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Epithelioid hemangioendothelioma

Report of a case with immuno-lectinhistochemical and ultrastructural demonstration of its vascular nature

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Summary. The epithelioid hemangioendothelioma (EHE) is a rare vascular tumour of borderline malignancy, first described as a separate entity in 1982 by Weiss and Enzinger. The abundant cytoplasm of endothelial cells mimiking epithelioid appearances, prominent cytoplasmic vacuolization and barely perceptible lumina even in reticulin stains may result in EHE being mistaken for a signet ring cell carcinoma. In our case, difficulties in differential diagnosis were enhanced by the location of the tumour within an inguinal lymph node. The usefulness of FVIIIR:Ag- and UEA I- histochemistry in ascertaining the endothelial nature of this tumour is demonstrated, in correlation with electron microscopic data. The different reaction sites of these markers are striking and typical: FVIIIR:Ag displays a granular or diffuse cytoplasmic reaction, whereas UEA I provides a linear staining of vacuoles and luminal surfaces.

Key words: Epithelioid hemangioendothelioma – Factor VIII-related antigen – Ulex europaeus I lectin

Introduction

In September 1982 a soft tissue tumour was referred to us which displayed a markedly epithelioid appearance. The tumour was described as a separate entity for the first time by Weiss and Enzinger in the same year (Weiss and Enzinger 1982). The case reported here is of some interest, mainly because of the considerable difficulties we had in clearly discriminating it from a carcinoma. Because of these problems of differential diagnosis, the tumour may serve as an example to illustrate the value of Ulex europaeus I lectin and Factor VIII-related antigen as markers for neoplasms derived from endothelial cells.

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Clinical history

This 55-year-old man was admitted to a general hospital presenting with a nodular induration in the right inguinal region. The swelling of the loin had progressively increased in size and become painful over the preceding 6 months. There was no prior history of any tumour-like lesion or major operation. Physical examination revealed a firm nodule in the right inguinal region of about walnut-size. The clinicians suspected a lymph node metastasis and therefore the tumour was excised.

Pathology

Gross appearance. The walnut-sized specimen consists of lobulated fatty tissue surrounding a firm tumour-like lesion measuring $1{,}5 \times 1 \times 1$ cm. The cut surface reveals a circumscribed grey-white nodule which is partially invested by delicate brown tissue.

Microscopic appearance. Histologically the lymph node shows partial destruction by a solid, sharply circumscribed, but not encapsulated tumour. The lesion involves the hilus of the node and the surrounding fatty tissue. The tumour (Fig. 1) is composed of anastomosing cords and small nests of predominantly rounded or polygonal cells containing an oval nucleus. The chromatin content of the nuclei is usually scanty, giving a vesicular appearance. Occasionally, the nucleus is marked by grooves. The nuclei exhibit 1 or 2 small but prominent nucleoli. Mitoses are not conspicuous. Focally, there is a transition into strands of slightly spindle shaped cells. A striking feature is the epithelioid or histiocytic aspect of the majority of cells, especially of those within cluster formations. The cytoplasm is abundant, sometimes pale, but most often eosinophilic and occasionally with a very delicate granularity. Cytoplasmic vacuolization is evident all over the different regions of the tumour, nuclei are sometimes displaced to the periphery and extended by great vacuoles resembling signet ring cells. The content of the vacuoles fails to stain with PAS, Alcian-blue or Hale's colloidal iron reaction (Fig. 2). In the peripheral region, strands of cells surrounded by a PAS-positive rim apparently form small clefts, which is also suggestive of abortive lumen formation or shrinkage artifacts. Anastomosing vascular channels containing erythrocytes are not seen. The tumour cells are embedded in a hyaline and myxoid matrix which is pronounced in central parts and includes isolated cells. The matrix is strongly Hale-positive (Fig. 3A) and contains scanty interspersed collagen fibers. The silver impregnation technique exhibits a dense network of reticulin fibers encompassing single cells as well as solid alveolar cell nests (Fig. 3B), but lumen-bearing channels are not evident.

The tumour reveals some features characteristic of metastasis: involvement of a lymph node, prominent clustering of epithelioid cells with abundant cytoplasm and marked cytoplasmic vacuolization. The failure to demonstrate epithelial mucin, however, excludes a signet ring cell carcinoma. The transition of the rounded cells to slightly spindle shaped ones, the increased cellularity and the focal arrangement of cells in randomly interlacing compounds are suggestive of an epithelioid mesenchymal tumour.

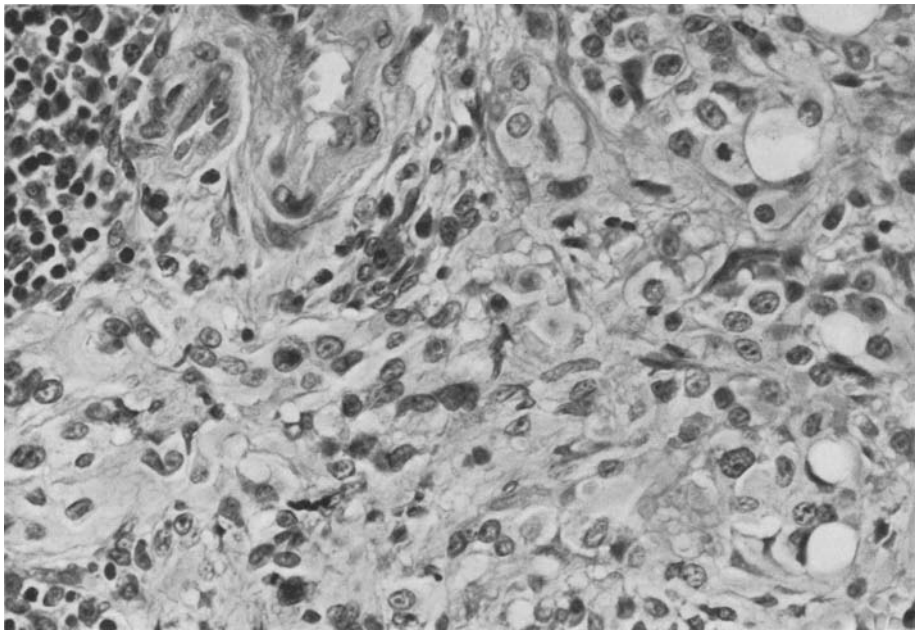


Fig. 1. Epithelioid hemangioendothelioma. Note the abundant cytoplasm and focal vacuolization of tumour cells. In the *upper left* residual lymph node structures. H.E. ×375

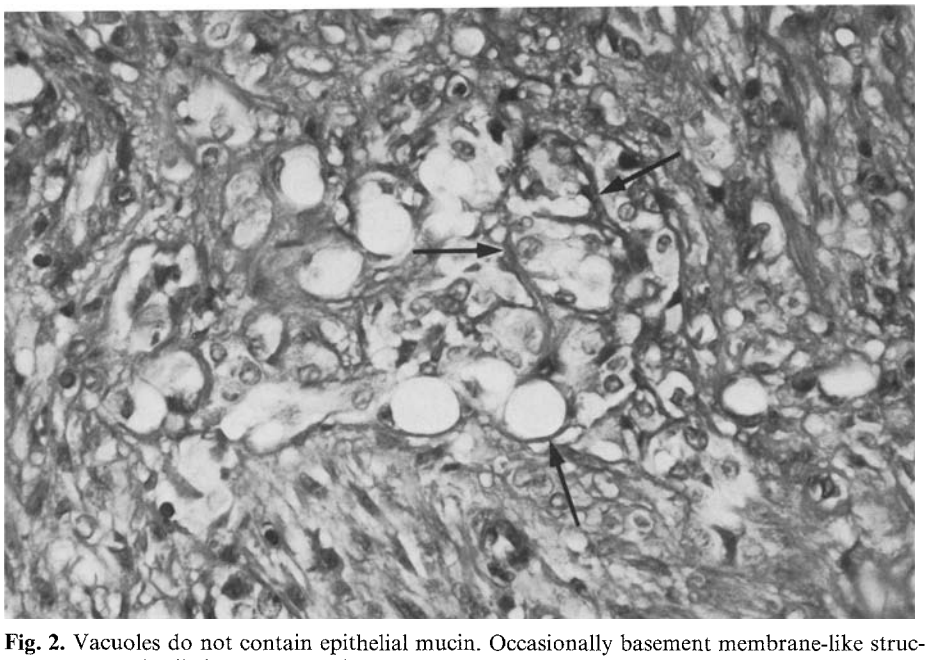


Fig. 2. Vacuoles do not contain epithelial mucin. Occasionally basement membrane-like structures surround cell clusters (*arrows*). PAS ×375

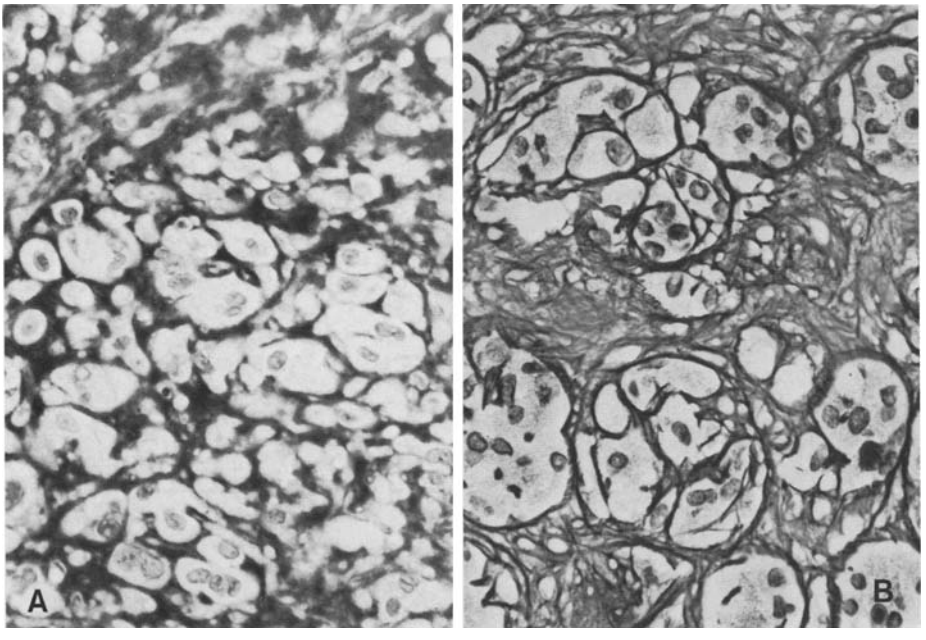


Fig. 3. **A** Cells are embedded in a hyaline matrix. Hale's colloidal iron reaction, × 375. **B** Tumour cells are surrounded by reticulin fibers. Lumina are not discernible in tumour cell clusters. Reticulin stain, × 375

Immuno-lectinhistochemical and ultrastructural studies

Because of the difficulties in classifying this tumour additional sections were prepared for identification of Factor VIII-related antigen (FVIIIR:Ag) and binding-sites of Ulex europaeus I lectin (UEA I) using peroxidase-labelling. Rabbit antiserum against FVIIIR:Ag (Dako-Copenhagen, Denmark) and the Biotin/Avidin-system (Vector laboratories-Burlingame, USA) were used, employing the method of Hsu et al. (1981). Peroxidase-conjugated UEA I was obtained from Medac (Hamburg, FRG) the incubation being performed according to Klein et al. (1983). The specifity of the reactions was tested by inhibition of the binding using absorption of the antibody with purified Factor VIII (Behring, Marburg, FRG) and by preincubating the lectin in 0,5 M α-L-fucose (Medac, Hamburg, FRG) in PBS.

In addition, tissue blocks formalin-fixed for 2 days, were processed for electron microscopy. The specimen was fixed in 3% glutaraldehyde in 0.067 M phosphate buffer (pH 7,2) for 6 h and postfixed in 1% osmium tetroxide in Palade's buffer (pH 7,2) for 4 h. Specimens were embedded in Epon and examined in a Philips EM 400 electron microscope.

Immunohistochemical localization of FVIIIR:Ag reveals a prominent diffuse cytoplasmic reaction product in some cells, irrespectively of the cell shape and the histological arrangement, thus contrasting with little or no reaction in adjacent cells (Fig. 4). The vacuoles are surrounded by either FVIIIR: Ag-reactive or non-reactive cytoplasm, but their content remains unstained.

UEA I gives a strong reaction with the tumour tissue (Fig. 5A). The matrix is labelled resulting in a pattern comparable with the Hale-reaction, as well as the cellular membranes and many vacuoles which give a linear positive

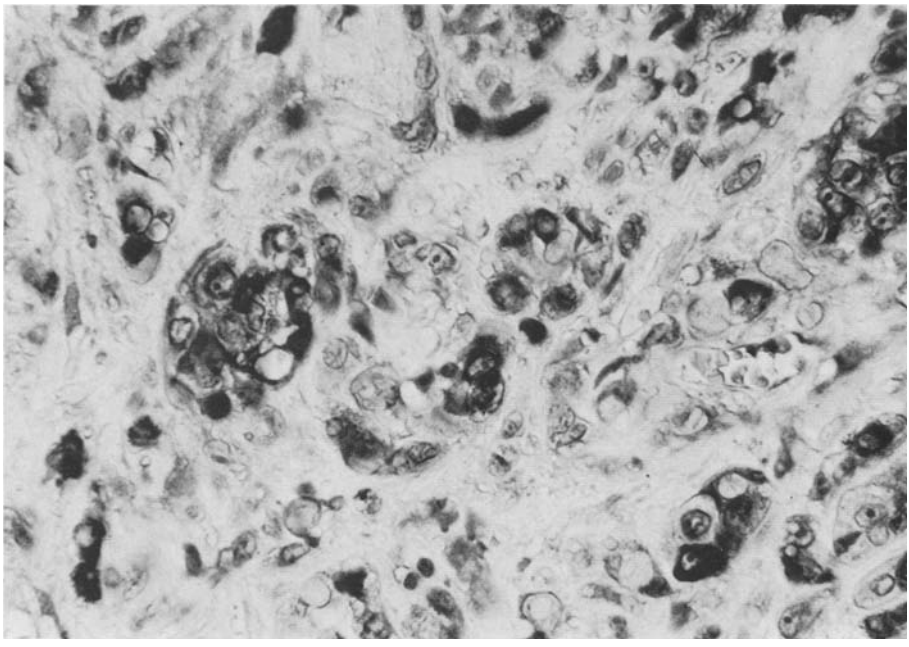
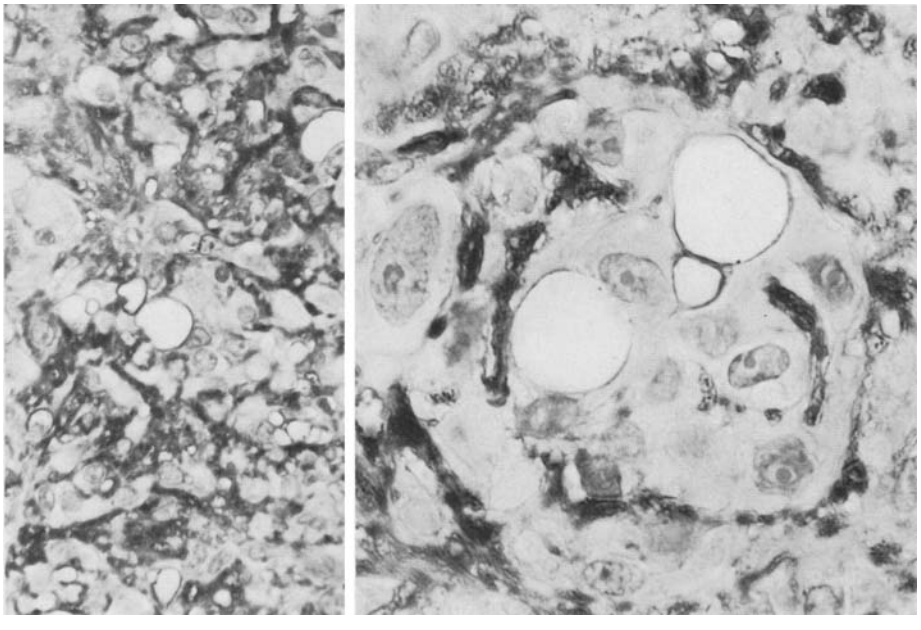


Fig. 4. Heavy cytoplasmic reaction product (black) in some cells with little or no reaction product in adjacent cells. FVIIIR: Ag-peroxidase-labelling, ×480



A **B**

Fig. 5. A Strong UEA I reaction product surrounding the cells and outlining vacuoles. UEA I-peroxidase-labelling, ×375. **B** Detail displaying a linear staining with UEA I at the luminal surface and at the rim of vacuoles, ×935

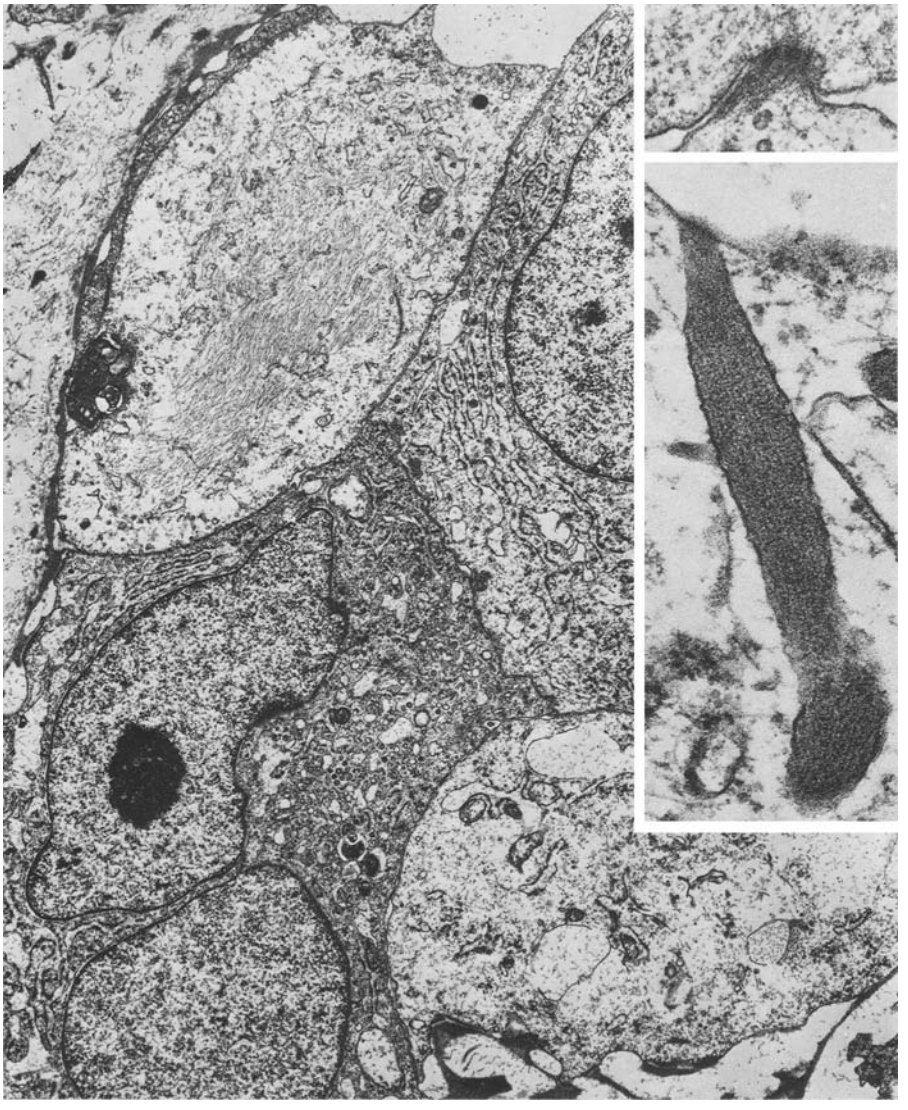


Fig. 6. Group of epithelioid cells with abundant cytoplasm surrounded by a basement membrane (*left* and *lower right*). Prominent cytoplasmic filaments at the *upper left*, × 8,500. *Upper inset:* Hemi-desmosome connecting two tumour cells, × 50,600. *Lower inset:* Weibel-Palade bodies containing a parallel array of internal tubules are occasionally present within tumour cells, × 64,400

reaction. Consequently, multiple minute clefts are discernible within the cell nests which simulated a solid pattern (Fig. 5B). The rim of vacuoles coated by the reaction product is made up by one or more cells; rarely erythrocytes are seen within lumina.

Ultrastructurally the tumour is composed of cohesive clusters of cells surrounded by a broad basement membrane (Fig. 6). The abundant cytoplasm

contains sparse organelles. There are numerous pinocytotic vesicles and inter-cellular tight junctions and hemidesmosomes. Some cells display many intermediate filaments devoid of dense bodies. In some cells rod shaped tubular bodies with a parallel array of internal tubules are identifiable (Fig. 6 inset).

All these histochemical and ultrastructural findings support the assumption that this tumour is derived from endothelial cells. They are compatible with the diagnosis of epithelioid hemangioendothelioma.

The patient is alive and well 3 years and 5 months after the excision of the tumour without any evidence for a recurrence or metastasis.

Discussion

Epithelioid hemangioendothelioma (EHE) is a rare vascular tumour, recently described as an entity (Weiss and Enzinger 1982). These authors stressed the difficulties of differential diagnosis, often leading to the erroneous interpretation of a carcinoma. There are some striking features simulating carcinoma: the abundant eosinophilic cytoplasm, prominent cytoplasmic vacuolization, growth in obviously solid clusters and the barely perceptible vascular lumina. In the present case the destruction of half a lymph node resembles the growth pattern of metastasis. Histochemical markers may therefore be a valuable aid in assessing the endothelial cell origin of this tumour.

UEA I is known as a marker for normal (Holthöfer et al. 1981) and neoplastic (Miettinen et al. 1983) endothelial cells. It is often characterized by a linear reaction product of the cell membrane, particularly at the luminal surface (Arnold et al. 1983). A diffuse cytoplasmic reaction product is seldom to be seen in endothelial tumours. In the present case the UEA I-labelling reveals a very striking linear staining, thus outlining otherwise non-detectable vascular channels. The specific coating of vacuoles by the reaction product confirms the view of Weiss and Enzinger (1982) that they may be considered to be primitive lumina formed by a single cell. Moreover, the hyaline stroma exhibits strong UEA I-labelling focally, a phenomenon not previously reported in the literature. It may represent an alteration typical of vascular tumours with a hyalinizing matrix.

The positive cytoplasmic staining of the tumour with antibodies against FVIIIR:Ag provides further evidence of its endothelial nature. Vacuoles embedded in the labelled cytoplasm support the concept that the inner membranes do not gain contact with the outer cellular membrane due to the loss of functional differentiation within neoplastic cells. The highly variable distribution of labelled cells interspersed with non or slightly labelled ones does not correlate with the morphological differentiation of these cells. Patchiness is a general phenomenon of FVIIIR:Ag-labelling, especially in malignant endothelial tumours (Burgdorf et al. 1981; Sehested and Hou-Jensen 1981).

Thus, the present neoplasm reflects the general properties of both the histochemical endothelial cell markers: FVIIIR:Ag which provides a focal

cytoplasmic labelling, whereas UEA I leads to a more regular distributed reaction outlining lumina. Because of these properties UEA I is claimed to be a specific and more sensitive marker for endothelial cells (Miettinen et al. 1983). It must be kept in mind, however, that the specificity of UEA I is directed towards α-L-fucose-residues, therefore also giving a positive staining result for epidermis (Hyun et al. 1984) and the glands of stomach, colon and the breast (unpublished observation). Due to this fucose-specifity, UEA I provides a stronger endothelial staining in patients with blood-group 0 than in those of other blood-groups. In order to prevent misinterpretation of tumours of uncertain origin, use combined with that of FVIIIR:Ag seems reasonable.

The ultrastructural features delineated here prove definitively the endothelial nature of the present neoplasm. The cells display many characteristics of normal endothelium, such as pinocytotic vesicles, junctional complexes, a broad basement membrane and particularly the so-called Weibel-Palade bodies (Weibel and Palade 1964). Consequently our observations are in accordance with the findings reported by Weiss and Enzinger (1982). Taking all features into account, this tumour is to be classified as EHE, which has probably developed in the hilus region of an inguinal lymph node. As remnants of a vein are visible in the central parts of the tumour, it is reasonable to assume that it began in a small vessel of the hilus.

According to the detailed study of Weiss and Enzinger (1982) the EHE is a vascular tumour of borderline malignancy. From the frequency of mitotic figures the histological findings in the present neoplasm indicate benign behaviour. However, the follow-up is too short for a final evaluation.

In addition to ultrastructural studies, UEA I and FVIIIR:Ag are valuable and easily applicable aids in differential diagnosis. They may reveal a higher than expected incidence of this tumour, and help to provide more information about its biological properties.

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