TITLE:

Angiomatoid fibrous histiocytoma with ALK expression in an unusual location and age group.

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ABSTRACT:
Angiomatoid fibrous histiocytoma (AFH) is a relatively rare soft tissue tumour of intermediate malignant potential, occurring most commonly in young adults, with a recognised propensity for local recurrence and occasional metastasis. A case of AFH occurring on the finger of a 60 year old male is described in which the unusual location and age group for this entity raised the original wrong diagnosis of an aneurysmal and cellular fibrous histiocytoma. Further workup demonstrated an *EWSR1-CREB1* translocation, confirming the correct diagnosis of AFH. Strong ALK expression using the antibody clone D5F3 was demonstrated in our case on immunohistochemistry, which is in concordance with recent findings of ALK positivity with this antibody in the majority of AFHs.

KEYWORDS:
Angiomatoid fibrous histiocytoma, *EWSR1,* ALK, soft tissue tumour.

INTRODUCTION

Angiomatoid Fibrous Histiocytoma (AFH) is a rare tumour of mesenchymal origin which represents approximately 0.3% [1] of all soft tissue tumours and typically occurs in young adults [2]. First described by Enzinger in 1979 as an unusual variant of malignant fibrous histiocytoma [3], AFHs have also historically been diagnosed as variants of vascular neoplasms or other fibrohistiocytic lesions due to their morphological heterogeneity and overlapping clinical and radiological features with these more common lesions. AFH is now recognised as a distinct entity due to characteristic chromosomal rearrangements [4], which places it within a group of neoplasms unified by the presence of identical gene fusions. We report the case of a digital AFH in a 60-year-old male. The atypical location and age group originally led to a wrong diagnosis of aneurysmal and cellular fibrous histiocytoma. Given the morphological features on histology, further molecular studies were performed which demonstrated an *EWSR1-CREB1* translocation, confirming the correct diagnosis of AFH. Strong ALK expression using the antibody clone D5F3 was demonstrated in our case on immunohistochemistry, which is in concordance with recent findings of ALK positivity with this antibody in the majority of AFHs [5, 6].

CASE REPORT

A 60-year-old healthy male presented with a one-year history of a discoloured nodule on the left ring finger causing intermittent discomfort. Past medical history included hypertension and he was also known to be a cystic fibrosis *CFTR* mutation carrier. Physical examination revealed a haemorrhagic nodule on the palmar surface of the proximal left ring finger, with overlying indurated scaly skin (Figure 1). Clinically, the differential diagnosis included a vascular malformation or sarcoma. After discussion with the patient, the decision was made for wide local excision under regional anaesthesia.

Histopathology demonstrated a dermal spindle cell tumour with sheet-like and storiform growth patterns and prominent pseudovascular spaces (Figure 2). There were conspicuous mitoses (average 10 per 10 HPF) and a focal area of more prominent cytological atypia containing hyperchromatic multinucleated cells. Prominent surrounding fibrosis, a moderately dense lymphoplasmacytic infiltrate and haemosiderin deposition were also noted (Figure 2). The pathological opinion offered on initial review favoured a diagnosis of cellular aneurysmal fibrous histiocytoma. The case was referred to our department for secondary review, and on immunostaining the tumour showed strong and diffuse expression of CD99 and EMA, and was negative for CD68, desmin, CD34, CD31, S100, CD10, AE1/3, MNF116, SMA, and Factor XIIIa. Staining with ALK clone ALK01 (Ventana Medical Systems, Tucson, AZ) showed weak to moderate patchy staining, however the ALK D5F3 (Ventana) clone demonstrated strong diffuse expression in tumour cells (Figure 3).

Given the morphology and immunohistochemistry results, formalin fixed curls were sent for FISH and real time PCR (RT-PCR) to distinguish between an aneurysmal fibrous histiocytoma and angiomatoid fibrous histiocytoma (AFH). Break apart FISH probes demonstrated a translocation involving *EWSR1* at 22q12 (Figure 4). RT-PCR demonstrated an *EWSR1-CREB1* t(2;22)(q33;q12) gene fusion. These findings supported a diagnosis of angiomatoid fibrous histiocytoma.

Our patient had a fairly uncomplicated post-operative period, with no restriction in finger movement but initial skin numbness which eventually resolved. As of fifteen months after surgery the wound is healed with no signs of recurrence or distant spread.

DISCUSSION

AFHs typically present as a slow growing, often painless nodule seated in the dermis or subcutis, which may show deep extension [4]. Clinically and radiologically they can mimic vascular neoplasms or a haematoma. They most commonly arise on the extremities, but can occur on the trunk or head and neck region. Rarer primary extrasomatic AFHs have been described in the brain, lung, retroperitoneum, and bone. Peak incidence lies within the first three decades, however they can occur at any age. Patients with tumours harbouring the *EWSR1-CREB1* fusion may present with fever, malaise and anemia as part of a paraneoplastic syndrome secondary to CREB-mediated interleukin 6 upregulation [7].

AFH was first recognized as a translocation associated neoplasm in the early 2000s [8-10]. At present three hallmark translocations are associated with AFH: t(2;22)(q33;q12) producing the *EWSR1-CREB1* fusion gene, t(12;22)(q13;q12) forming the *EWSR1-ATF1* fusion gene, and t(12;16)(q13;p11) resulting in the *FUS-ATF1* fusion gene. *EWSR1-CREB1* is the most commonly demonstrated fusion gene in AFH [10], followed by *EWSR1-ATF1* [4]. In the clinical setting, testing is done by fluorescence in situ hybridisation (FISH) and/or real time polymerase chain reaction (RT-PCR). *EWSR1-CREB1* and *EWSR1-ATF1* fusion genes are not unique to AFH and have been demonstrated in a heterogeneous group of tumours which includes clear cell sarcoma of soft tissue, clear cell sarcoma-like tumour of the gastrointestinal tract, primary pulmonary myxoid sarcoma, hyalinizing clear cell carcinoma of the salivary gland, clear cell odontogenic carcinoma [11, 12], and most recently a subgroup of intracranial myxoid mesenchymal tumours [13]. The members of this group demonstrate distinct clinical presentations, pathological features and prognoses with limited overlap. However, the intracranial myxoid tumors appear to show histological and immunohistochemical features similar to those observed in AFH. Therefore, there is current debate whether these tumors are a new entity or simply represent an intracranial location of the myxoid variant of AFH. Ewing sarcoma breakpoint region 1 gene (*EWSR1)* has been well documented as a 'promiscuous' fusion gene partner, and is involved in several reciprocal neoplasia associated fusions [14]. The *EWSR1* gene commonly contributes the 5' component of these chimeric genes, resulting in a fusion transcript comprising the N-terminal transcriptional activation domain of *EWSR1* fused to the C-terminal DNA-binding domain of one of its various transcription factor fusion partners. This results in deregulation of the activity of the 3' fusion partner, which is believed to be a major driving factor in the tumorigenesis of these translocation associated neoplasms [15].

AFHs are grossly multinodular tumours with a variable amount of haemorrhage and pseudocystic spaces [1, 16]. Histologically these tumours demonstrate ovoid to spindle shaped cells with histiocyte-like morphology forming sheets and fascicles with focal whirling. Intralesional haemorrhage and haemosiderin deposition is often prominent. The triad of pseudoangiomatous spaces, a thick fibrous capsule, and a prominent lymphoplasmacytic infiltrate and/or cuff represents an important clue to the diagnosis of AFH. Tumour cells are usually bland with vesicular nuclei, although foci showing increased nuclear atypia and multinucleated forms as in our case have been well described [4, 16, 17]. Other reported unusual features include an absence of pseudoangiomatoid spaces, marked myxoid change

[18], prominent sclerotic stroma, tumour necrosis, and one case report describing focal Ewing sarcoma-like small cell histology [16]. Tumour cells are typically positive for CD99 as in our case, as well as desmin and/or EMA in approximately 60% of cases. Immunostaining for SMA, CD34, S-100 and cytokeratins are most often negative. Recently, immunohistochemical expression of anaplastic lymphoma kinase (ALK) in the majority of AFHs has been demonstrated in an initial series by Cheah et al, and subsequently confirmed by Van Zwam et al [5, 6]. Curiously, the expression of ALK appears not to be associated with ALK translocations or increased copy number, and the mechanism of expression remains unknown. Our case therefore further supports the utility of ALK immunohistochemistry in supporting the diagnosis of AFH. It is worth noting that in our case initial testing with the ALK01 clone (Ventana) resulted in only weak staining, with subsequent use of the D5F3 clone (Ventana) antibody demonstrating strong cytoplasmic staining. This finding is in accordance with the cases reported by Cheah et al [5], and D5F3 is currently FDA approved as a companion diagnostic to ALK therapies, after several studies demonstrating D5F3 to have a higher sensitivity for lung adenocarcinoma harbouring ALK rearrangements [19-21]. This may be due to D5F3 being a rabbit monoclonal antibody which is generally shown to have higher sensitivity and specificity than mouse monoclonal antibodies including the Ventana ALK01 antibody [22].

Due to histological variation encountered in AFH, the differentials to consider may be quite diverse. Aneurysmal benign fibrous histiocytoma (ABFH) was an important differential considered in this case, as a tumour which occurs on the distal extremities, can clinically mimic vascular lesions and microscopically also demonstrates spindle cells with pseudovascular blood filled spaces [23]. However, ABFH usually demonstrates classic features of fibrous histiocytomas including scattered foamy and giant cells as well as peripheral collagen entrapment and overlying epidermal hyperplasia [24]. ABFHs do not typically demonstrate the same degree of surrounding fibrosis or inflammatory infiltrate as in AFH. Vascular neoplasms are frequently in the clinical differential for AFHs, but immunohistochemical demonstration of endothelial differentiation using CD31, CD34 and ERG [25] is helpful in distinguishing histologically similar tumours such as spindle cell haemangioma and angiosarcoma. AFHs may be misdiagnosed as nodular Kaposi's sarcoma, but in difficult cases HHV8 immunostaining will normally provide accurate distinction [1]. As described by Schaefer and Fletcher, AFHs with prominent extracellular mucin may raise the differential of other myxoid soft tissue tumours such as cellular myxoma, low grade fibromyxoid sarcoma (LGFMS) and extraskeletal myxoid chondrosarcoma (EMC) [18]. As *EWSR1* fusions are also present in EMC and *FUS* translocations characterise LGFMS, careful correlation of FISH results and use of RT-PCR to determine involved fusion partners is prudent when evaluating lesions with myxoid features. Finally, long standing AFHs may show fibrosis alternating with myxoid areas and prominent haemosiderin deposition, raising the differential of a schwannoma with ancient change [16]. However the latter are typically S100 and SOX-10 positive.

Wide local excision is the mainstay of treatment, with margin status an important prognostic factor. Adjuvant chemotherapy and or radiotherapy is indicated for unresectable lesions and metastatic disease. Case reports describing the efficacy of targeted therapeutics against IL-6 have also been published in the literature [7]. The majority of AFHs follow a benign course, however case series have demonstrated up to 15% local recurrence risk [4, 26]. Interestingly, recurrence rates are higher for head and neck and extrasomatic tumours, which may represent the influence of several factors such as depth of invasion, infiltrative margins and status of resection margins [4]. Large case series report metastases

in less than 5% of patients [4, 27, 28], predominantly to lymph nodes but also occurring in the lungs, liver, and brain.

In summary, our case of AFH demonstrates the utility of molecular studies in establishing a more robust diagnosis in non-classical cases, especially where the age of the patient and location are not typical. ALK expression in AFH is recently described and is again demonstrated in this case, providing further support for its use in the diagnostic workup of suspected AFH.

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FIGURE LEGENDS

Figure 1. Haemorrhagic soft tissue mass over left ring finger.

Figure 2. Angiomatoid fibrous histiocytoma - histopathology: (A) low power of tumour in dermis; (B) pseudovascular spaces without endothelial lining, (C) prominent haemosiderin laden macrophages and plasma cell infiltrate, (D) cellular regions of bland ovoid cells with vague whirling pattern, (E) Mitotic figures were noted throughout the lesion, (F) tumour cells with bizarre nuclear contours, multinucleation and hyperchromasia were seen focally.

Figure 3. Angiomatoid Fibrous Histiocytoma - immunohistochemistry: (A) EMA expression (B) ALK (D5F3 clone) expression (C) CD99 expression (D) Desmin negative in tumour cells.

Figure 4: FISH studies with an EWSR1 break-apart probe show separation of green and red signals, indicating a translocation involving EWSR1 at 22q12.

Figure 1

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Figure 2

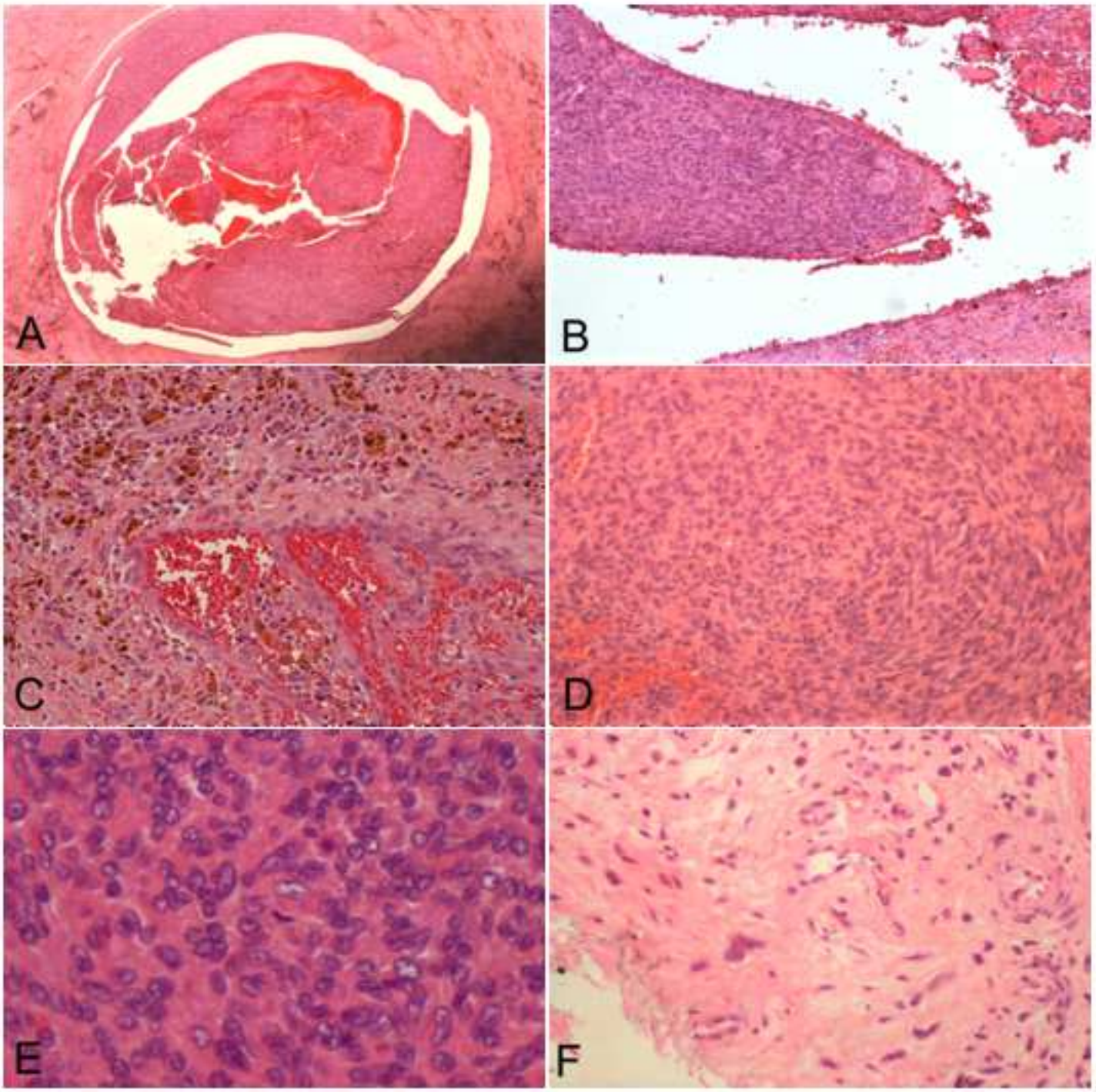

Figure 3

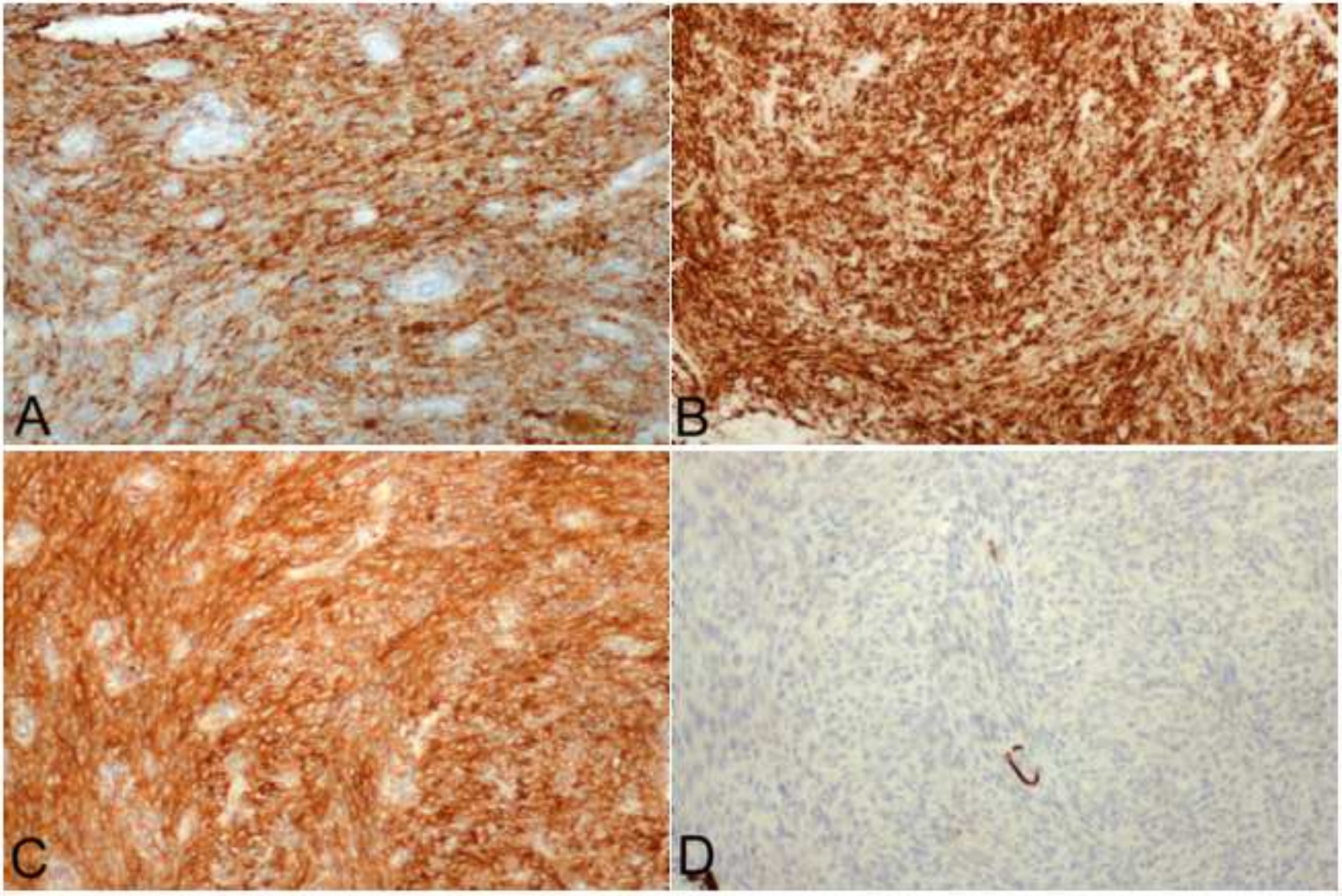

Figure 4

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