

# Immunoreactivity of Wilms tumor 1 (WT1) as an additional evidence supporting hemangiomatous rather than inflammatory origin in the etiopathogenesis of angiolymphoid hyperplasia with eosinophilia

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**ABSTRACT** **Background:** Angiolymphoid hyperplasia with eosinophilia (ALHE) is a rare vascular proliferative disorder mainly located in the periauricular region. The etiopathogenesis of ALHE is unknown, and it is still controversial as to whether the entity represents a benign vascular neoplasm or an inflammatory process.

**Aim:** Recently, the intracytoplasmic staining pattern of Wilms tumor 1 (WT1) on immunohistochemistry has highlighted true vascular neoplasms, such as microvenular hemangioma, tufted angioma, and spindle cell hemangioma, which has made it helpful to distinguish ALHE from vascular malformations, as there is a negative staining pattern in the other entities. We aimed to investigate the immunoreactivity of ALHE specimens for WT1 as well as glucose transporter protein 1 (GLUT1) immunohistochemistry, an important and sensitive marker for the diagnosis of infantile hemangioma, which recently has been described to label other hemangiomas, such as verrucous hemangioma.

**Material and methods:** Clinical data and histopathological specimens from patients diagnosed with ALHE were reviewed, and immunohistochemical staining and microscopic analysis for WT-1 and GLUT1 were performed.

**Results:** Intracytoplasmic endothelial staining of WT1 was detected in 19 of 20 ALHE specimens. GLUT1 was not detected in any ALHE specimen.

**Conclusions:** We conclude that ALHE may represent a true hemangioma (i.e., benign vascular neoplasia) characterized by an eosinophil- and lymphocyte-rich inflammatory component as opposed to the reactive inflammatory dermatosis with a positive intracytoplasmic staining pattern for WT1. As far as we are aware, WT1 staining for ALHE has not been described to date.

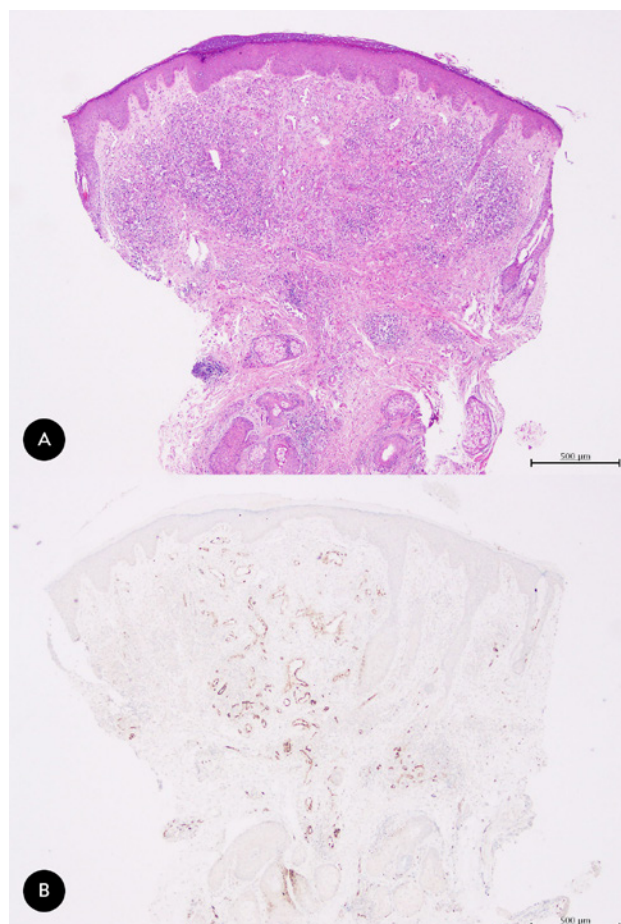
## Introduction

Angiolymphoid hyperplasia with eosinophilia (ALHE) is an uncommon, idiopathic vascular proliferative disorder characterized by dermal or subcutaneous red to brown papules or nodules, commonly located in the head and neck regions. ALHE is mainly a disorder of young adulthood to middle age, although children and elderly patients with the disorder have also been described.

The precise etiopathogenesis of ALHE is unknown and it is controversial whether it is a true vascular neoplasm (i.e., a hemangioma variant with overlying eosinophilic and lymphocytic infiltration related to cytokine expression) or a vascular proliferative disorder (secondary to an inflammatory tissue response). In this investigation, we assessed the immunoreactivity for Wilms tumor 1 (WT1) in ALHE specimens, which shows a cytoplasmic staining pattern in benign neoplasms such as microvenular hemangioma, tufted angioma, and spindle cell hemangioma, to highlight the pathogenesis of this rare entity [1,2]. In these tissues, we also assessed glucose transporter protein 1 (GLUT1) immunohistochemistry, an important and sensitive marker for the diagnosis of infantile hemangioma, which recently has been described to label some other hemangiomas such as verrucous hemangioma [3].

## Material and Methods

Clinical data and histopathological specimens from patients (n = 20) diagnosed with ALHE (between 2006 and 2016) were obtained from the Acibadem University School of Medicine Pathology Department and the Mayo Clinic Dermatopathology Department. Approval was obtained from the Institutional Review Board (IRB) of the Mayo Clinic regarding the ethical concerns for the study. The diagnosis of ALHE was histopathologically confirmed with the characteristic features of epithelioid endothelial cells with cytoplasmic vacuoles and a perivascular inflammatory cell infiltrate composed of numerous eosinophils, lymphocytes, and histiocytes. Immunohistochemical staining and microscopic analysis for WT1 and GLUT1 were performed for all ALHE specimens. The method used for immunostaining was the streptavidin-biotin-amplified system. The slides were submitted for subsequent steps of deparaffinization and rehydration. Sections were sliced (6 µm thick) and air-dried for 30 minutes. Then the sections were fixed in cold acetone for 10 minutes. After blocking endogenous peroxidase using 0.2% sodium azide for 5 minutes, they were washed with phosphate buffered saline for 15 minutes. Subsequently, the sections were incubated with primary antibodies for 1 hour. The primary antibodies were WT-1 (Cell Marque, California, USA) and GLUT1 (Cell Marque, California, USA) with a dilution of 1:100. After incubation, the sections were rinsed with



**Figure 1.** Histopathologic panoramic view of ALHE (hematoxylin and eosin stain) (a) and positive staining pattern for WT1 (b) on low power magnification.

distilled water and tap water. The tissue was counterstained with Mayer's hematoxylin. All slides were covered with a cover slip. The staining intensity was evaluated as negative (-), weak (+), moderate (++) and strong staining (+++) with a primary intensity score of tumor cell staining as a positive control for the immune markers.

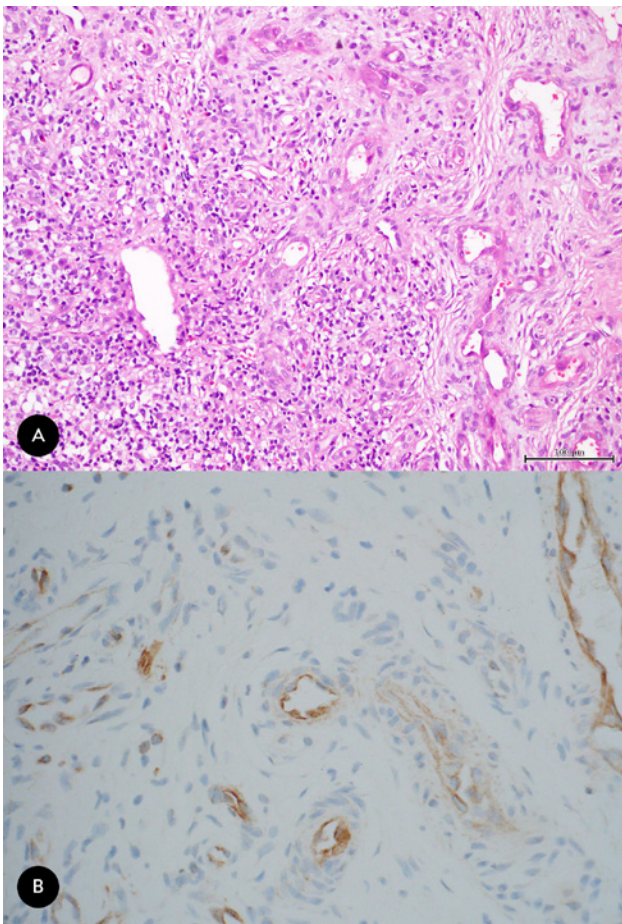
## Results

### Clinical findings

Clinical data for 20 patients with histopathologically confirmed ALHE were evaluated. The age of the patients ranged from 9 and 93 years of age at the time of biopsy procedure with a median age of 48. A slightly increased male (n = 12) to female (n = 8) ratio was identified. Most of the lesions were located on the head and neck (n = 15), followed by upper extremities (n = 4) and in a lower extremity (n = 1).

### Immunohistochemical findings

Nineteen of 20 specimens were positive for WT1 intracytoplasmic staining (Figure 1). Moderate staining intensity (n = 10) was more frequent than weak (n = 5), and strong (n = 4)

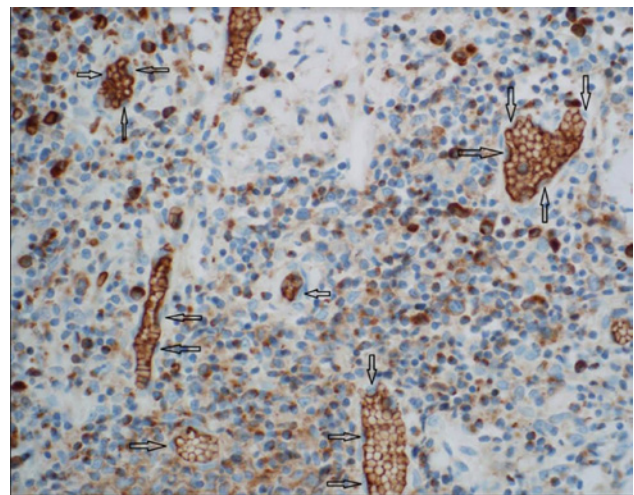


**Figure 2.** Details of the same specimen with epithelioid endothelial cells showing characteristic cytoplasmic vacuoles and associated eosinophilic and lymphocytic inflammation (a) Intracytoplasmic staining pattern for WT1 is identified on high power magnification revealing a strong staining intensity (b).

patterns. The staining pattern for WT1 was intracytoplasmic in endothelial cells, similar to that reported previously for various benign vascular neoplasms such as microvenular hemangioma and verrucous hemangioma, which revealed an intracytoplasmic staining pattern as well [1,2] (Figure 2). GLUT1 immunohistochemistry was negative for all ALHE specimens despite positive controls (Figure 3). Table 1 summarizes the results of the immunohistochemical stains for WT1 and GLUT1 in 20 histopathologically proven ALHE specimens.

## Discussion

ALHE was first described by Wells and Whimster in 1969 and was originally considered to represent late-stage Kimura's disease, but it is now widely accepted as a separate entity [4]. ALHE is a benign vascular proliferation characterized by dull, single or multiple nodules mainly located in the head and neck region. Involvement of the oral mucosa, arm, hand, shoulder, genital region, breast, parotid gland, orbit, colon, bone, and parapharyngeal spaces has also been described.



**Figure 3.** The lack of staining with GLUT1 in endothelial cells (arrows). Erythrocytes in blood vessels serve as the positive control.

**TABLE 1. Immunohistochemical results of WT1 and GLUT1 immunoreactivity.**

Staining Intensity	WT1 (n)	GLUT1 (n)
Strong staining (+++)	4	0
Moderate staining (++)	10	0
Weak staining (+)	5	0
Negative staining (-)	1	20

This rare condition has also been termed epithelioid hemangioma, histiocytoid hemangioma, inflammatory angiomatous nodule, and pseudo- or atypical pyogenic granuloma [5]. The lesions commonly arise in the third and third decade of life with a slight predilection in females. Histopathologically, ALHE presents as an ill-defined, dermal, lobulated mass composed of numerous vascular spaces of varying luminal diameter lined by large rounded endothelial cells with conspicuous eosinophilic cytoplasm and cytoplasmic vacuoles representing primitive lumina. A prominent inflammatory infiltrate composed largely of lymphocytes, numerous eosinophils, and histiocytes surrounding the vessels are identified [6].

The etiology of ALHE is unclear, and it is still controversial whether the disorder represents a benign vascular neoplasm or an inflammatory process. Association with arteriovenous shunts in 43% of cases of ALHE strengthens the conclusion that the entity may represent a vascular neoplastic proliferation [7]. ALHE has also been reported to develop within a port wine stain in a patient, and the authors highlighted the role of increased serum renin levels in the pathogenesis of this case in which the histopathology specimen revealed expression of angiotensin converting enzyme and angiotensin II receptors [8].

In a retrospective study of 116 ALHE patients, 10 cases (9%) were found to be associated with antecedent trauma



(frostbite, surgery, laceration, frictional trauma, and other unspecified) with a time course between injury and the onset of lesions ranging from 7 months to 20 years (median, 30 months) [8]. This phenomenon has also been described in pyogenic granulomas, another vascular proliferative disorder, which developed after thermal burn, lightning, or mine injury [9]. In one patient, multiple ALHE lesions on the volar surfaces on both wrists and antecubital fossae, situated over a superficial vein and corresponding to a site of recent venipuncture, also supports the role of trauma in the etiopathogenesis of this entity [10].

ALHE that arises during pregnancy suggests that hormonal changes may also take place in the etiopathogenesis of this condition, a phenomenon also described in other vascular neoplasms such as hobnail hemangioma, pyogenic granuloma, liver, pancreatic, and spinal epidural hemangiomas [11].

Immunohistochemistry for WT1 antigen permits differentiation of vascular neoplasms and malformations; the former show cytoplasmic staining pattern, as in our study, whereas the latter is negative for this marker. In a large study (n = 167), cytoplasmic WT1 was detected in 117 various vascular neoplasms, and was not detected in 50 cases of capillary, venous and lymphatic malformations [1].

In a true vascular neoplasm such as microvenular hemangioma, immunoreactivity for WT1 was found in 9/10 tumors, which is similar to the results described herein [12]. Despite the detailed dermatopathological evaluation of the WT1 negative case, the diagnosis was consistent with ALHE in our study. We suggest that WT1 expression could be lost in a subset of hemangiomas such as ALHE, microvenular hemangioma and verrucous hemangioma reminding the phenomenon that MART-1 (melanoma antigen recognized by T cells 1) antibody, which is a highly sensitive marker, is lost in some melanoma specimens. In another study, WT1 was detected in 9/9 cases of microvenular hemangioma [2]. In a comparative study of WT1 expression in 23 vascular tumors and 20 vascular malformations, cytoplasmic staining of WT1 was detected in cases of infantile hemangiomas (8/9), angiosarcomas (9/9), pyogenic granulomas (2/2), and epithelioid hemangioendothelioma (1/1), whereas complete vascular malformations consisting of port wine stains, venous, and lymphatic malformations were negative for WT1. Cytoplasmic WT1 staining pattern has been described in complete cases with tufted angioma (n = 8) and a single case of spindle cell hemangioma [1]. WT1 reactivity was also shown in extracutaneous hemangiomas such as anastomosing hemangioma of the kidney, histopathologically characterized by irregular fenestrated vascular spaces and reminiscent of the splenic red pulp with tightly packed, capillary-sized vessels with small lumen [13].

In a recent study, cytoplasmic immunoreactivity of WT1 was detected in complete cases with verrucous hemangioma

suggesting that it is a vascular neoplasm rather than a vascular malformation [3]. In another study, WT1 was found to be focally positive in 14/74 cases of verrucous hemangioma [14]. In this study, a positive control for WT1 is omitted and a high false-negative rate may explain the discrepancy, which may also result from differences in immunohistochemical techniques and reagents.

In our study, cytoplasmic staining of WT1 was detected in the vascular endothelial cells of 19/20 specimens. This result is in keeping with the context that the entity may represent a true vascular neoplasm rather than a vascular proliferative response to inflammation. This concept is supported by the fact that various anti-inflammatory medications are not effective in most cases of ALHE, but that the best therapeutic response would be excision/destructive treatment modalities [15-17].

A case report of a congenital ALHE with a blaschkoid segmental distribution in the anogenital region also suggests that the vascular proliferation may be unlikely to represent a secondary phenomenon of reactive inflammatory process, which is not expected to develop during intrauterine period, but rather a true vascular neoplasm such as congenital involuting/noninvoluting hemangioma [18]. This phenomenon also supports our point of view regarding these study results.

Rapid remission of severe pruritus related to ALHE with pulsed dye laser in a patient, who was persistent to topical, intralesional, and oral corticosteroid medications suggests that the inflammatory component of this entity would be secondary to vascular neoplastic proliferation. The proliferating endothelial cells of ALHE express adhesion molecules ICAM-1, ELAM-1, and VLA-1, -3, -5, which is considered to result in an inflammatory response [19]. In a systemic review of ALHE including 416 studies representing 908 patients, treatment failure was found to be lowest for excision and pulsed dye laser compared with anti-inflammatory treatment strategies [20]. We suggest that this phenomenon supports the conclusion that eosinophil and lymphocytic inflammatory component would be related to vascular endothelial cells expressing proinflammatory cytokines such as ICAM-1. The efficacy of imiquimod, an immune response modifier that induces interferon-alpha, in ALHE, as well as other vascular neoplasms such as retiform hemangioendothelioma, infantile hemangioma and proliferating hemangioma of infancy also supports this point of view [21,22].

GLUT1 is an erythrocyte-type glucose transporter protein expressed in juvenile hemangiomas. It is a member of the facilitative cell-surface glucose transporter family, which includes five other isoforms originally identified in human erythrocyte membranes. GLUT1 is also expressed in brain capillary endothelium, where it plays a critical role in the transport of glucose across the blood-brain barrier. GLUT1 staining has been identified in a variety of normal cell types

including placental trophoblasts and perineural cells as well as in a subset of mesenchymal neoplasms such as epithelioid sarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma [23].

GLUT1 is a useful marker for differentiation of infantile hemangiomas and other vascular neoplasms such as congenital hemangioma, tufted angioma and kaposiform hemangioendothelioma, being positive in the infantile hemangiomas and negative in the other entities [24]. A recent research highlighted that complete cases of microvenular hemangioma (n = 9) and congenital hemangiomas (n = 16) were found to be devoid of GLUT1 expression [25]. In a study, the positivity of GLUT1 was described in complete specimens with verrucous hemangioma, which was negative in our study [3]. As far as we are aware, staining findings regarding this marker for ALHE have not been described to date.

GLUT1 was not detected in various benign oral vascular lesions including; 19 hemangiomas, 9 varices, 48 pyogenic granulomas, and 17 vascular malformations, which suggests that most of benign vascular proliferations (other than infantile hemangiomas, such as ALHE) are negative for this marker [26]. The limitation of our study is the small patient numbers related to rarity of ALHE.

In conclusion, ALHE may represent a true hemangioma (i.e., benign vascular neoplasia) that shows an eosinophil- and lymphocyte-rich inflammatory component instead of a reactive inflammatory dermatosis with positive intracytoplasmic staining pattern for WT1.

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