reported more than 90% sensitivity for the EWS FISH [8,11,15]. In their series, Tanas et al., found that 74% of the cases were positive for *EWS* break-apart probe [14]. However, in our patients only three out of seven cases (43%) showed an abnormal result by FISH. This low percentage of positivity may be explained by poor preservation of archival tissue. Only one of the solid forms of AFH in our series was positive by FISH.

Most large series with prolonged follow up confirm the indolent

Table 3 Histology, immunohistochemistry and FISH results.

Case	Size (cm)	Capsule	Hemorrhagic /Cystic	Lymphoid aggregates	Hemosiderin	Atypia (0-3)	Mitosis /10hpf	Necrosis	CD99	Desmin	SMA	CD68	S100	CYK	FISH
1	2.5	no	no	yes	no	1	1	no	pos	Neg	neg	pos (focal)	neg	neg	pos
2	2.2	yes	yes	no	yes	2	0	no	pos	Pos	neg	pos	neg	neg	pos
3	2.5	yes (partial)	yes	no	yes	1	1	no	pos	Neg	neg	pos	neg	neg	neg
4	3.5	no	no	yes (small)	no	2	0	no	pos	Neg	neg	pos	neg	neg	neg
5	4.0	yes (partial)	yes	no	yes	1	0	no	neg	pos (focal)	neg	pos	neg	neg	neg
6	N/A	yes (partial)	no	yes	yes	1	0	no	pos	Pos	neg	pos	neg	neg	neg
7	N/A	yes	yes	no	yes	2	3	no	pos	Neg	neg	pos	neg	neg	pos
8	N/A	yes	yes	yes	no	2	3	no	NA	NA	NA	NA	NA	NA	NA

SMA: Smooth Muscle Actin; **CYK:** Cytokeratin; **Pos:** Positive; **Neg:** Negative; **NA:** Not Available

behavior of these tumors with variable risk for local recurrence (2-12%), but only rare metastasis and death [2,3,4]. Chen et al., showed that visceral/extrasomatic tumors tend to be larger, present at an older age, have associated systemic symptoms and carry higher risk for local recurrence [11] In general, the degree of atypia and mitosis in AFH do not correlate with clinical behavior; however, irregular tumor borders and head and neck location may be associated with increased local recurrence [3]. The mainstay of therapy is wide local excision and careful follow up [2,7]. All cases with clinical follow up in our series, including the solid forms, behaved in a benign fashion with no recurrences or metastases

Summary

AFH is a soft tissue tumor with variable morphology. The fact that this tumor has the potential for metastasis and that it may be confused with other soft tissue tumors with worse prognosis

makes it important that an accurate diagnosis be made. Often times one or more of the characteristic histologic features will be absent, leading to further diagnostic difficulty. The solid form, in particular, can be diagnostically challenging and may be mistaken for metastatic malignancy. The constellation of morphologic features, immunostains and FISH testing, when necessary, should lead to the correct diagnosis. *EWSR1* FISH probes are readily available in many institutions and, if positive, can aid in the diagnosis of equivocal cases of AFH.

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This study had been approved by Wake Forest Internal Review Board (IRB)

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