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Special Techniques for the Study of Vessels and Vascular Proliferations

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The recognition of vascular lesions is most often straightforward; however, sometimes the use of additional techniques becomes necessary to secure a more definitive diagnosis, notably when the vascular nature of a neoplasm mimics another category of neoplasm. For example, epithelioid hemangioendotheliomas and epithelioid angiosarcomas may simulate poorly differentiated carcinomas, even to the extent of positivity for the conventional epithelial marker, cytokeratin (1,2). In still other cases the vascular nature of a neoplasm may be obscured by a prominent inflammatory infiltrate (3,4). Special techniques can help clarify the true nature of these problematic neoplasms.

Before the era of immunohistochemical techniques, much reliance was placed on histochemical stains. Stains for reticulin and elastic fibers were the gold standard to detect the presence of vascular differentiation. Enzymatic stains were less often employed. Most of these techniques are currently outdated and seldom used. This chapter discusses the evaluation and application of the common markers currently used for the detection of vascular neoplasms. Molecular techniques receive less attention.

1. IMMUNOHISTOCHEMICAL STAINS

The most common markers used to assert the vascular nature of a lesion are von Willebrand factor (vWF; formerly factor VIII-related antigen), CD34 (human hematopoietic progenitor cell antigen), CD31 (platelet endothelial cell adhesion molecule-1), and *Ulex europaeus* lectin. More recently, vascular endothelial growth factor receptor-3 (VEGFR-3) and GLUT1 have been added to the armamentarium of immunohistochemical stains for the characterization of vascular lesions.

vWF was one of the first immunohistochemical markers to be developed, and it has been utilized now for more than 15 years (5). The factor is an intrinsic secretory component of endothelial cells. It has limited sensitivity but is present in most types of nonneoplastic endothelium, as well as in serum and body fluids. Although it is a good marker for epithelioid hemangioendotheliomas, it is absent in most angiosarcomas. Being widespread in distribution, it is usually present in areas of hemorrhage, exudation, and necrosis and thus can create heavy background staining that compromises interpretation (6).

The *Ulex europaeus* lectin selectively binds a terminal fucosyl residue of the H blood group antigen to a unique endothelial glycoprotein. Although this marker generated initial optimism because of its great sensitivity, it was subsequently discovered that the targeted sugar is also present in normal epithelium, as well as in a wide range of epithelial tumors (7).

CD34 (human hematopoietic progenitor cell antigen) is a 105–120-kDa transmembrane glycoprotein normally present in human hematopoietic progenitor cells (8). It was initially developed for the characterization of acute leukemias, in which the expression of this antigen is usually correlated with a poor prognosis. It was subsequently found to also be present in endothelial cells, although those of lymphatic vessels express this antibody in a less uniform fashion (9). The most commonly used monoclonal antibodies to CD34 are My10 and QB-END 10, two reagents with comparable reactivities (6,10). It has been validated that CD34 will intensely label mature, well-formed vessels but can be nonreactive or weakly so with immature or poorly formed vessels. Thus, CD34 can be nonreactive in granulation tissue, papillary intravascular endothelial hyperplasia, and bacillary angiomatosis (11). On the other hand, it appears to be a dependable marker for Kaposi's sarcoma and angiosarcomas (6,12,13). A shortcoming of CD34 is its affinity for other tissues of mesenchymal origin; thus, it stains fibrocytes, fat and perivascular cells. Consequently, many neoplasms including dermatofibrosarcoma protuberans, solitary fibrous tumor, epithelioid sarcomas and gastrointestinal stromal tumors are positive for CD34 (14–17).

CD31 is a 130-kDa glycoprotein that mediates platelet cell adhesion to endothelial cells (PECAM1). As one of the cell adhesion molecules, it belongs to the immunoglobulin gene superfamily. Although normally present in endothelial cells, it is also a constituent of platelets, monocytes/macrophages, and subsets of lymphocytes, plasma cells, and hematopoietic stem cells (6). The two monoclonal antibodies most commonly utilized for detection of CD31 are JC/70A and EN4 (18,19). CD31, the most sensitive and specific endothelial marker, is consistently present in angiosarcomas, hemangioendotheliomas, Kaposi's sarcomas, and hemangiomas (13). Among the nonvascular tumors, an occasional carcinoma or epithelioid sarcoma may show weak staining with this reagent, seemingly because of the partial crossreaction with related homologous adhesion molecules, such as carcinoembryonic antigen (CEA) (6).

VEGFR-3 is a tyrosine kinase receptor. Its expression is limited almost exclusively to endothelial cells lining adult lymphatic vessels, as detected by the monoclonal antibody 9D9F9 (20). Although this antibody is highly sensitive for the detection of Kaposi's sarcoma, in which it marks even the spindle component of this neoplasm, it is also reactive with other vascular neoplasms including angiosarcomas, kaposiform hemangioendotheliomas, Dabska's tumor, hobnail hemangioma (Fig. 1), and a few cases of infantile hemangiomas (21,22).

GLUT1 is an erythrocyte-type facilitative glucose transport protein. It is a member of at least six structurally related proteins, each with a characteristic tissue distribution. Interestingly, it is present in the endothelium of the microvasculature of blood-tissue barriers, such as those of the central nervous system, retina, placenta, ciliary muscle, and endoneurium of peripheral nerves, but it is absent in the vascular endothelium of normal

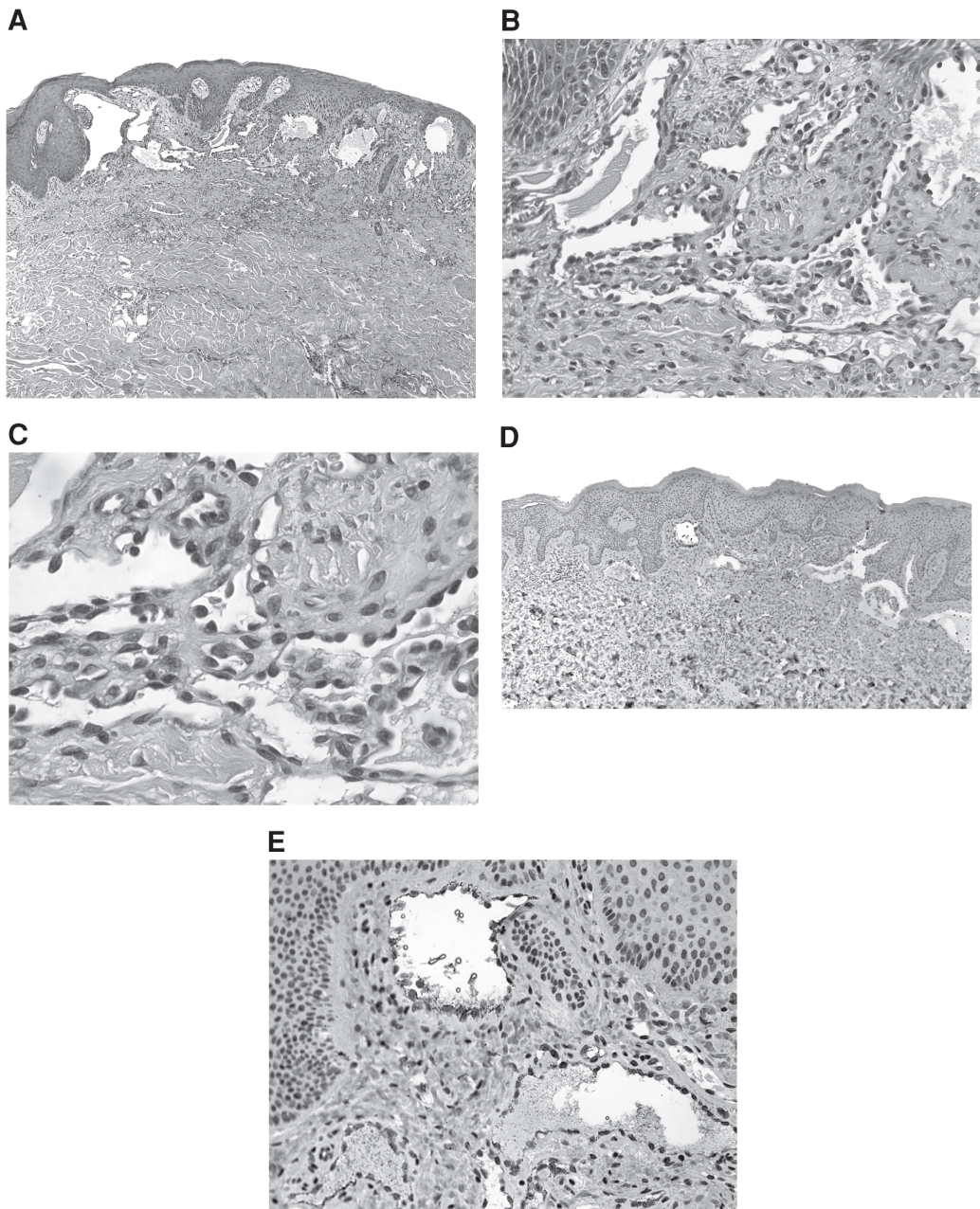


Fig. 1. Hobnail hemangioma stained with hematoxylin-eosin and VEGFR-3. **(A)** At scanning magnification there are dilated vascular structures on the superficial dermis. **(B)** Many of these vascular structures show thin walls and are lined by a discontinuous layer of endothelial cells, giving a lymphatic appearance to the channels. **(C)** Higher magnification shows that some endothelial cells protrude within the lumina with a hobnail appearance. **(D)** Scanning power view of a section of the same case stained with VEGFR-3. **(E)** Higher magnification demonstrates that endothelial cells lining the lumina express immunoreactivity for VEGFR-3.

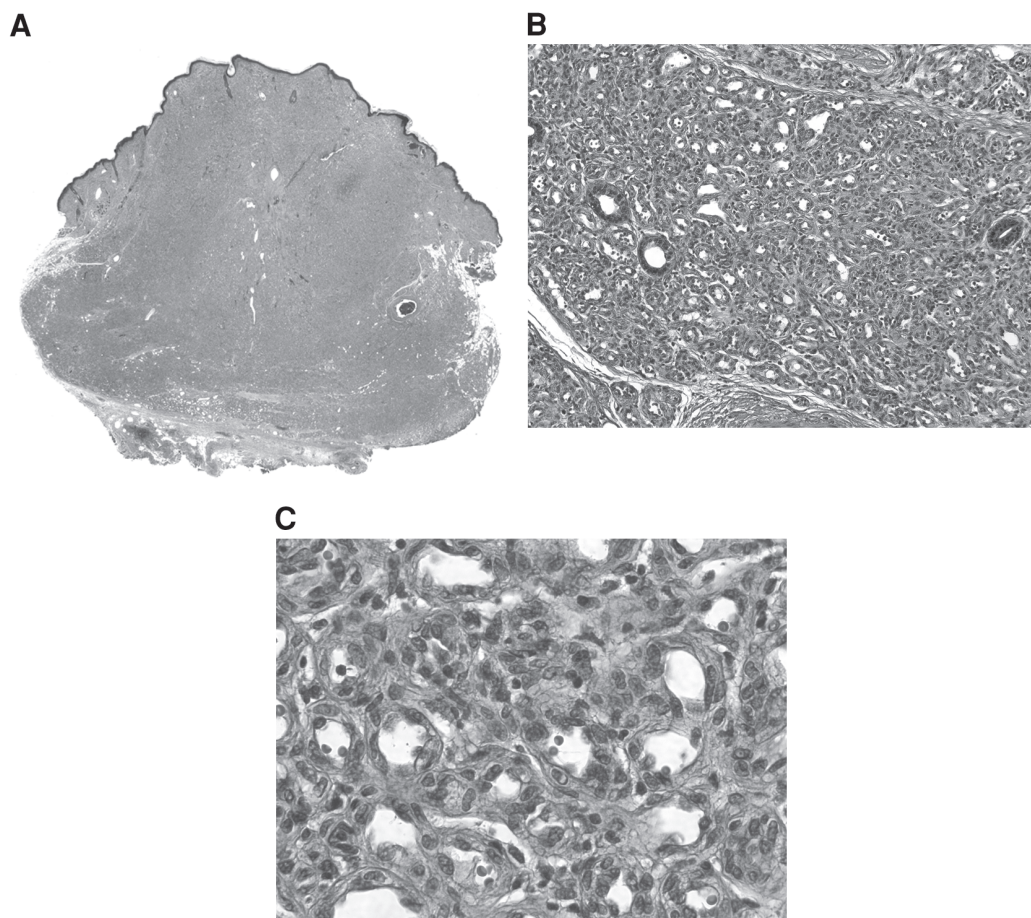


Fig. 2. Infantile hemangioma stained with hematoxylin-eosin and GLUT-1. **(A)** Scanning power view of the lesion showing a cellular proliferation involving the entire thickness of the dermis. **(B)** Higher magnification demonstrates numerous vascular channels. **(C)** Still higher magnification demonstrates that the vascular channels exhibit a capillary appearance and are lined by a single layer of endothelial cells.

vessels of the skin and subcutaneous tissue (23,24). This marker has also been found in infantile hemangiomas at different stages of their evolution (Fig. 2). Its specificity for this category of vascular proliferations is noteworthy, since other lesions including vascular malformations, pyogenic granulomas, kaposiform hemangioendotheliomas, and epithelioid hemangioendotheliomas do not express this marker (23,24).

2. MOLECULAR TECHNIQUES

During the past few years seminal discoveries have led to valuable applications of new techniques for the identification and characterization of neoplasms. Techniques such as the polymerase chain reaction, Southern and Northern blot analysis, and *in situ* hybridization are almost routine in many centers. The enhanced knowledge in cancer genetics is contributing significantly to the prognosis, diagnosis, and treatment of divergent neo-

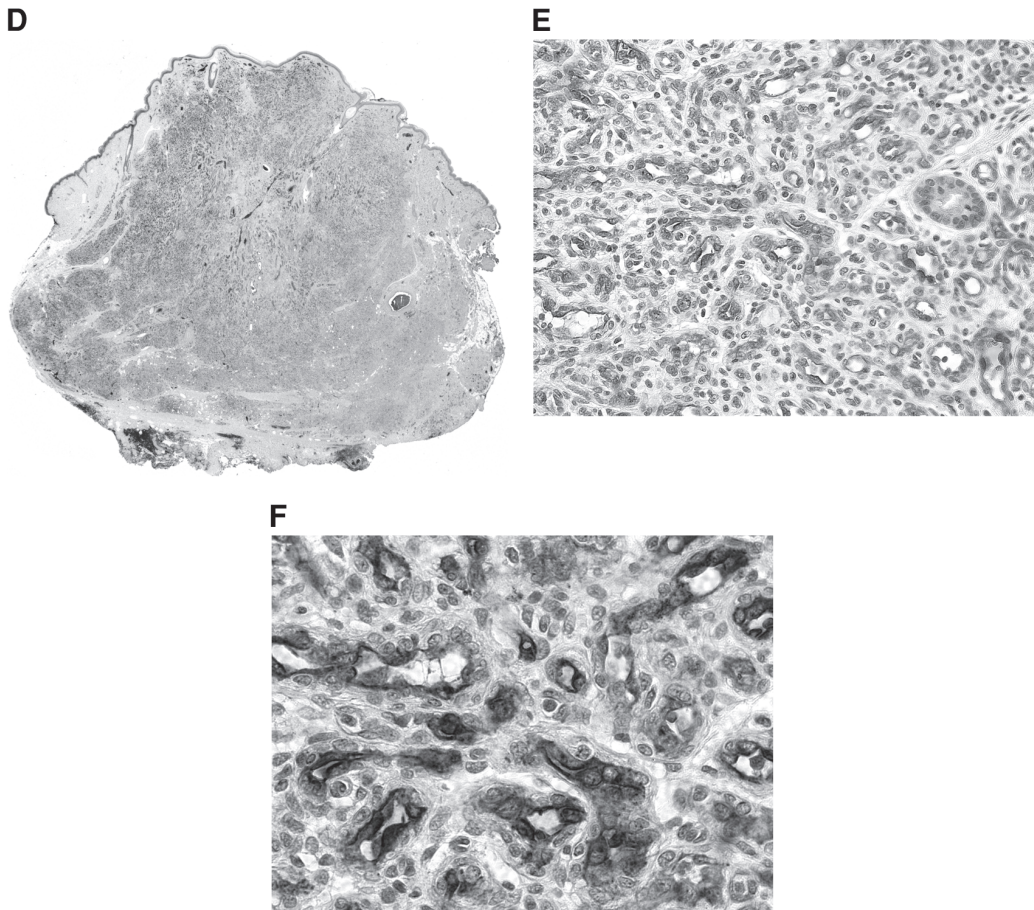


Fig. 2. Infantile hemangioma stained with hematoxylin-eosin and GLUT-1. **(D)** Scanning power view of the same lesion stained with GLUT-1. **(E)** Numerous cells in the dermis express GLUT-1 immunoreactivity. **(F)** Higher magnification shows GLUT-1 immunoreactivity in endothelial cells lining the capillary blood vessels.

plasms. Although these specific molecular contributions have predominantly favored hematopoietic malignancies, some soft tissue neoplasms have also benefited.

With regard to vascular neoplasms, the most important development has been the association of Kaposi's sarcoma with human herpes virus 8 (HHV-8). Confirmatory observations during the last few years have found a strong correlation between the presence of this virus and the presence of the neoplasm. HHV-8, also known as Kaposi's sarcoma-associated herpes virus (KSHV), is a γ -herpes virus. Following the initial report by Chang et al. (25) the recognitions of this association in laboratories around the world were promptly confirmed. HHV-8 is widespread in African and American Indian populations and is of limited prevalence in Northern Europe. An intermediate prevalence is found in Mediterranean countries, Asia, North America, and Central America (26). A pathogenetic role of HHV-8 in KS is unequivocal. The viral genome contains cellular genes that stimulate cell growth and angiogenesis; the virus is present in all clinical variants of KS, and, among all high-risk groups, seroconversion precedes the develop-

ment of KS. The virus is detectable in both endothelial and spindle cells, even in early lesions (27).

3. CYTOGENETIC STUDIES

Only limited cytogenetic studies have been carried out on vascular neoplasms; the most relevant related to angiosarcomas, and Kaposi's sarcomas. Although no consistent chromosomal abnormalities have been manifested in all cases of angiosarcoma the most common concern the number of chromosomes, with ranges from hypodiploid to hypertriploid. Notable are the abnormalities: trisomy of chromosome 5, translocation (5;15), additions in chromosomes 8 and 20, and losses of chromosomes 7, 22, and Y (27,28).

Most commonly, KS manifests: abnormalities in chromosome 7, sometimes with additions, translocation (7;13), multiple additions and deletions. A few cases have also shown abnormalities related to chromosomes 10 and 12 (29,30).

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