Rare angiolymphoid hyperplasia with eosinophilia examined through fine needle aspiration cytology, histopathology and immunophenotypic characterization: A case report

XIAOHUA WANG[1*], XIANG SHI[2*], DAHUA SUN[3] and MEIPING WAN[4]

Departments of [1]Obstetrics, [2]Pathology, [3]Thyroid and Breast Surgery and [4]Traditional Chinese Medicine, Qianjiang Central Hospital, Qianjiang, Hubei 433100, P.R. China

Received March 22, 2023; Accepted June 15, 2023

DOI: 10.3892/etm.2023.12093

Abstract. Angiolymphoid hyperplasia with eosinophilia (ALHE), a rare benign proliferative tumor, mainly occurs in several countries in Asia and it is characterized by true vascular branching hyperplasia with infiltration of a large number of lymphocytes and eosinophils in the stroma. The present case report analyzed the clinical symptoms and fine-needle aspiration cytology, histopathological and immunohistochemical results of a patient with ALHE, and summarized the clinicopathological diagnostic features of the disease. To the best of our knowledge, this was the first study to comprehensively report the cytological, histopathological and immunophenotypic characteristics of ALHE, which could help clinicians fully understand this rare type of proliferative tumor.

Introduction

Angiolymphoid hyperplasia with eosinophilia (ALHE) is also known as epithelioid hemangioma or histiocytic hemangioma. Although these types of neoplastic lesions belong to the same category of diseases, each one is characterized by a unique onset site (1). ALHE is a relatively rare disease that commonly occurs in the head and neck, and more particularly in the auricle. The incidence of ALHE is high in Asian countries (2), such as China, Japan and South Korea, with a wide age distribution, and is most common among middle-aged and elderly male patients. The various other names of this particular disease are mainly associated with lesions occurring at other soft tissue sites, while several differences in their histopathology (for instance, different degrees of vascular endothelial proliferation and the number of lymphocytes) may also be observed (3,4). Based on previous limited reports, ALHE is considered to be a benign vascular proliferative neoplastic lesion characterized by inflammatory reactions (5). Surgical resection is considered the most appropriate therapy approach for treating ALHE. However, its early and accurate diagnosis is a prerequisite for its effective treatment (5). Therefore, the current report thoroughly analyzed the clinical features and cytological, histopathological and immunophenotypic data from a patient with ALHE, thus providing clinicians with more diagnostic data.

Case report

A 65-year-old male patient visited Qianjiang Central Hospital (Qianjiang, China) in December 2020 with a history of a long nodular tumor on the right auricle for >1 year. The initial nodules were small; however, they subsequently increased slowly to ~5 cm. (Fig. 1). The nodules were often accompanied by itching and scratching and were prone to bleeding. No other symptoms of the disease were observed in other parts of the body. The patient did not undergo any other auxiliary tests or treatment before attending the hospital for treatment. The patient visited the Qianjiang Central Hospital for treatment since the itching and recurrent bleeding from the nodular tumor was not tolerable. The patient was subjected to histopathological biopsy and fine-needle aspiration cytology (10-ml fine-needle syringe) of the auricle tumor and routine blood laboratory examination (Sakura Blood Analyzer).

A blood routine examination revealed a significant increase in the number of eosinophils compared with normal range limits ($1.1x10^9$/l; normal range, 0.05-$0.5x10^9$/l). The ratios of the other blood cell types (neutrophils, basophils, lymphocytes, monocytes) were within normal limits (reference interval for commonly used clinical testing items based on the Chinese population (6). Only 2 ml blood was extracted from the puncture during the nodular tumor fine-needle aspiration.

Correspondence to: Professor Meiping Wan, Department of Traditional Chinese Medicine, Qianjiang Central Hospital, 22 Zhang Hua Street, Qianjiang, Hubei 433100, P.R. China
E-mail: wanmeiping@126.com

Professor Dahua Sun, Department of Thyroid and Breast Surgery, Qianjiang Central Hospital, 22 Zhang Hua Street, Qianjiang, Hubei 433100, P.R. China
E-mail: sundahua@126.com

*Contributed equally

Key words: angiolymphoid hyperplasia with eosinophilia, fine needle aspiration cytology, histopathology, immunohistochemistry

Following rapid staining with Liu's ready-to-use staining solution (BASO Diagnostics Inc.) at room temperature, the sample was stained with solution A for 10 sec, solution B was added for 10 sec, followed by thorough rinsing with clean water), a high number of red blood cells was observed under the microscope, accompanied by raised eosinophil and lymphocyte counts and several epithelioid cells (Fig. 2).

Subsequently, hematoxylin and eosin (H&E) staining was performed. The initial processing steps for H&E staining were the same as the immunohistochemical steps. Following deparaffinization and rehydration as mentioned below, sections (thickness, 4 μm) were treated with hematoxylin reagent for 5 min at 37˚C and then treated with 1% hydrochloric acid-ethanol solution for 1 sec. Subsequently, the sections were stained with eosin reagent for 3 min at 37˚C. The slides were dehydrated and mounted, and images were acquired under a light microscope. Microscopical observation of tissue specimens obtained via histopathological biopsy showed that the tissue was covered with squamous epithelium, while abundant proliferative branching blood vessels were observed in the superficial and middle layers of the dermis. Additionally, the vascular endothelial cells exhibited epithelioid and shoe nail-like structures, with eosinophilic cytoplasm protruding into the lumen. At the same time, rare mitotic figures were identified. Furthermore, a large number of lymphocytes and several histiocytes, eosinophils and plasma cells infiltrating the peripheral stroma were observed (Fig. 3).

In addition, immunohistochemical staining was performed to determine the expression of specific proteins using the corresponding antibodies (Fig. 4). Fresh tumor tissues were soaked in 4% neutral formaldehyde at 37˚C for 24 h and then dehydrated with different concentrations of alcohol and paraffin-embedded. Adhesion slides with 4-μm sections were kept at 60˚C for 4 h. Thereafter, the slides were deparaffinized with xylene and hydrated with an ethanol gradient. Subsequently, the slides were placed in citrate buffer (0.01 mol/l) under high pressure for 3 min at 120˚C for antigen retrieval and then the slides were incubated with 0.3% H_2O_2 for 30 min at 37˚C. Subsequently, the sections were rinsed in PBS-1% Tween 20 and were blocked with 10% goat serum (Zhongshan Goldenbridge Bio) for 30 min at 37˚C. Subsequently, immunohistochemistry was performed using a rabbit polyclonal anti-CD31 (cat. no. 11265-1-AP), anti-ETS-related gene (ERG; cat. no. 14356-1-AP), anti-smooth muscle actin (anti-SMA; cat. no. KHC0053) and anti-Ki-67 (cat. no. 27309-1-AP) (all 1:200 and from ProteinTech Group, Inc.) and a mouse monoclonal anti-CD3 (cat. no. KHC0013), anti-CD20 (cat. no. KHC0018), anti-CD68 (cat. no. KHC0006) and CD138 (cat. no. KHC0020) (all 1:100 and from ProteinTech Group, Inc.), at 4˚C overnight. After completing the previous steps, the sections were incubated with a secondary antibody (goat anti-rabbit/mouse IgG-HRP multimer; SAP-9100; Zhongshan Goldenbridge Bio) for 20 min at 37˚C and then 3,3'-diaminobenzidine reagent was added followed by hematoxylin staining for 1-3 min at 37˚C. The slides were dehydrated and then mounted in neutral resins and image acquisition was performed using a light microscope. The results showed that the blood vessels of the patient with ALHE expressed CD31, ERG and SMA, while infiltrating lymphocytes mainly expressed the T-cell marker CD3 and a small amount of the B-cell marker CD20.

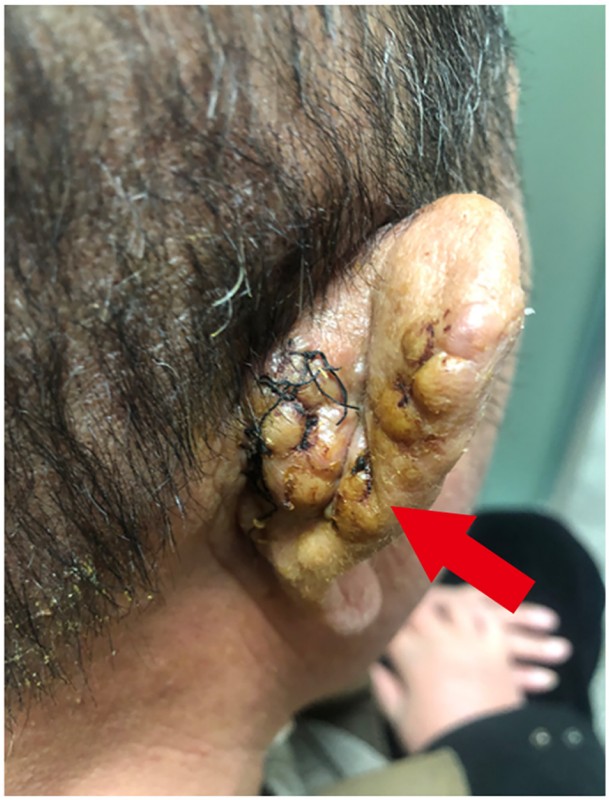

Figure 1. Clinical presentation of ALHE. ALHE mainly occurs in the auricle, presenting as a multinodular mass, often accompanied by itching and bleeding. ALHE, angiolymphoid hyperplasia with eosinophilia.

The lesion tissue contained several tissue cells expressing CD68 and a small number of plasma cells expressing CD138. Immunohistochemical staining for the proliferation marker Ki-67 revealed that the tumor itself was in a low proliferative state. By contrast, the inflammatory cells in the peripheral stroma exhibited strong proliferation (Fig. 4).

Tumor resection was used as low-risk primary surgery: After the patient's skin was disinfected with iodophor, 1% lidocaine hydrochloride was used for local anesthesia by subcutaneous injection, and the epidermis and subcutaneous tissue were cut layer by layer with the scalpel to completely remove the tumor tissue, and then the incision was sutured. After completely removing the tumor via surgical resection, the patient with ALHE did not undergo any other drug treatment, radiotherapy or chemotherapy. In addition, no recurrence or other symptoms were recorded at the 2-year follow-up.

Discussion

ALHE was first reported by Wells and Whimster in 1969 (7). Due to the relatively rare nature of the disease, there is insufficient research on its pathogenesis. The disease mainly occurs in Asia, with a slightly higher incidence in male patients compared with that in female patients and a wide range of age of onset (8). The tumor, which is mainly distributed in the head and neck, and more specifically around the ear and auricle, is presented as dermal papules or nodules, commonly accompanied by itching and scratching; therefore, the patients are prone to bleeding (9). The early stage of the disease is characterized

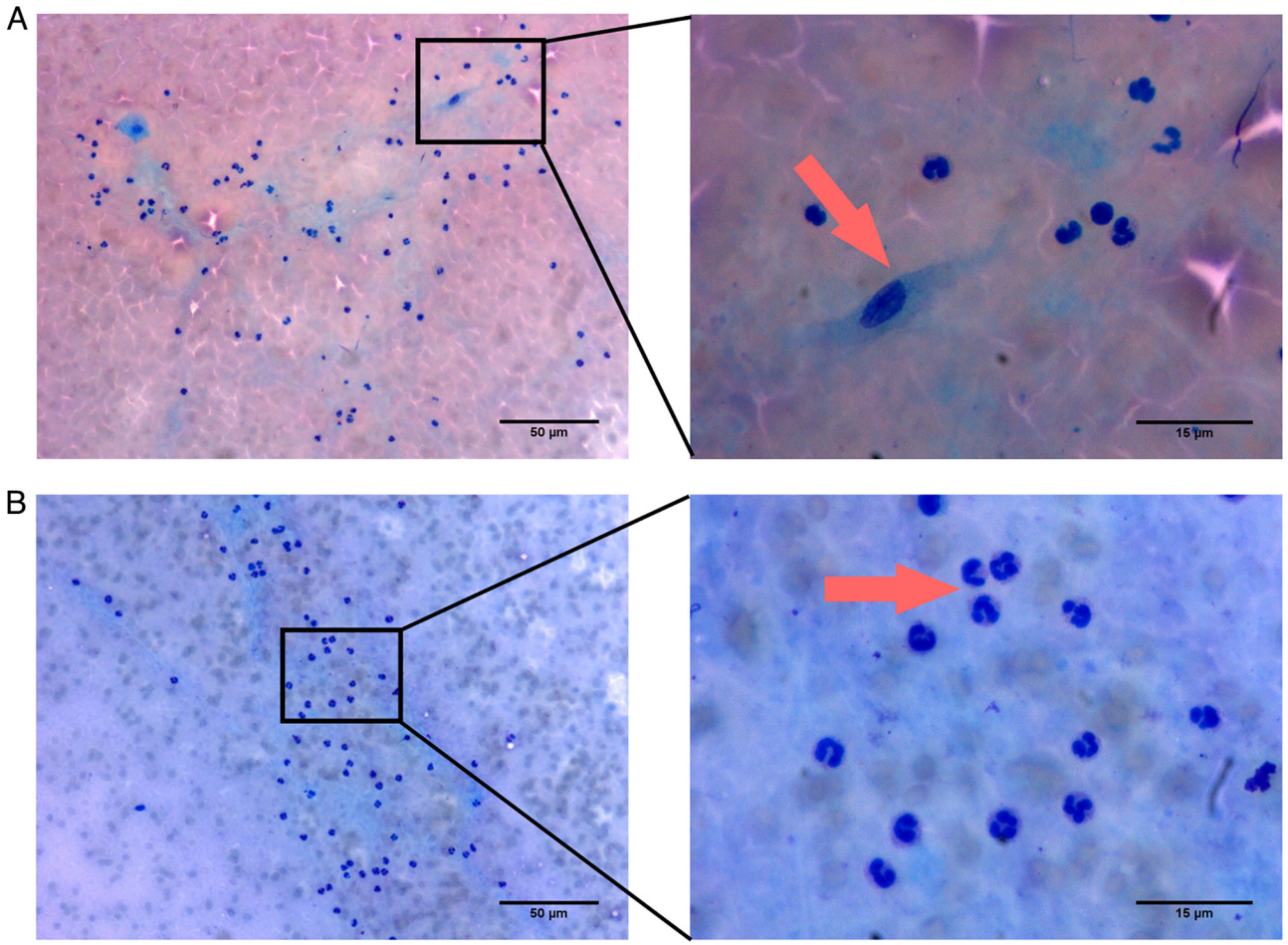

Figure 2. Cytological characteristics of fine needle aspiration (Liu's rapid staining). (A and B) A large number of red blood cells, some eosinophils, lymphocytes and epithelioid cells can be seen under the microscope. (A) Morphological characteristics of vascular endothelial cells in fine needle aspiration cytology, the arrow points at a vascular endothelial cell. (B) morphological characteristics of abundant eosinophils in fine needle aspiration cytology; the arrow points at an eosinophil (scale bars, 50 μm; 15 μm in the magnified windows).

by vascular proliferative lesions, while the late stage presents significant lymphoproliferative lesions, which needs to be differentiated morphologically from Kimura's disease (8,10). To the best of our knowledge, the present study is the first one to comprehensively elaborate on the diagnostic points of ALHE combined with fine needle aspiration cytology, histopathology and immunohistochemical characterization.

Based on fine-needle aspiration cytology, ALHE shares similar characteristics with other vascular tumors. Therefore, the naked-eye appearance of the aspirate is not significantly different from other hemangiomas. This finding suggests that ALHE is essentially a type of vascular tumor disease. Microscopic analysis revealed that the ALHE sample was not only rich in red blood cells, but it also exhibited abundant eosinophils and several lymphocytes and epithelioid cells in the background. The above findings are uncommon in other hemangiomas, which usually only show dense proliferation of small blood vessels, while eosinophils and lymphocytes are not seen when there is no inflammatory response (11); by contrast, these characteristics are common in ALHE. In ordinary hemangiomas, significant endothelial cell proliferation does not occur as in ALHE, and it is not easy to obtain endothelial cells through fine needle puncture, whereas this is easy to achieve in ALHE. Therefore, the aforementioned result of fine-needle aspiration cytology could be used as a key point to differentiate ALHE from other diseases.

In terms of histopathology, microscopic examination of ALHE samples commonly reveals significant vascular proliferation, accompanied by a tendency to lobulation. The vascular walls could be thickened and vascular endothelial cells could appear epithelioid or histiocytic, with abundant cytoplasm and deep eosinophilic color, and their edges are scallop-shaped or spike-like (12,13). In addition, the excessive proliferation of endothelial cells could block the vascular lumen (14). A large number of lymphocytes, several tissue cells, eosinophils and plasma cells can be also observed infiltrating the peripheral fibrous stroma (15). It has been suggested that eosinophils may affect the progression of this disease by inducing the production of nitrous oxide and eosinophilic cationic proteins (16). The aforementioned pathological and morphological features were also observed in the current case report. ALHE and Kimura's disease are commonly presented with subcutaneous nodular masses on the head and face and exhibit several similarities that should be differentiated, they usually have proliferation of vascular endothelial cells and lymphocytes, and eosinophil infiltration can also be obvious, but there are certain differences in the location of the disease, the diversity of inflammatory cells and fibrosis (17). Kimura's disease,

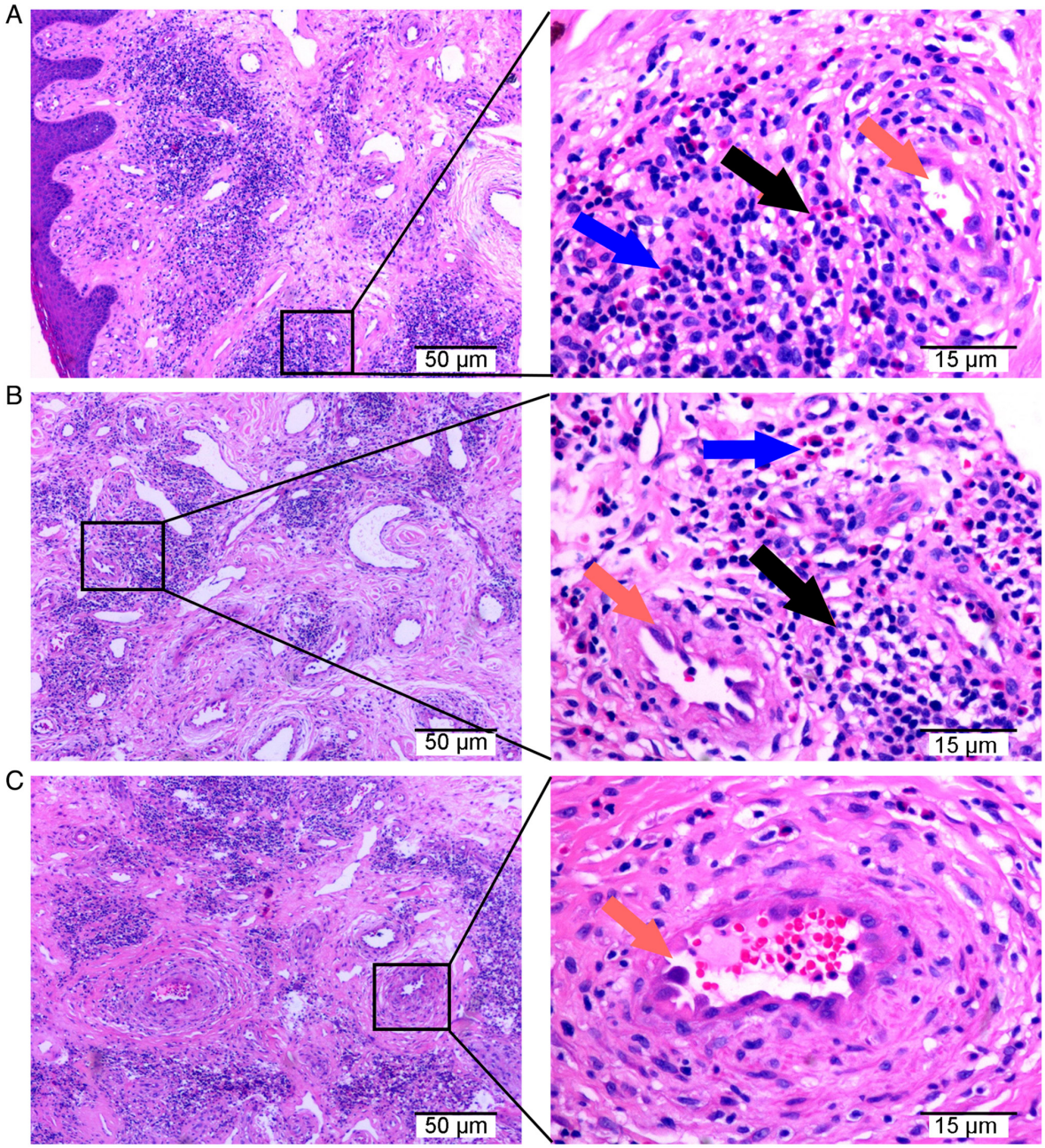

Figure 3. Histopathological characteristics of angiolymphoid hyperplasia with eosinophilia (hematoxylin and eosin staining). Histopathological biopsy under the microscope showed that the tissue was covered with squamous epithelium, and abundant proliferative branched blood vessels were seen in the superficial to middle layers of the dermis, while the vascular endothelial cells were epithelioid and shoe nail-like. A large number of lymphocytes, some histiocytes, eosinophils and plasma cells were seen infiltrating the peripheral stroma. (A) Hyperplasia of vascular endothelial cells and abundanT lymphocytes and eosinophil on histopathology. (B) Hyperplasia of vascular endothelial cells and abundanT lymphocytes and Eosinophil in another lesion area. Red, black and blue arrows point to vascular endothelial cells, lymphocytes and eosinophils, respectively. (C) Significant shoe nail-like proliferation of vascular endothelial cells. The red arrow points to a shoe nail-like vascular endothelial cell (scale bars, 50 μm; 15 μm in the magnified windows).

also known as eosinophilic proliferative lymphogranuloma, is often accompanied by enlarged lymph nodes, a significant increase in the eosinophil count in peripheral blood and elevated serum IgE levels, while it can be associated with bronchial asthma (18). It has been suggested that this disease is an immune-mediated inflammatory response disease (19). Although ALHE is mainly characterized by benign vascular hyperplasia, it is currently considered to be a true vascular tumor rather than an inflammatory disease (20).

In terms of immunohistochemical phenotype, the present study indicated that the cells in the vessels with hyperplasia highly expressed the vascular endothelial-related markers ERG and CD31, while those in the thick vascular wall strongly expressed SMA. In addition, the proliferative lymphocytes showed high expression levels of CD3 and low levels of the B lymphocyte-related marker CD20, thus indicating that the disease was mainly characterized by T-cell proliferation. In a mixed pattern, several CD68-expressing tissue cells and scattered CD138-expressing plasma cells were observed. Ki-67 was highly expressed in the inflammatory zone, while the proliferative ability of vascular endothelial cells of the tumor was reduced. The increase in T-cell count suggested that a

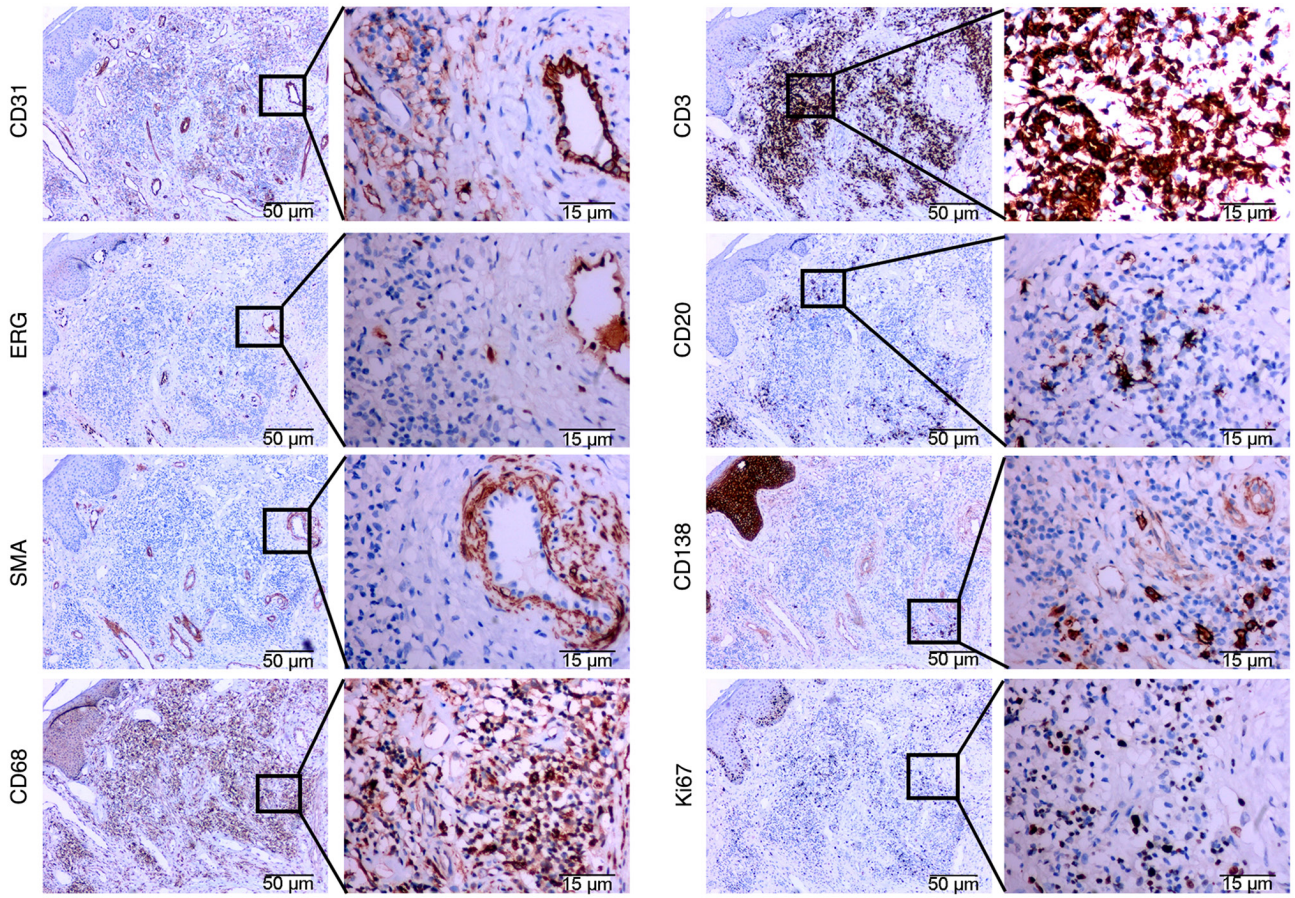

Figure 4. Expression of immune indicators in angiolymphoid hyperplasia with eosinophilia. The proliferative blood vessels strongly express CD31, ERG and SMA immune markers, while the surrounding matrix is characterized by a large number of CD68-expressing histiocytes and some CD138-expressing plasma cells. The proliferative lymphocytes mainly express the T-cell marker CD3 and a small amount of CD20. The proliferation marker Ki-67 shows lower expression in the tumor itself and higher expression in inflammatory regions (scale bars, 50 μm; 15 μm in the magnified windows). ERG, ETS-related gene; SMA, smooth muscle actin.

cellular immune response could occur at advanced stages of the disease, because sensitized T cells are typically involved in delayed immune responses (21). However, the present study showed that the serum levels of IgE were not increased, and no allergy- or asthma-related diseases were identified, thus indicating that ALHE could be a non-immune response-mediated disease. In addition, Ki-67 expression suggested that ALHE involved a tumor with low proliferative activity, thus indicating that ALHE could be a benign disease.

The considerable expression of vascular-related immunohistochemical markers strongly suggested that ALHE is a vascular tumor lesion. The Ki-67 proliferation index of the ALHE lesion was low, indicating that ALHE is a low-grade benign tumor. The large number of T lymphocytes present in the current case did not exhibit malignant features, but some previous studies found that ALHE may be accompanied by abnormal proliferation of T lymphocytes and have the potential to further develop into peripheral T-lymphocyte tumors (22,23).

Although the present case report elaborated on the characteristics of ALHE based on several pathological examination methods, including fine needle aspiration cytology, histopathology and immunophenotypic characterization, certain limitations still exist. The rarity of ALHE results in only one case being included in the current study, which makes it impossible to describe other rare pathological features of ALHE through large sample size characterization. Furthermore, due to the insufficient sample size, further research is not possible to further investigate and explain the formation of abundant eosinophils and T lymphocytes observed in the present case. According to a relevant previous report (23), T lymphocytes in ALHE may develop into T-cell lymphoma; therefore, further research on T lymphocytes, especially at the molecular level is necessary. In addition, in the comparison between ALHE and Kimura's disease, the lack of suitable images for comparative analysis makes the identification analysis less intuitive. Although these limitations have no impact on the conclusion of the main topic of the present report, collecting more cases through multi-center cooperation and conducting research on the molecular mechanism will improve the understanding of this disease.

The classic cytological and histopathological changes of ALHE are known, and it is relatively easy for clinical and pathological physicians to diagnose this disease by understanding these characteristics. However, due to the rarity of ALHE and the relatively small number of reported cases, the pathogenesis of the disease remains unclear. It has been suggested that ALHE may be related to vascular injury, bacterial infection and abnormal hormone levels, among other causes (3). However, it is crucial to conduct multicenter

epidemiological investigations to clarify the etiology of ALHE. In addition, to the best of our knowledge, there are currently no reports on the molecular pathology of ALHE. With the current advances in genetic research, it is hoped that more cases can be collected, and the pathogenesis of ALHE will be elucidated at the genetic level through second-generation sequencing technology. Once the associated genes are found, a targeted drug approach may become another treatment scheme in addition to surgery.

In summary, ALHE is a relatively rare disease, and its etiology remains unclear. The results of the present case report suggested that ALHE could represent a benign tumor originating from vascular cell proliferation, also characterized by local immune reactions at the advanced stages of the disease. To uncover the mechanism underlying the pathogenesis of ALHE, further research with larger sample sizes is needed. Currently, ALHE is considered a benign lesion, with a good outcome after surgical resection. Overall, a clear diagnosis of the disease could be achieved after the combined analysis of the clinical features of patients with ALHE and the typical pathological changes.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XW, XS and MW collected disease data. XS and MW participated in surface fine needle aspiration surgery, while DS and XW participated in tumor resection surgery. DS and MW retrieved the relevant literature. XW and XS wrote and reviewed the article. XW, XS, DS and MW confirm the authenticity of all the raw data. All authors have read and confirmed the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Qianjiang Central Hospital [approval no. 2022 (11001); Qianjiang, China].

Patient consent for publication

Written informed consent was obtained from the patient for their information to be published in this case report.

Competing interests

The authors declare that they have no competing interests.

References

1. Guo R and Gavino AC: Angiolymphoid hyperplasia with eosinophilia. Arch Pathol Lab Med 139: 683-686, 2015.
2. Sun Z, Jing L and GuangF Z: A case of angiolymphoid hyperplasia with Eosinophilia. China Leprosy Skin Dis J 32: 426-427, 2016.
3. Halteh P, Magro C and Lipner SR: Angiolymphoid hyperplasia with eosinophilia of the nail unit. Dermatol Online J 23: 13030/qt0wp8p8bb, 2017.
4. Su HH, Shan SJ, Elston DM, Guo Y and Men JL: Congenital blaschkoid angiolymphoid hyperplasia with eosinophilia of the anogenital region. Am J Dermatopathol 38: 305-306, 2016.
5. Fernandes BF, Al-Mujaini A, Petrogiannis-Haliotis T, Al-Kandari A, Arthurs B and Burnier MN Jr: Epithelioid hemangioma (angiolymphoid hyperplasia with eosinophilia) of the orbit: a case report. J Med Case Rep 1: 30, 2007.
6. Hong S, WenX C and PanB S: Establishing a reference range for commonly used clinical testing items based on the Chinese population. China Health Standard Management 4: 17-21, 2013.
7. Wells GC and Whimster IW: Subcutaneous angiolymphoid hyperplasia with eosinophilia. Br J Dermatol 81: 1-14, 1969.
8. Hashida Y, Higuchi T, Nakajima K, Ujihara T, Murakami I, Fujieda M, Sano S and Daibata M: Human polyomavirus 6 with the Asian-Japanese genotype in cases of Kimura disease and angiolymphoid hyperplasia with eosinophilia. J Invest Dermatol 140: 1650-1653 e4, 2020.
9. Fernandez-Flores A and Cassarino DS: Three unusual histopathological presentations of angiolymphoid hyperplasia with eosinophilia. J Cutan Pathol 44: 300-306, 2017.
10. Zhuang S, Chen X, Chen W, Li C, Wang T, Lin Q and Wang D: A retrospective study of 20 patients with Kimura's disease from China. Acta Otolaryngol 142: 357-362, 2022.
11. Kita AE and Long JL: Hemangioma. Ear Nose Throat J 95: 19-20, 2016.
12. Velamala P, Gupta P, Gupta N, Radotra BD and Rajwanshi A: Cytologic features of angiolymphoid hyperplasia with eosinophilia: Highlighting the diagnostic clues. Diagn Cytopathol 49: E7-E13, 2021.
13. Yuki CS, Young PJ, Terrano D and Nguyen X: Angiolymphoid hyperplasia with eosinophilia in a patient with coccidioidomycosis. Cutis 110: E32-E34, 2022.
14. Bahloul E, Amouri M, Charfi S, Boudawara O, Mnif H, Boudawara T and Turki H: Angiolymphoid hyperplasia with eosinophilia: report of nine cases. Int J Dermatol 56: 1373-1378, 2017.
15. Trivedi A, Norris I, DeWitt CM and Goldman GD: Angiolymphoid hyperplasia with eosinophilia treated with Mohs micrographic surgery. Dermatol Online J 25: 13030/qt1t75v80t, 2019.
16. Ohta N, Okazaki S, Fukase S, Akatsuka N, Aoyagi M and Yamakawa M: Serum concentrations of eosinophil cationic protein and eosinophils of patients with Kimura's disease. Allergol Int 56: 45-49, 2007.
17. Chen AJ, Li FZ and Fang S: Angiolymphoid hyperplasia with eosinophilia: Presentation with pleural effusion and characteristics of Kimura's disease. J Dtsch Dermatol Ges 19: 275-277, 2021.
18. Parikh S, Sharma S and Shah JS: Kimura's disease: A confounding condition. Indian J Otolaryngol Head Neck Surg 74 (Suppl 3): S5342-S5346, 2022.
19. Kumar N and Bhoriwal S: Kimura's disease: A diagnostic dilemma. Indian J Med Res 152: S92-S93, 2020.
20. Zou A, Hu M and Niu B: Comparison between Kimura's disease and angiolymphoid hyperplasia with eosinophilia: Case reports and literature review. J Int Med Res 49: 3000605211040976, 2021.
21. Zhang XM, Liu CY and Shao ZH: Advances in the role of helper T cells in autoimmune diseases. Chin Med J (Engl) 133: 968-974, 2020.
22. Kempf W, Haeffner AC, Zepter K, Sander CA, Flaig MJ, Mueller B, Panizzon RG, Hardmeier T, Adams V and Burg G: Angiolymphoid hyperplasia with eosinophilia: Evidence for a T-cell lymphoproliferative origin. Hum Pathol 33: 1023-1029, 2002.
23. Chen JF, Gao HW, Wu BY, Tsai WC and Chiang CP: Angiolymphoid hyperplasia with eosinophilia affecting the scrotum: A rare case report with molecular evidence of T-cell clonality. J Dermatol 37: 355-359, 2010.