Virchows Arch A (1987) 410:309–316

Virchows Archiv A

Epithelioid sarcoma and epithelioid hemangioendothelioma: an immunocytochemical and lectin-histochemical comparison*

Mark R. Wick and J. Carlos Manivel**

Division of Surgical Pathology, Department of Laboratory Medicine and Pathology, University of Minnesota School of Medicine, Minneapolis, Minnesota, USA

Summary. Epithelioid sarcoma (ES) and epithelioid hemangioendothelioma (EH) both occur preferentially in the soft tissues, and may be confused with one another microscopically. We compared 8 examples of each tumor immunohistochemically, using formalin-fixed tissue, the ABC method, unconjugated *Ulex europaeus* I agglutinin (UEA), rabbit antibody to UEA, and monoclonal antibodies to epithelial membrane antigen (EMA), cytokeratin (CK), factor VIII-related antigen (FVIIIRAG), and blood group isoantigens A, B, and H (BGI). Six of 8 cases of ES, and 7 of 8 epithelioid hemangioendotheliomas bound UEA; similarly, 6 of 8 ES cases were reactive for BGI, as were 4 of 8 examples of EH. All epithelioid sarcomas were positive for CK, and 7 displayed EMA, whereas these antigens were lacking in EH. Conversely, 5 of 8 cases of EH contained FVIIIRAG, which was absent in all examples of ES. These findings underscore the nonspecificity of UEA-binding and BGI-expression as markers of endothelial differentiation. Moreover, they suggest that sole reliance upon these immunohistologic reactants for the identification of vascular tumors may result in diagnostic error. Inasmuch as ES and EH differ in biological behavior, such a mistake would be significant. Thus, we advocate the inclusion of immunostains for EMA, CK, and FVIIIRAG in the evaluation of histologically-similar cases of epithelioid sarcoma and epithelioid hemangioendothelioma.

Key words: *Ulex europaeus* I, Blood group isoantigens

Introduction

The clinicopathological similarities between epithelioid sarcoma (ES) (Enzinger 1970) and epithelioid hemangioendothelioma (EH; "low-grade epithelioid angiosarcoma") (Weiss and Enzinger 1982) are several. Both lesions tend to occur in the superficial soft tissue of the extremities in adults, and are composed of clusters or sheets of polygonal cells with varying degrees of nuclear atypia (Enzinger and Weiss 1983). Cytoplasmic vacuolization and hemorrhage in ES may lead to an appearance simulating that of a vascular neoplasm; conversely, many cases of EH do not demonstrate overt vasogenesis, and are mistaken for epithelial tumors (Chase and Enzinger 1985). These points of overlap are not accompanied by biological likenesses between the two lesions. Epithelioid sarcoma recurs and metastasizes more frequently than epithelioid hemangioendothelioma; 30% of patients with the former neoplasm suffer tumor-related fatality, compared with 12% of cases of EH (Chase and Enzinger 1985; Weiss and Enzinger 1982).

In view of these data, we undertook an immunohistochemical comparison of ES and EH, in an effort to determine points of immunophenotypic similarity and difference between the two tumors. The results of this study constitute the basis of this report, and will be used as a focus for discussion of the differential diagnosis of each neoplasm.

* Presented at the Seventy-fifth Annual Meeting of the International Academy of Pathology (United States-Canadian Division), New Orleans, Louisiana, USA, March 11, 1986

** Dr. Wick is a recipient of a Career Development Award in Oncology from the American Cancer Society

Offprint requests to: M.R. Wick, Box 76, Mayo Memorial Building, University of Minnesota Hospitals, 420 Delaware Street, S.E. Minneapolis, MN 55455, USA

Table 1. Immunohistochemical reagents used in the study of epithelioid sarcoma and epithelioid hemangioendothelioma

Reagent	Source	Dilution
Unconjugated *Ulex europaeus* I lectin	Vector Laboratories	1:3,600
Anti-*Ulex europaeus* I	DakoPatts Co., Inc.	1:3,600
Anti-blood group isoantigens A, B, and H[a]	DakoPatts Co., Inc.	1: 80
Anti-cytokeratin (Hybridoma clones AE1/AE3)[a]	Hybritech Laboratories	1: 200
Anti-epithelial membrane antigen[a]	DakoPatts Co., Inc.	1: 160
Anti-factor VIII-related antigen[a]	DakoPatts Co., Inc.	1: 40
Biotinylated goat antirabbit globulin	Vector Laboratories	–[b]
Biotinylated horse antimouse globulin	Vector Laboratories	–[b]
Avidin-biotin-peroxidase complex	Vector Laboratories	–[b]

[a] Monoclonal (hybridoma) antibodies; anti-*Ulex* is of rabbit origin
[b] Vectastain Rabbit and Mouse Kits

Materials and methods

Cases of epithelioid sarcoma and epithelioid hemangioendothelioma were retrieved from the surgical pathology files at the University of Minnesota, seen between 1974 and 1984. Examples of each tumor were chosen for further consideration on the basis of their histological similarity to one another.

Formalin-fixed, paraffin-embedded microscopic sections from each case were dewaxed in Americlear (Scientific Products, IN, USA) and absolute ethanol, and incubated in 0.03% methanolic hydrogen peroxide for 30 min. After rehydration in graded alcohol solutions, distilled water, and phosphate-buffered saline (PBS; pH 7.4), they were incubated with monoclonal antibodies to epithelial membrane antigen (EMA), cytokeratin (CK), factor VIII-related antigen (FVIIIRAG), and blood group isoantigens A, B, and H (BGI), and with unconjugated *Ulex europaeus* I agglutinin (UEA) for 18 h at 4 degrees C. Protease digestion was not employed. UEA was applied with and without the prior incubation of sections with blood group antibodies, for 2 h at ambient temperature. In further assessment for UEA binding, rabbit anti-UEA was applied for 2 h at room temperature, the following day. All sections were subsequently developed with the avidin-biotin-peroxidase complex (ABC) method, as previously described (Hsu et al. 1981) (Table 1).

Chromogenic development was accomplished by immersion in 3,3′-diaminobenzidine solution with 0.003% hydrogen peroxide, for 10 min or until optimal staining was obtained. Sections were then dipped in 0.125% osmium tetroxide, rinsed in tap water, and counterstained with Harris' hematoxylin or methyl green.

Negative controls consisted of sections stained with nonimmune rabbit serum or mouse ascites fluid, in lieu of primary antibodies. Sections of stock tumors, known to contain the determinants of interest, served as positive controls.

Results

Clinical findings. All cases (8 each of ES and EH) occurred in the soft tissues. Among patients with ES, 5 were males and 3 were females, ranging in age from 10 to 76 years at diagnosis (mean 38). There were 3 men and 5 women with EH, who varied in age from 23 to 41 years (mean 34). Three cases of ES showed at least one recurrence after initial excision, and 3 demonstrated metastases to regional lymph nodes or lungs. All patients with EH were free of recurrence and metastatic spread.

Light microscopic features. Each of the 16 tumors were composed of sheets and nests of polygonal cells, separated by variably-dense fibrovascular stroma. Epithelioid sarcomas commonly displayed a central area of necrosis, and "pseudopalisading" of surrounding tumor cells. Epithelioid hemangioendotheliomas manifested a relationship to large arteries of veins in 5 cases. The neoplastic cells of ES contained oval to round nuclei, occasional nucleoli, and a moderate amount of amphophilic cytoplasm. Those of EH were similar, except that the cytoplasm was commonly vacuolated. Mitoses were infrequent in both lesions (Figs. 1 and 2).

Two cases of ES demonstrated regional intratumoral hemorrhage, with a consequent similarity to vascular tumors (Fig. 3). Neither tumor type demonstrated cellular pleomorphism or spindle-cell change.

Immunohistochemical observations. Seven cases of EH, and 6 of ES bound UEA, in a combined cell-membranous and cytoplasmic pattern (Figs. 4A and B). Similarly, 6 ES cases displayed BGI with the same cellular distribution (Fig. 5A), compared with 4 of epithelioid hemangioendotheliomas (Fig. 5B). Cases of ES showing immunoreactivity for BGI were the same as those which bound UEA. All epithelioid sarcomas diffusely expressed cytoplasmic CK, and 7 showed membrane-based EMA-reactivity (Figs. 6A and B), whereas these determinants were not observed in EH. Conversely, 5 cases of the latter manifested cytoplasmic FVIIIRAG-positivity (Fig. 7), which was lacking in epithelioid sarcomas. Prior incubation of sections from ES cases with antibodies to blood group antigens abolished the binding of UEA, but similar treatment of tissue from epithelioid hemangioendotheliomas did not interfere with affinity for this lectin. Negative and positive controls stained appropriately.

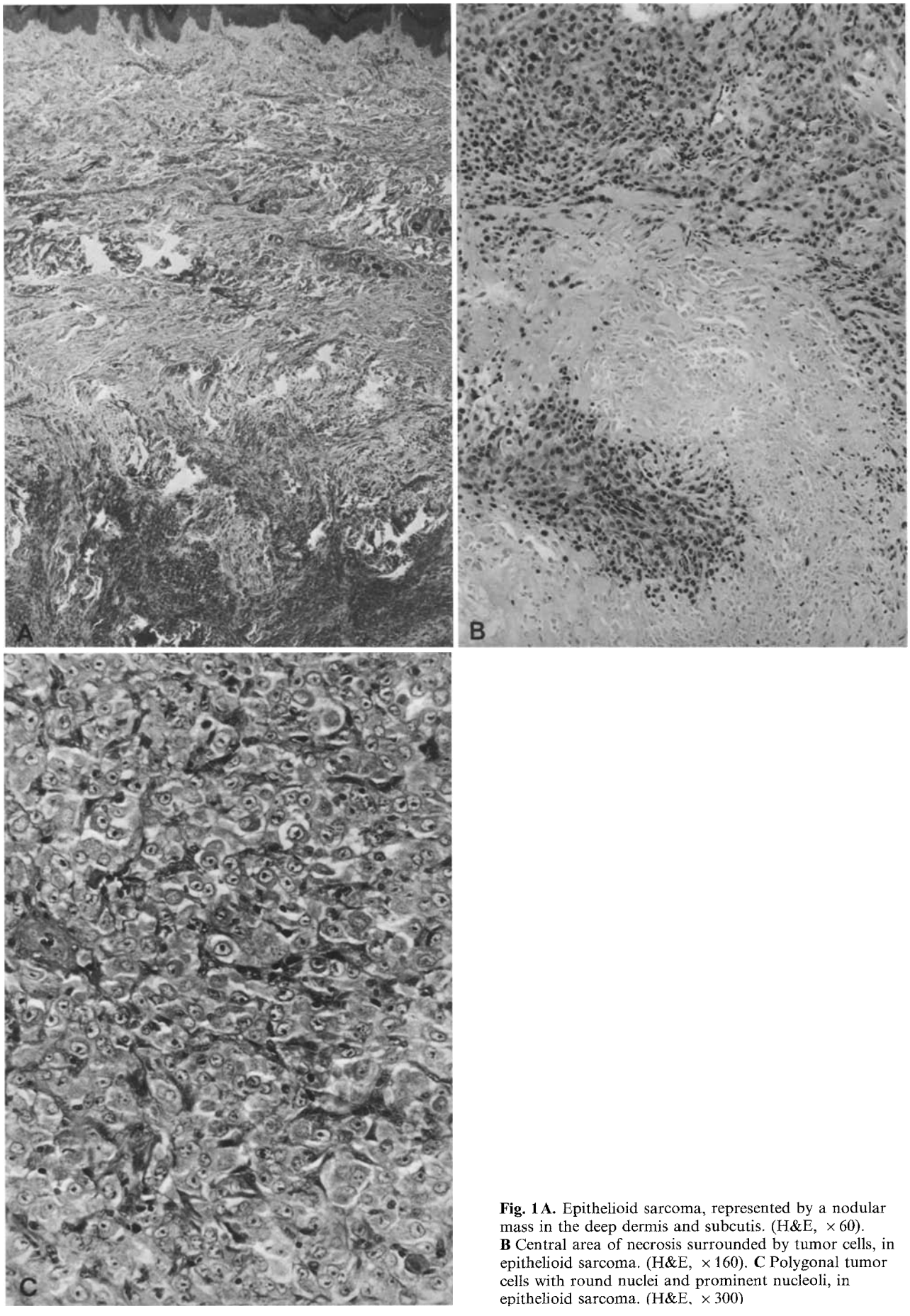

Fig. 1 A. Epithelioid sarcoma, represented by a nodular mass in the deep dermis and subcutis. (H&E, ×60). **B** Central area of necrosis surrounded by tumor cells, in epithelioid sarcoma. (H&E, ×160). **C** Polygonal tumor cells with round nuclei and prominent nucleoli, in epithelioid sarcoma. (H&E, ×300)

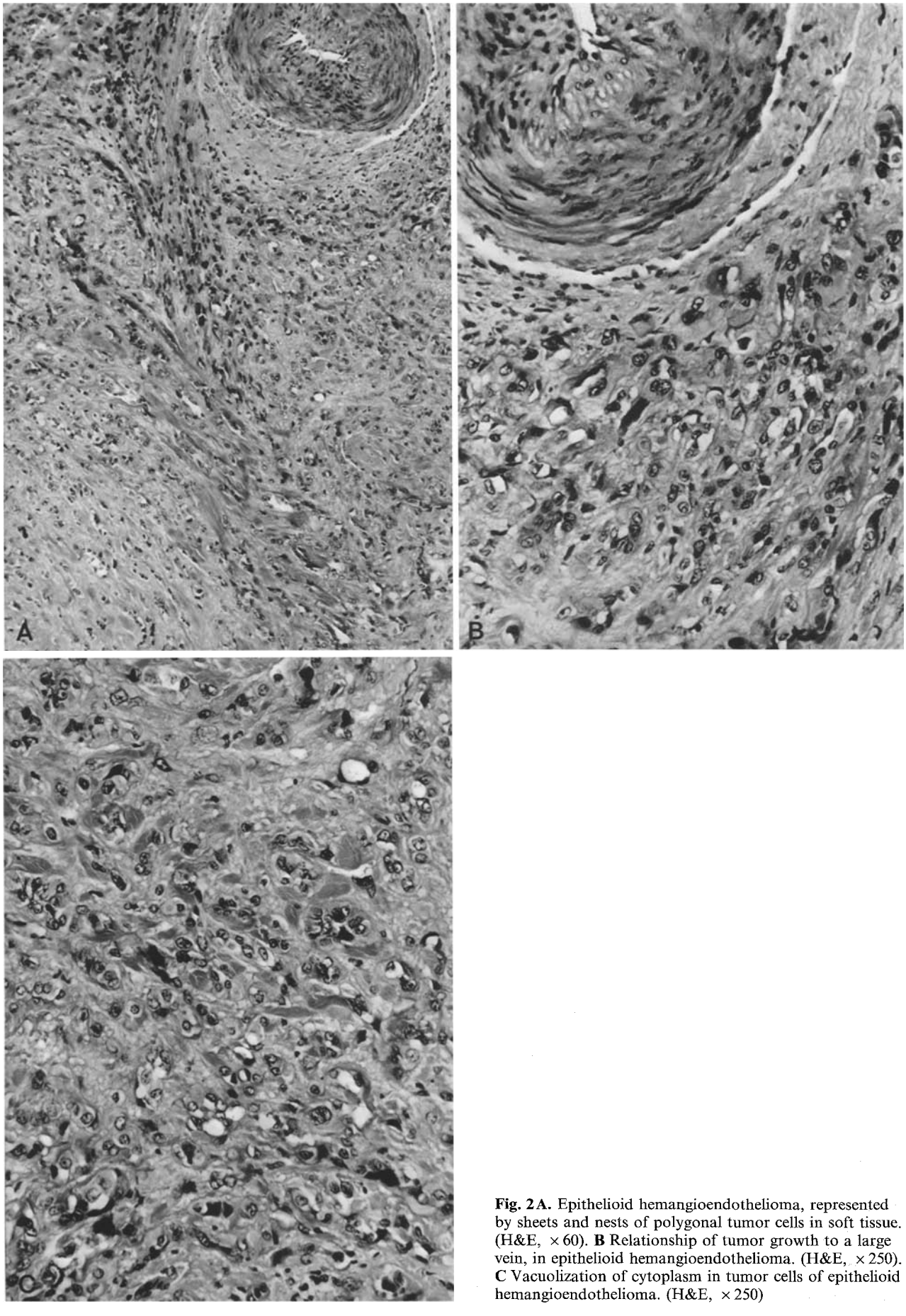

Fig. 2A. Epithelioid hemangioendothelioma, represented by sheets and nests of polygonal tumor cells in soft tissue. (H&E, ×60). **B** Relationship of tumor growth to a large vein, in epithelioid hemangioendothelioma. (H&E, ×250). **C** Vacuolization of cytoplasm in tumor cells of epithelioid hemangioendothelioma. (H&E, ×250)

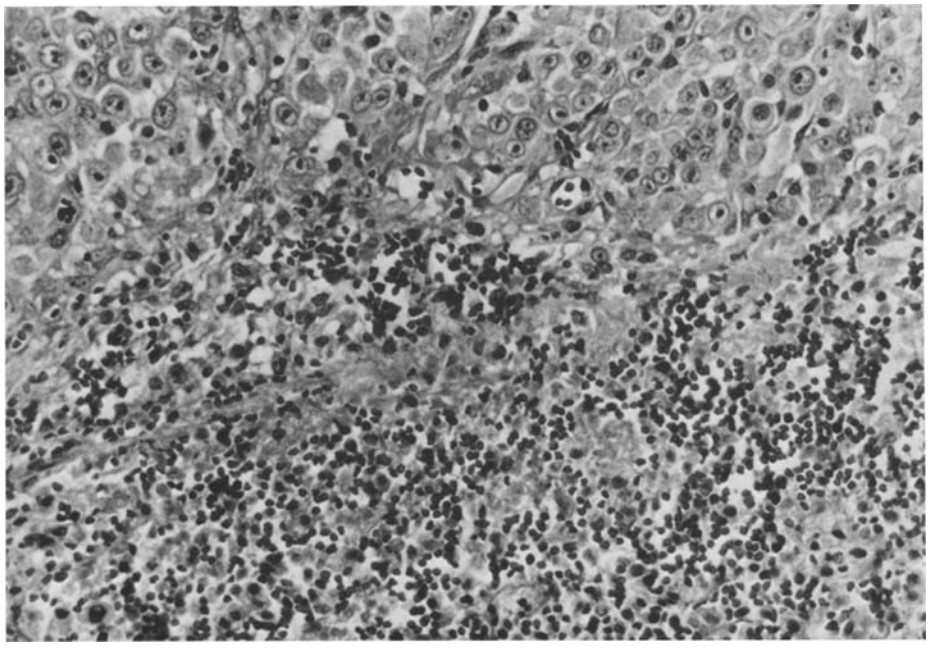

Fig. 3. Epithelioid sarcoma containing intralesional hemorrhage (*bottom*), simulating a vascular neoplasm. (H&E, ×250)

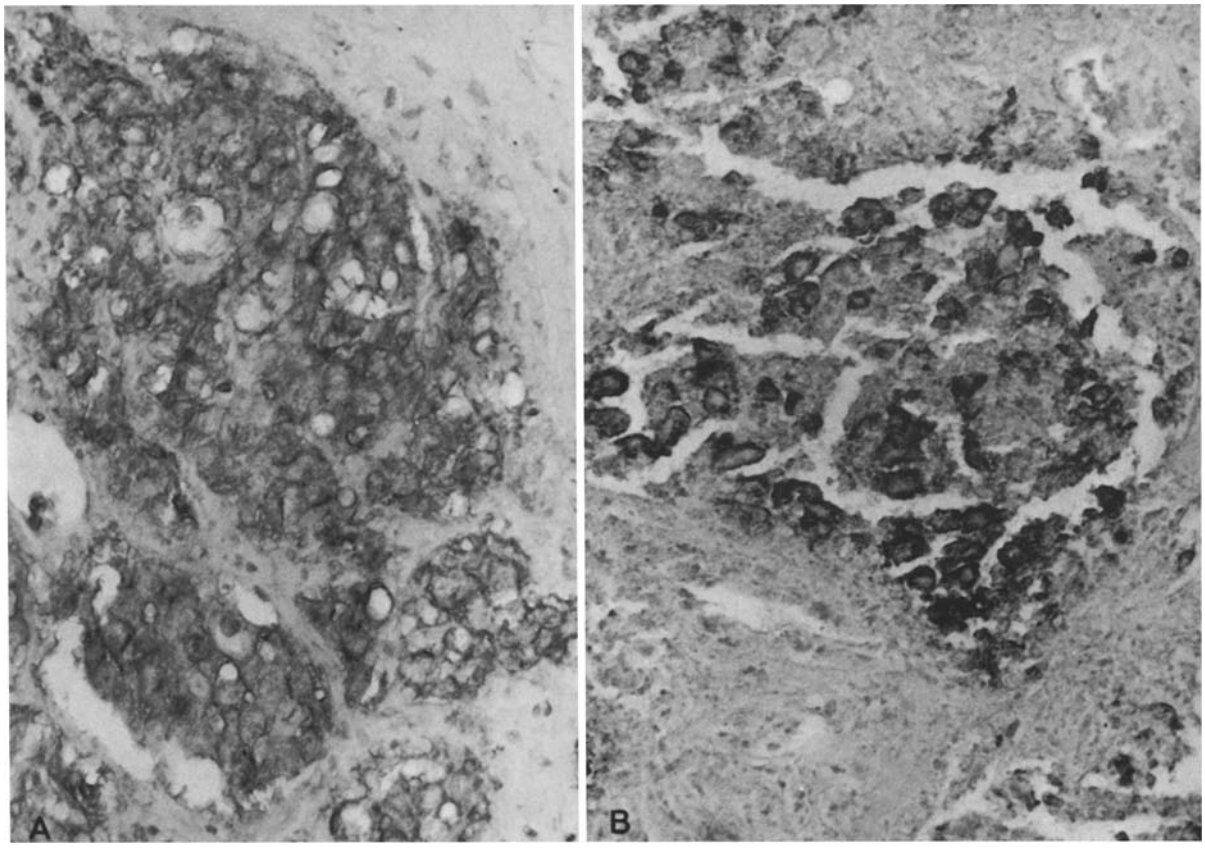

Fig. 4A. Membranous and cytoplasmic affinity for *Ulex europaeus* I agglutinin, in epithelioid sarcoma. (ABC/methyl green, ×160). **B** Reactivity for *Ulex europaeus* I lectin binding, in epithelioid hemangioendothelioma. (ABC/methyl green, ×160)

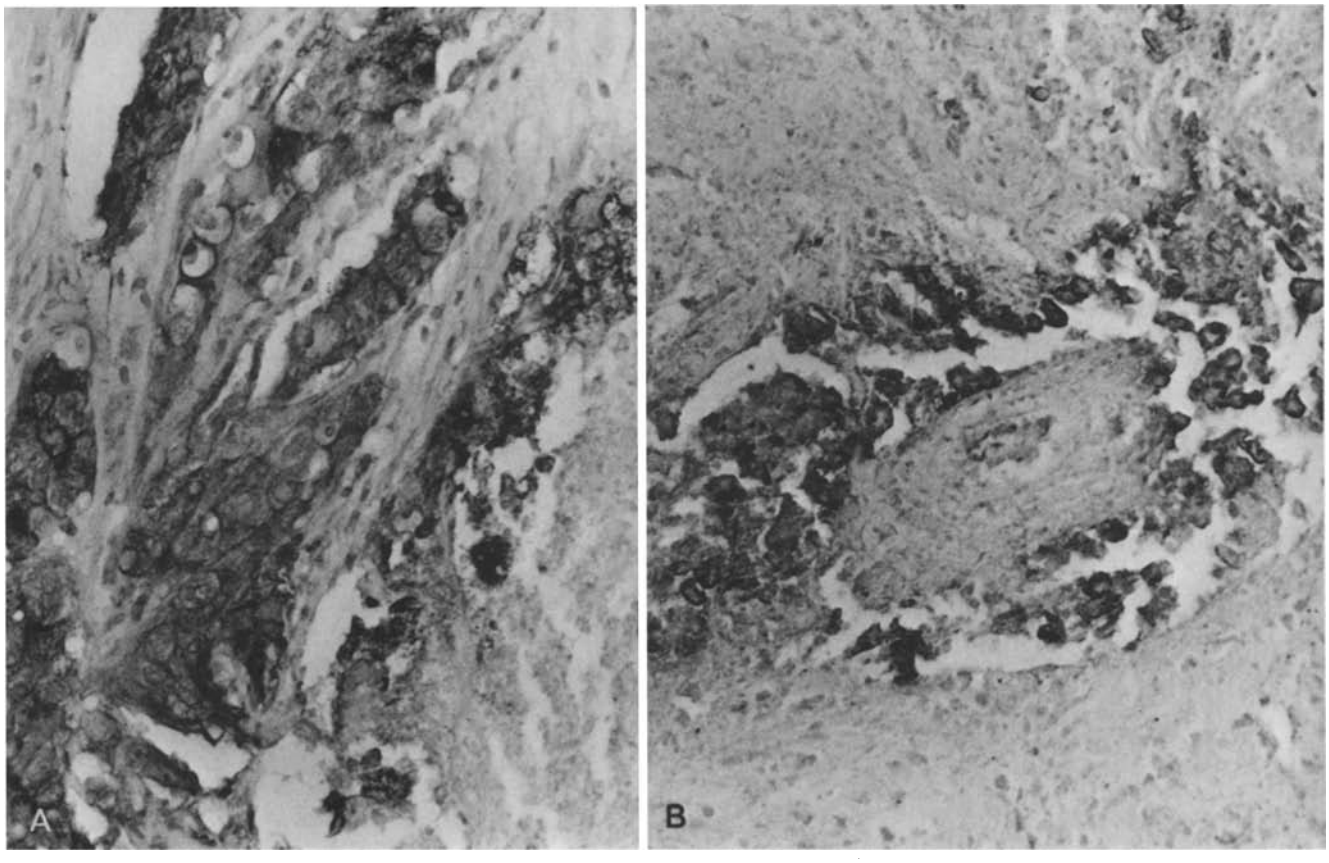

Fig. 5A. Immunoreactivity for blood group isoantigens in epithelioid sarcoma. (ABC/methyl green, ×250). **B** Expression of blood group isoantigens by cells of epithelioid hemangioendothelioma. (ABC/methyl green, ×160)

Discussion

The results of this study indicate that *Ulex europaeus* I and blood group isoantigens cannot be used to distinguish between epithelioid sarcoma and epithelioid hemangioendothelioma. In recent years, both of these reactants have been proposed as markers for vascular neoplasms (Berry and Amerigo 1980; Holthofer et al. 1982; Miettinen et al. 1983; Ordonez and Batsakis 1984), but the fact that they are also present in epithelial tumors (Ordonez and Batsakis 1984) has been inappropriately neglected. We have demonstrated that more cell-specific antigens such as CK, EMA, and FVIIIRAG must be included in the assessment of EH and ES, to avoid diagnostic errors.

Ulex europaeus I recognizes L-fucose residues, which are present on normal and neoplastic human endothelial cells, regardless of the blood type of the patient (Holthofer et al. 1982; Miettinen et al. 1983). L-fucose also constitutes a major sugar residue in A, B, and H blood group substances (Goldstein and Hayes 1978). The latter are expressed by endothelial and epithelial cells, and by tumors composed of these elements (Limas et al. 1979). These characteristics explain the affinity of UEA for vascular neoplasms, carcinomas, and carcinoma-like sarcomas, and correlate with the expression of BGI by epithelioid sarcomas, as demonstrated by blood-group antibody blocking studies. Epithelioid hemangioendotheliomas bound this lectin in spite of prior blocking of BGI.

Cytokeratin, epithelial membrane antigen, and factor VIII-related antigen vary in the level of their expression by neoplasms, but no cross-over in reactivity for these determinants has been observed in vascular and epithelial tumors (Moll et al. 1982; Pinkus and Kurtin 1985; Mukai and Rosai 1984). Universal reactivity for CK was seen in epithelioid sarcomas in our study, and 7 of 8 displayed EMA. Positivity for FVIIIRAG was observed in all of a limited number of EH cases studied in previous reports (Weiss and Enzinger 1982; Angervall et al. 1985), but it was not uniformly detected in this series. Nevertheless, only one of our 8 epithelioid hemangioendotheliomas lacked UEA binding, BGI, and FVIIIRAG. These data suggest that combined results of UEA- or BGI-reactivity with

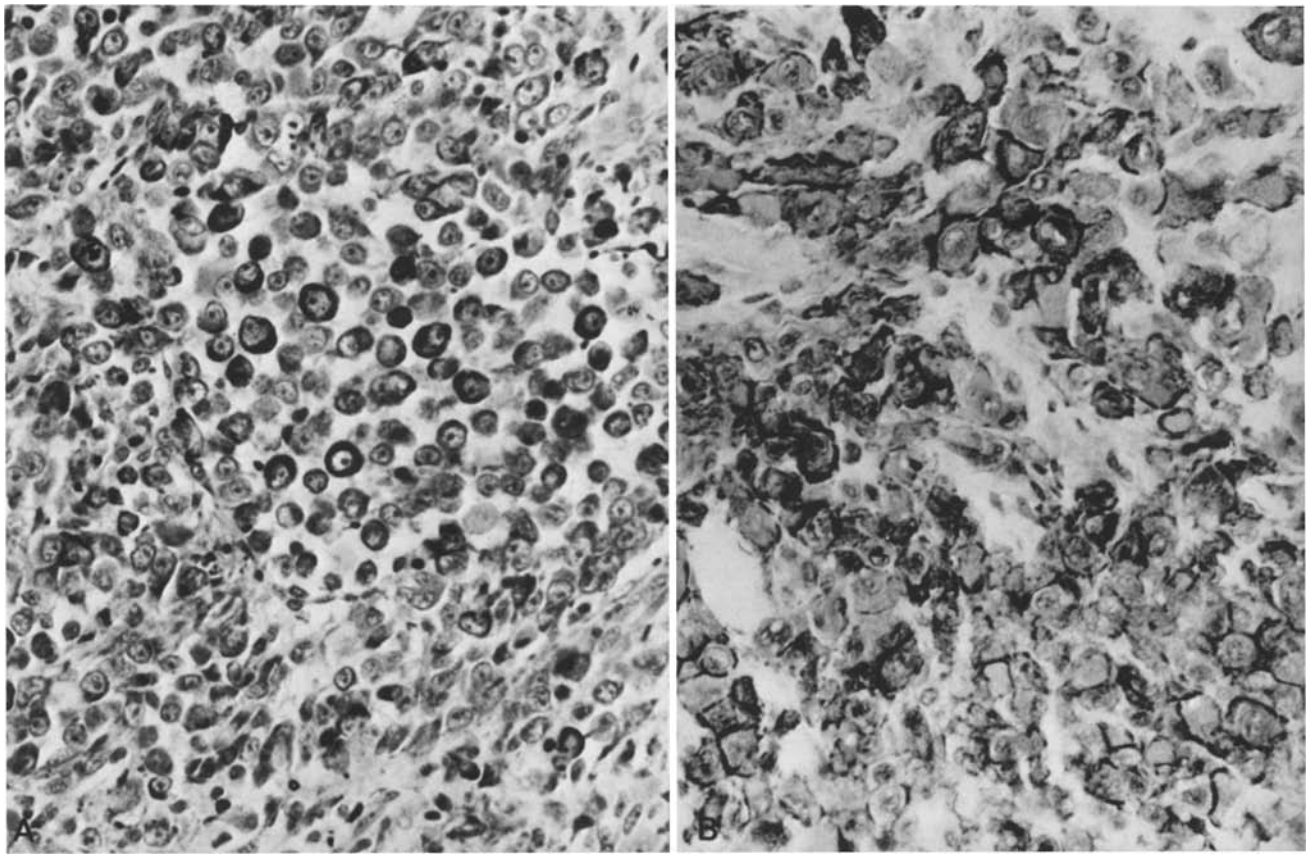

Fig. 6A. The cytoplasm of tumor cells shows dark immunoreactivity for cytokeratin, in this epithelioid sarcoma. (ABC/hematoxylin, ×160). **B** Membrane-based reactivity for epithelial membrane antigen, in epithelioid sarcoma. (ABC/hematoxylin, ×200)

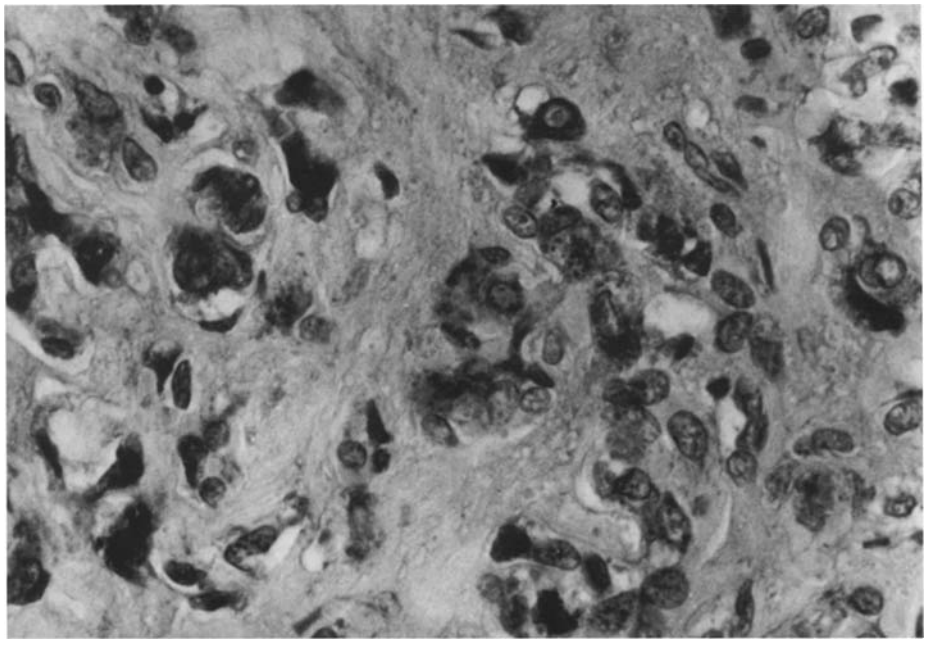

Fig. 7. Darkly-staining cytoplasmic positivity for factor VIII-related antigen, in tumor cells of epithelioid hemangioendothelioma. (ABC/hematoxylin, ×250)

EMA- and CK-negativity may be utilized to support a diagnosis of EH. Conversely, the presence of either EMA or CK in a primary polygonal-cell soft tissue tumor suggests an interpretation of epithelioid sarcoma.

We do not mean to imply that immunohistochemical studies are always necessary in the recognition of ES and EH. Both have sufficiently characteristic pathologic features in most cases, so as to make their separation possible on conventional microscopy alone. However, occasional examples, such as those included in this series, bear striking resemblances to one another. Obviously, it is under the latter circumstances that immunostaining is most rewarding in differential diagnosis. In light of the afore-mentioned differences in the biological behavior of these two tumors, accuracy in this process is highly desirable.

Acknowledgment. The authors are grateful to Dr. Juan Rosai, from whose consultation files many of the cases in this report were obtained.

References

Angervall L, Kindblom L-G, Karlsson K, Stener B (1985) Atypical hemangioendothelioma of venous origin. A clinicopathologic, angiographic, immunohistochemical, and ultrastructural study of two endothelial tumors within the concept of histiocytoid hemangioma. Am J Surg Pathol 9:504–516

Berry CL, Amerigo J (1980) Blood group antigens in vascular tumours. Evaluation of the immunoperoxidase technique. Virchows Arch [Pathol Anat Histol] 388:167–174

Chase DR, Enzinger FM (1985) Epithelioid sarcoma. Diagnosis, prognostic indicators, and treatment. Am J Surg Pathol 9:241–263

Enzinger FM (1970) Epithelioid sarcoma: a sarcoma simulating a granuloma or a carcinoma. Cancer 26:1029–1041

Enzinger FM, Weiss SW (1983) Soft tissue tumors, CV Mosby, St Louis, Missouri, USA, pp 409–413, 787–795

Goldstein IJ, Hayes CF (1978) The lectins: carbohydrate binding proteins of plants and animals. Adv Carbohydr Chem Biochem 35:127–140

Holthofer H, Virtanen I, Kariniemi AL, Hormia H, Linder E, Miettinen M (1982) *Ulex europaeus* I lectin as a marker for vascular endothelium in human tissue. Lab Invest 47:60–66

Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Limas C, Lange P, Fraley EE, Vessella RL (1979) A,B,H antigens in transitional cell tumors of the urinary bladder. Correlation with the clinical course. Cancer 44:2099–2107

Miettinen M, Holthofer H, Lehto V-P, Miettinen A, Virtanen I (1983) *Ulex europaeus* I lectin as a marker for tumors derived from endothelial cells. Am J Clin Pathol 79:32–36

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalogue of human cytokeratin polypeptides. Patterns of expression of cytokeratins in normal epithelia, tumors, and cultured cells. Cell 31:11–24

Mukai K, Rosai R (1984) Factor VIII-related antigen: an endothelial marker. In: DeLellis RA (ed) Diagnostic immunohistochemistry. Masson, New York, USA, pp 253–261

Ordonez NG, Batsakis JG (1984) Comparison of *Ulex europaeus* I lectin with factor VIII-related antigen in vascular lesions. Arch Pathol Lab Med 108:129–132

Pinkus GS, Kurtin PJ (1985) Epithelial membrane antigen – a diagnostic discriminant in surgical pathology: immunohistochemical profile in epithelial, mesenchymal, and hematopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum Pathol 16:929–940

Weiss SW, Enzinger FM (1982) Epithelioid hemangioendothelioma. A vascular tumor often mistaken for a carcinoma. Cancer 50:970–981

Accepted September 24, 1986