Pathology - Research and Practice 215 (2019) 152497

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Case report

Ectopic hamartomatous thymoma in an immunocompromised male

David Waters[a], Farres Obeidin[a], Borislav A. Alexiev[a], M Sambasiva Rao[a], Terrance D. Peabody[b], Mark Agulnik[c], Anjana Yeldandi[a]

[a] Northwestern University, 303 E Chicago Ave Ward 3-140 W127, Chicago, 60611, United States
[b] Department of Orthopedic Surgery, Northwestern University Feinberg School of Medicine, 675 N Saint Clair St, Chicago, IL, 60611, United States
[c] Department of Internal Medicine - Medical Oncology, Northwestern University Feinberg School of Medicine, 675 N Saint Clair St, Chicago, IL, 60611, United States

ARTICLE INFO

Keywords:
Ectopic hamartomatous thymoma
Head and neck pathology
Soft tissue pathology
Immunocompromised

ABSTRACT

Ectopic hamartomatous thymoma (EHT) is a rare benign neoplasm classically occurring in the lower neck of adult males. Here we present a case of EHT occurring in a 43-year-old immunocompromised male and a brief review of existing literature. The patient presented with a palpable mass overlying the left clavicle which, on imaging, showed a solitary nodule possibly eroding the cortical bone. A biopsy predominantly showed spindle cells that were immunopositive for keratin AE1/AE3 as well as weakly positive for CD99, SMA, and CD34. A diagnosis of synovial sarcoma was favored; at which point surgical resection was performed. The resected mass was well-demarcated with a tan-yellow cut surface. Microscopically, the lesion was composed of a mixture of spindle cells, glands, and mature adipose tissue. The spindle cells were plump with bland nuclei, and the epithelial component showed morphology similar to glands of salivary or breast tissue with a bilayered appearance (luminal and basal). No pleomorphism, mitotic figures, or necrosis was present. Immunohistochemical stains were performed and showed the spindle cells to express a myoepithelial phenotype (cytokeratin AE1/AE3, p63, calponin positive). The glands showed SMA and p63 positivity in the basal cells (similar to salivary gland and breast). Overall, given the clinical context, histomorphologic, and immunohistochemical profile, a diagnosis of EHT was made. At 12 months of follow-up there was no evidence of recurrence.

1. Introduction

Ectopic hamartomatous thymoma (EHT), also known as branchial anlage mixed tumor (BAMT), is a rare benign neoplasm of uncertain cell origin [1,2]. The tumor was originally described in 1982 by Smith and McClure and further named by Rosai et al in 1984 [3,4]. Multiple studies have attempted to determine the exact origin of this tumor without definitive classification [1,2]. Due to the lack of known origin and tri-phasic tissue composition, these tumors are often misdiagnosed. EHTs often present with a classic clinical course, arising as a lump in the lower neck near the sternoclavicular junction in middle aged males (median age 46 years) [1–6]. Previous reports have not linked EHT occurrence with any form of predisposition such as viral infection or state of immunodeficiency; however, studies have not been widely performed to investigate predisposing factors due to the theoretical nature of the tumor as a developmental remnant [2,5,6]. Here we describe a case of an EHT arising in an immunocompromised male with HIV.

2. Clinical history

A 43-year-old male presented with a slowly enlarging mass overlying the left proximal clavicle that he had first noted 15 years prior. He noted slow enlargement of the mass and worsening pain since that time. His past medical history was significant for HIV/AIDS on anti-retroviral therapy (undetectable viral load) complicated by HIV dementia (stage 1), arthralgia, and chronic fatigue. Additionally, he had diabetes mellitus type 2, complicated by peripheral neuropathy and retinopathy. There was no family history of cancer or social history of any smoking or alcohol use. On physical exam, a mass was palpated overlying the proximal left clavicle near the sternoclavicular joint. A chest CT (Image 1) showed a solitary 1.4 cm soft tissue nodule adjacent to the proximal left clavicle with associated subtle clavicular cortical erosion. The remainder of the CT was unremarkable. Given the solitary nature of the lesion and the patients increased risk of malignancy, an interventional radiology guided biopsy was performed.

The biopsy showed limited material for diagnosis, show predominantly spindle cells with scant cytoplasm arranged in a vague fascicular pattern. Focal gland formation and epithelioid cells were also

E-mail address: d.waters8557@gmail.com (D. Waters).

https://doi.org/10.1016/j.prp.2019.152497
Received 21 March 2019; Received in revised form 27 May 2019; Accepted 8 June 2019
0344-0338/

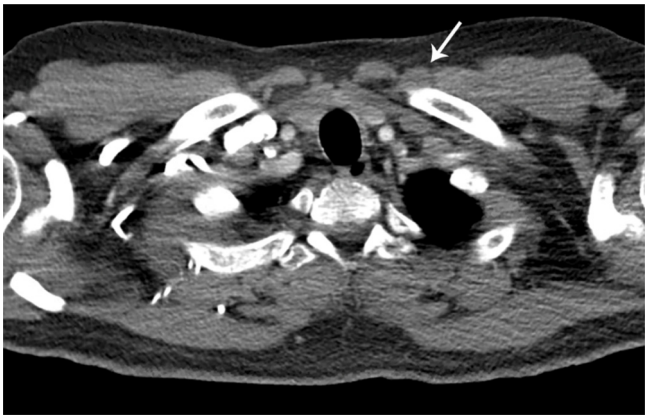

Image 1. A chest CT showed a solitary 1.4 cm soft tissue nodule adjacent to the proximal left clavicle (arrow) with associated subtle clavicular cortical erosion.

appreciated. Multiple immunohistochemical stains were performed and the spindle cell element showed strong immunopositivity for keratin AE1/AE3, as well as weak positivity for CD99, SMA, and, CD34. The lesion was immunonegative for S100, MUC4, and Myo-D1. Due to the spindle cell nature of the lesion, and the patient's HIV status, a HHV8 latency-associated nuclear antigen (LANA) stain was performed and was negative (excluding Kaposi sarcoma). Given this immunoprofile and biphasic morphologic appearance, synovial sarcoma was favored and confirmatory molecular testing for fusion transcripts were sent.

Given the possible diagnosis of synovial sarcoma, the clinical team decided to proceed with surgical resection, followed by radiation therapy based on the size and location of the lesion. While the patient was awaiting surgical excision, molecular testing on the biopsy failed to identify any of the most common synovial sarcoma fusion transcripts (*SYT-SSX1, SYT-SSX2*, *SYT-SSX4*, *SYT-SSX7*, and *SYT-SSX8*). The surgery proceeded as scheduled and the resection specimen was received by pathology.

3. Material and method

Tissue sections of the entire tumor were fixed in 10% buffered formalin and embedded in paraffin blocks. Routine microscopic sections were taken at 4-μm-thick sections and stained with hematoxylin and eosin. Immunohistochemical stains were performed using an automated immunostainer (Leica Bond-III, Leica Biosystems, Buffalo Grove, IL) and BondRefinePolymerTM biotin-free DAB detection kit. Immunohistochemical stains were also performed on appropriate immunohistochemical control tissues and evaluated for appropriate staining. The antibodies applied to the resection specimen are listed in Table 1. A positive nuclear, cytoplasmic and/or membranous expression in 10% or more of neoplastic cells was defined as 'positive'.

Table 1
Immunohistochemistry antibodies (performed on the resection specimen).

Antibody	Manufacturer	Species	Clone
AE1/AE3	Dako	Mouse	AE1/AE3
Calponin	Ventana	Rabbit	EP798Y
Cam 5.2	Cell Marque	Mouse	Cam 5.2
CD5	Leica	Mouse	4C7
CD34	Leica	Mouse	QBEnd 10
CD45	Leica	Mouse	X16/99
CD99	Ventana	Mouse	O13
Desmin	Ventana	Mouse	DE-R-11
MUC4	Epitomics	Rabbit	EP256
p63	Ventana	Mouse	4A4
S100	Ventana	Mouse	4C4.9
Smooth Muscle Actin	Cell Marque	Mouse	16/f5
Smooth Muscle Myosin	Cell Marque	Mouse	SMMS-1
TdT	Leica	Mouse	SEN28

4. Results

4.1. Macroscopic findings

Macroscopic examination revealed a tan-yellow, lobular, homogenous, and well-circumscribed mass measuring 1.4 × 1.0 x 0.8 cm (Fig. 1). The surrounding tissue was comprised predominantly of skeletal muscle.

4.2. Histology and immunohistochemical findings

Histologically, the tumor was well-circumscribed and solid, showing predominantly a moderately cellular proliferation of spindled cells arranged in a fascicular architecture (Fig. 2a). The spindle cells were predominantly plump with eosinophilic cytoplasm and contained bland nuclei with occasional prominent nucleoli (Fig. 2b-f). A second smaller, more delicate population of spindle cells were present surrounding the fascicles of plump spindle cells. Other areas showed discrete gland formation composed of cuboidal cells within a fibrotic stroma (Fig. 2b-e). The nuclei were round and monotonous, with prominent nucleoli and a moderate amount of eosinophilic cytoplasm. Some of the glands showed a distinct epithelial/luminal cell layer and basal cell layer, similar to salivary gland or breast morphology. The smaller population of thinner spindle cells were also present surrounding the glands. A third component of mature adipose tissue was also seen at the periphery of the tumor (Fig. 2a-d). Overall, the tumor composition was approximately 80% spindle cell component, 10% glandular component, and 10% adipocyte component. No pleomorphism, necrosis, or mitotic figures were readily identified in any of the tumor components. A scant rim of chronic inflammation was appreciated, with rare intratumoral lymphocytes seen. No Hassal's corpuscles were appreciated.

Immunohistochemical stains were performed to better classify the lesion. The lesional, plumper spindle cells were immunopositive for cytokeratin AE1/AE3, cytokeratin CAM5.2, calponin, p63, and smooth muscle actin, and immunonegative for S100, CD34, smooth muscle myosin, desmin, CD99, TdT, CD5, and MUC4. These stains highlight the myoepithelial differentiation of the spindle cell component of the tumor (Fig. 3a-d). The smaller, more delicate spindle cell population was positive for CD34 and CD99, and negative for cytokeratins. The glandular/epithelial component stained positively with the tested cytokeratins; epithelial basal cells showed staining with smooth muscle actin and p63 (Fig. 3a, c–d). S100 stain highlighted the adipocytic component. CD45 stained only the intratumoral lymphocytes. Approximately 50% of the lymphocytes were positive for CD5 (T cells), and none of these cells were positive for TdT or CD99.

Overall, given the clinical presentation, triphasic morphology, benign nuclear features, and immunoprofile consistent with myoepithelial nature, the diagnosis of spindle cell neoplasm most consistent with EHT was made.

4.3. Patient follow-up

At a 12 month follow-up after the excision, the patient is doing well on antiretroviral therapy, and post-operative imaging is negative for any sign of tumor recurrence.

5. Discussion

The World Health Organization (WHO) defines EHT as a "benign tumor of the lower neck showing an admixture of spindle cells, epithelial islands and adipose cells suggesting branchial origin" [1]. They further emphasize the classic clinical presentation of a well circumscribed mass in the region of the sternoclavicular joint, with a strong male predominance (> 10:1). Our present case showed all of these

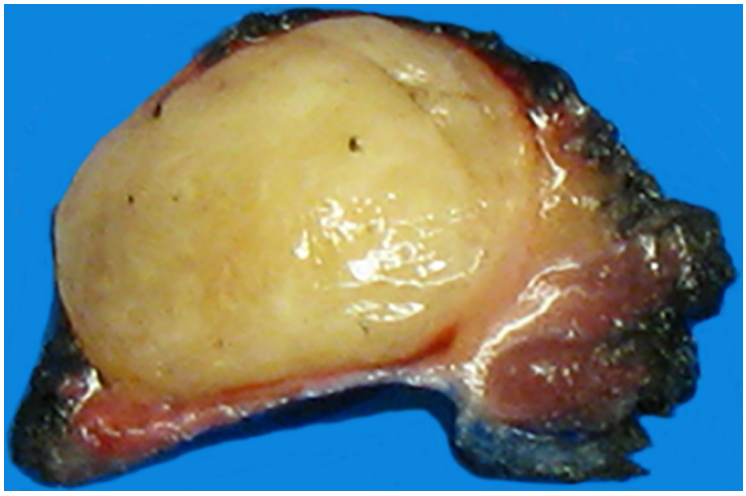

Fig. 1. Macroscopic examination showed a well circumscribed mass with a tan-yellow, homogenous cut surface.

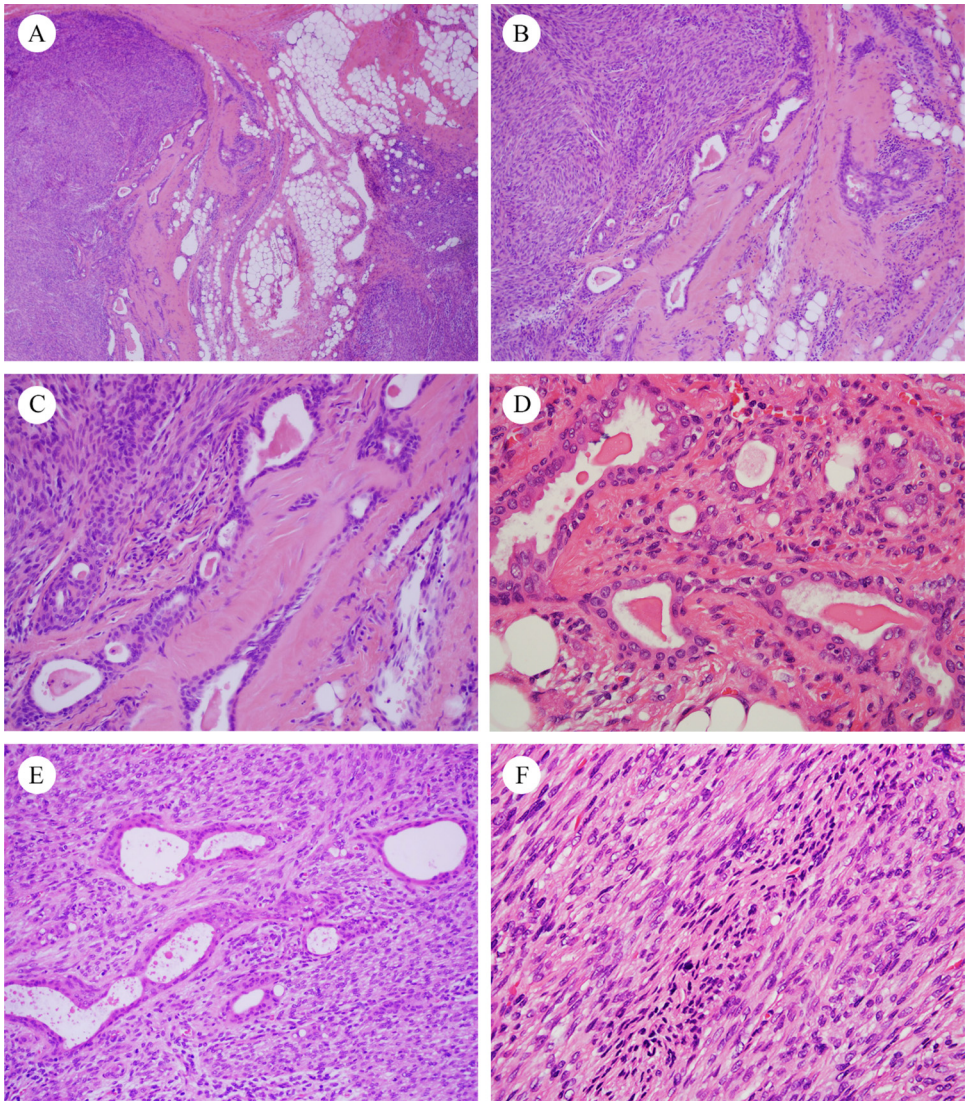

Fig. 2. A. Ectopic hamartomatous thymoma. Sections show a well-circumscribed, triphasic tumor with predominantly spindle cells forming vague fascicles. H&E stain, 40 × . B–C. Higher power showed discrete gland formation and adipocytes. H&E stain, 100x and 200 × . D. Areas of the glandular component were lined by a simple cuboidal lining with round, monotonous nuclei, with inconspicuous nucleoli. H&E stain, 400 × . E. Other areas showed glands with both a distinct luminal, and basal cell layer, surrounded by thin, delicate spindle cells. H&E stain, 200x F. The spindle cells were predominantly plump with bland nuclei and occasional prominent nucleoli. A smaller population of thinner spindle cells were also present surrounding the plumper population. H&E stain, 400 × .

classic features. Histologically, the tumor is composed of three tissue components: bland plump spindle cells in a fascicular or lattice-like growth pattern, epithelial islands with squamous/syringomatous/glandular/cystic differentiation, and adipose tissue [1]. In this case, the epithelial islands were exclusively glandular structures. An expanded immunophenotype is not currently endorsed, but it is noted that both the spindle and epithelial component stains positively with cytokeratins and smooth muscle actin as was seen in this case [1].

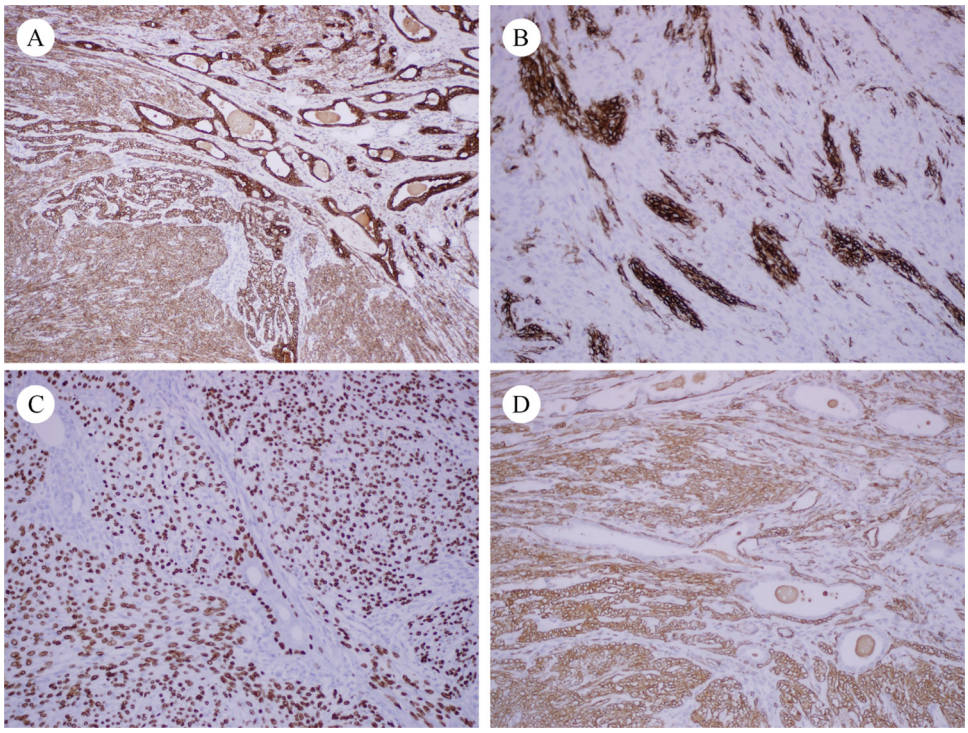

Fig. 3. A. Ectopic hamartomatous thymoma. AE1/AE3 keratin stain is strongly positive in the epithelial component and positive, but less intensely, in the spindle cell component. AE1/AE3 stain, 100 × . B. CD34 stain highlights the thinner fibroblast-like spindle cells surrounding the negative plumper myofibroblasts and the epithelial components. CD34 stain, 200 × . C. p63 stain highlights the nuclei of spindle cells, as well as the basal layer of the epithelial component (similar to salivary gland and breast). p63 stain, 200 × . D. Smooth muscle actin stain shows cytoplasmic staining in a similar distribution to p63. Smooth muscle actin stain, 2000 × .

Overall, EHT is a rare benign neoplasm with less than 80 cases published in the English literature [7]. Within the multiple available literature reviews and case series, only exceptionally rare malignant cases have been reported [2,5–8]. Numerous studies have failed to elucidate a true thymic origin and have proposed an alternative nomenclature such as 'branchial anlage mixed tumor', 'thymic anlage tumor', and most recently 'biphenotypic branchioma' [2,6,7]. Despite these attempts at proposing a standardized nomenclature, the true histogenesis remains controversial. However, most believe the tumor to be derived from an embryologic remnant. Due to this possible embryologic origin, studies have not investigated any relation to immune status. Multiple sites of origin have been proposed including branchial pouches, heterotopic salivary gland tissue, and the cervical sinus of His [2,6,7]. Given the paucity of data on these tumors, the favored name by the WHO is still EHT [1]. Our case concurrently delves into the histogenesis dilemma of this tumor by showing no morphologic signs of relation to thymic tissue. The immunohistochemical findings are consistent with a myoepithelial cell type, similar to previous reports [2,6,7]. This case also showed no tumoral expression of CD5 in the tumor components or TDT or CD99 expression in the intratumoral lymphocytes. Overall, in this case, the morphology and immunohistochemical expression is most consistent with the work of Fetsch et al., who reported the characteristics of 21 cases and suggested a branchial origin of the tumor (branchial anlage mixed tumor), such as the cervical sinus of His [2].

Despite the uncertain origin, the differential diagnosis is well-described. This differential includes many tumors with biphasic morphology such as synovial sarcoma, peripheral nerve sheath tumors with glandular differentiation, mixed tumors of skin adnexal and salivary gland origin, soft tissue myoepithelioma, spindle epithelial tumor with thymus-like differentiation (SETTLE) and teratoma [2,5–8]. Other considerations included spindle cell (sarcomatoid) carcinoma and dendritic fibromyxolipoma [2]. In this case, due to the patients HIV positivity, mycobacterial spindle cell pseudotumor is also a consideration.

As in this case, the most common diagnostic dilemma is with the differential of biphasic synovial sarcoma given the keratin positivity of the spindle cell component. However, EHT should be negative for low molecular weight cytokeratins such as cytokeratin 7 and cytokeratin 8/18 (commonly expressed by synovial sarcomas), but positive for high molecular weight cytokeratins such as cytokeratin 5/6 [9]. Cytokeratin 5/6 highlights the myoepithelial nature of the lesion and is only rarely positive in synovial sarcomas, approximately 5–6% of cases [9]. EHTs should also not express BCL-2, show no cytologic atypia, and have minimal mitotic activity [6]. Recently, TLE1 has been found to be sensitive for synovial sarcomas, but testing has not been performed in EHT's to look for expression [10]. TLE1 was not performed in this case due to the negative fusion transcripts for synovial sarcoma. Additionally, EHT is negative for the typical transcripts commonly found in synovial sarcoma such as t(x;18)(p11.2; q11.2) [11].

Other considered tumors are more easily excluded by morphology and location. Mixed tumors of skin and salivary glands often show chondroid or cartilaginous foci and both soft tissue myoepitheliomas and dendritic fibromyxolipomas show prominent myxoid stroma, both features that are absent in EHT [2]. Others may be excluded by immunohistochemical staining, such as peripheral nerve sheath tumors showing positivity for S100 or SOX10 [12]. Spindle cell carcinoma should, by definition, show evidence of malignancy including a high degree of atypia and numerous mitotic figures. In this case, mycobacterial spindle cell pseudotumor was considered due to the patients immunocompromised statues; however, this tumor is composed of spindled histiocytes expressing histiocytic markers CD58 or CD163, as opposed to myoepithelial cells [13]. SETTLE is exclusively seen within the thyroid gland, contains no adipocytic component, and typically is limited to adolescents (median age 12.9 years); however, this tumor does have low metastatic potential [14]. In this case, no lesion was present within the thyroid.

Multiple studies have elucidated a 'classic' phenotype to distinguish EHT from the other tumors listed in the differential diagnosis. The myoepithelial nature of the spindle cell component is well established, and thus, the cells stain with numerous markers including cytokeratins, calponin, p63, and smooth muscle actin [2,6]. Immunostains for BCL2, STAT6, and S100 are negative in EHT, with the exception of S100 in the adipocytic component [6]. As such, differentiation with other myoepithelial-rich neoplasms may be difficult. Salivary gland mixed tumors typically show a prominent chondromyxoid background not seen in

EHT and present in a different location [15]. Soft tissue myoepithelioma may also show epithelioid or plasmacytoid nests with a spindle cell background but has a more myxoid stroma and lacks an adipocytic component [16]. Positive staining for S100 or SOX10, as well as identification of EWSR1 or PLAG1 gene rearrangements may be needed to definitively exclude these lesions [15,16]. In this case, the 'classic' immunophenotype was demonstrated in conjunction with a consistent clinical presentation and benign triphasic histomorphology to make the final diagnosis.

6. Conclusion

The distinction of EHT from other spindle cell lesions is important for appropriate therapy. Awareness of this benign entity and its classic clinical presentation are helpful in distinguishing this lesion from imitators, in particular synovial sarcoma. In immunocompromised patients, the differential diagnosis is broadened (including mycobacterial pseudotumor), second to the increased clinical concern for malignancy. The treatment involves surgical excision only and tumor recurrence is extremely rare, most likely attributed to incomplete resection. The index of suspicion should be high for any lower neck mass associated with the clavicle or sterno-clavicular joint in a male patient, displaying and admixture of bland spindled cells, benign epithelial islands, and adipose tissue (triphasic tumor).

Funding

No external funds were obtained for this work.

Disclaimer

All authors have seen and approved the manuscript, and contributed significantly to the work. The manuscript has not been previously published, nor is it being considered for publication elsewhere.

References

[1] J.K.C. Chan, Ectopic hamartomatous thymoma, in: C. Fletcher, J. Bridge, P. Hogendoorn, F. Mertens (Eds.), World Health Organization Classification of Tumours of Soft Tissue and Bone, IARC Press, Lyon, France, 2013, pp. 201–202.
[2] J.F. Fetsch, W.B. Laskin, M. Michal, et al., Ectopic hamartomatous thymoma: a clinicopathologic and immunohistochemical analysis of 21 cases with data supporting reclassification as a branchial anlage mixed tumor, Am. J. Surg. Pathol. 28 (2004) 1360–1370.
[3] P.S. Smith, J. McClure, Unusual subcutaneous mixed tumor exhibiting adipose, fibroblastic, and epithelial components, J. Clin. Pathol. 35 (1982) 1074–1077.
[4] J. Rosai, C. Limas, E.M. Husband, Ectopic hamartomatous thymoma: a distinctive benign lesion of lower neck, Am. J. Surg. Pathol. 8 (1984) 501–513.
[5] H. Jing, J. Wang, H. Wei, et al., Ectopic hamartomatous thymoma: report of a case and review of the literature, Int. J. Clin. Exp. Pathol. 8 (2015) 11776–11784.
[6] A. Weissferdt, N. Kalhor, F. Petersson, et al., Ectopic hamartomatous thymoma-new insights into a challenging entity, Am. J. Surg. Pathol. 40 (2016) 1571–1576.
[7] K. Sato, L. Thompson, K. Miayai, et al., Ectopic hamartomatous thymoma: a review of the literature with report of new cases and proposal of a new name: biphontypic branchioma, Head Neck Pathol. 12 (2018) 202–209.
[8] S. Parihar, R. Gohil, R. Oparka, et al., Benign anlage tumour: a very unusual neck mass, BMJ Case Rep. (2016) 1–3.
[9] M. Miettinen, J. Limon, A. Niezabitowski, et al., Patterns of keratin polypeptides in 110 biphasic, monophasic, and poorly differentiated synovial sarcomas, Virchows Arch. 437 (3) (2000) 275–283.
[10] W.C. Foo, M.W. Cruise, M.R. Wick, et al., Immunohistochemical staining for TLE1 distinguishes synovial sarcoma from histologic mimics, Am. J. Clin. Pathol. 135 (6) (2011) 839–844.
[11] J. Przybyl, R. Sciot, P. Rutkowski, et al., Recurrent and novel SS18-SSX fusion transcripts in synovial sarcoma: description of three new cases, Tumor Biol. 33 (6) (2012) 2245–2253.
[12] J.R. Karamchandani, T.O. Nielsen, M. van de Rijn, et al., Sox10 and S100 in the diagnosis of soft-tissue neoplasms, Appl. Immunohistochem. Mol. Morphol. 20 (5) (2012) 445–450.
[13] R.N. Miranda, J.D. Khoury, L.J. Medeiros, Mycobacterial spindle cell pseudotumor, Atlas of Lymph Node Pathology, Springer, New York, NY, 2013, pp. 33–34.
[14] S. Ippolito, C. Bellevicine, D. Arpaia, et al., Spindle epithelial tumor with thymus-like differentiation (SETTLE): clinical-pathological features, differential pathological diagnosis and therapy, Endocrine 51 (3) (2016) 402–412.
[15] C. Martins, I. Fonseca, L. Roque, et al., PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization and immunocytochemistry, Mod. Pathol. 18 (8) (2005) 1048–1055.
[16] C.R. Antonescu, L. Zhang, N.E. Chang, et al., EWSR1-POU5F1 fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone, and visceral lesions, showing common involvement of the EWSR1 gene, Genes Chromosomes Cancer 49 (12) (2010) 1114–1124.