PREFACE

Why a book on cutaneous vascular proliferations? There are several compelling reasons to justify the existence of a book on this topic. One of the most important is that cutaneous vascular proliferations are exceedingly common and affect a large number of individuals of both sexes and within a wide age range. They make up a broad spectrum of lesions with morphological and biological variations, ranging from hamartomas to highly malignant, aggressive neoplasms. Although the diagnosis of some vascular lesions is straightforward, many entities pose significant problems in diagnosis, classification, and treatment. Within the past two decades there has been an increase in the number of patients affected with Kaposi's sarcoma, related to the epidemic of the acquired immunodeficiency syndrome (AIDS). As a consequence, a number of variants and vascular lesions that simulate Kaposi's sarcoma, both clinically and histopathologically, have been described. In addition, other vascular entities not related to Kaposi's sarcoma have been introduced in the literature. All of these have added confusion to an already complicated field. Since there are no recent textbooks on this subject, we felt an update was overdue.

The aim of *Pathology of Vascular Skin Lesions: Clinicopathologic Correlations* is to provide a comprehensive and in-depth review of all vascular proliferations involving the skin and subcutaneous tissue, including recently described entities. Although our work is primarily directed to pathologists, dermatologists, and dermatopathologists, its wide scope will make it useful to pediatricians and plastic surgeons as well.

Pathology of Vascular Skin Lesions: Clinicopathologic Correlations is divided into three parts. The first part covers classification and nomenclature of vascular neoplasms, an area that is still controversial. We propose a new classification with the hope that it will bring more order into a chaotic arena. We recognize that this classification may have some pitfalls and limitations, but we also believe that it is the most logical way to approach the study of vascular proliferations.

In order to know what is abnormal, a student of the field should first know what is normal, which is the reason for including a chapter on normal embryology, histology, and anatomy of the skin vasculature. Another chapter is devoted to the use of special techniques for the study of vascular proliferations.

In the second part, we include benign proliferations ranging from hamartomas and malformations to benign neoplasms. The final part of the book deals with malignant vascular proliferations, ranging from Kaposi's sarcoma to angiosarcomas. It includes some new entities, too.

The whole of *Pathology of Vascular Skin Lesions: Clinicopathologic Correlations* was conceived in terms of a clinicopathologic correlation. The clinical and morphologic

aspects of each entity are described in detail, including their differential diagnosis, prognosis, and therapy. Each chapter is fully illustrated with both clinical and histopathologic photographs, and we include color versions of all illustrations on the accompanying CD-ROM. Additionally, there is a complete and updated list of references for each particular section. We hope that you find this book interesting and useful.

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2 Special Techniques for the Study of Vessels and Vascular Proliferations

Contents

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 fucosil 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

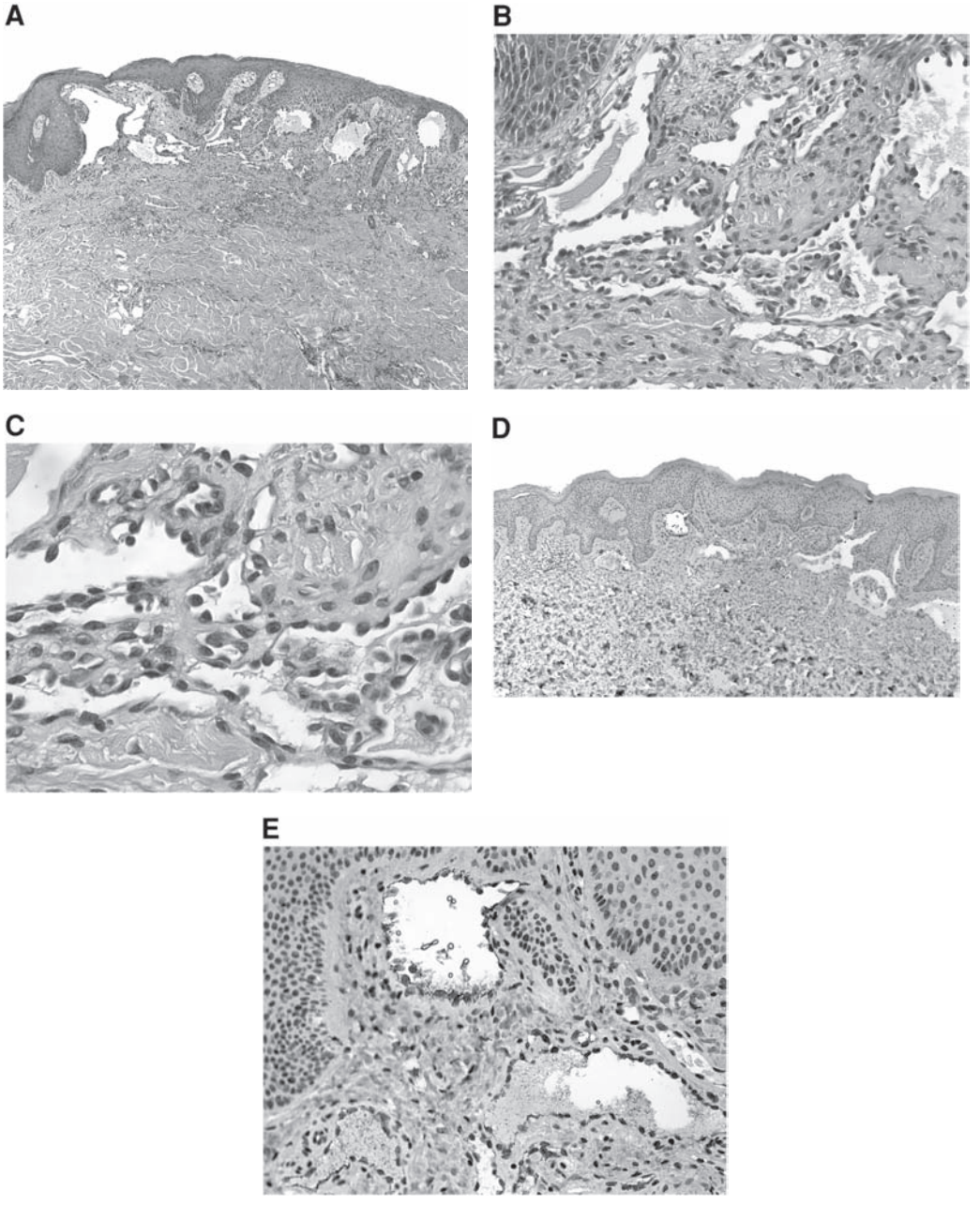

Fig. 1. Hobnail hemangioma stained with hematoxylin-eosin and VEGFR-3. **(A)** At scanning magnification there are dilated vascular structures on the superficial dermis. **(B)** Many of these vascular structures show thin walls and are lined by a discontinuous layer of endothelial cells, giving a lymphatic 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.

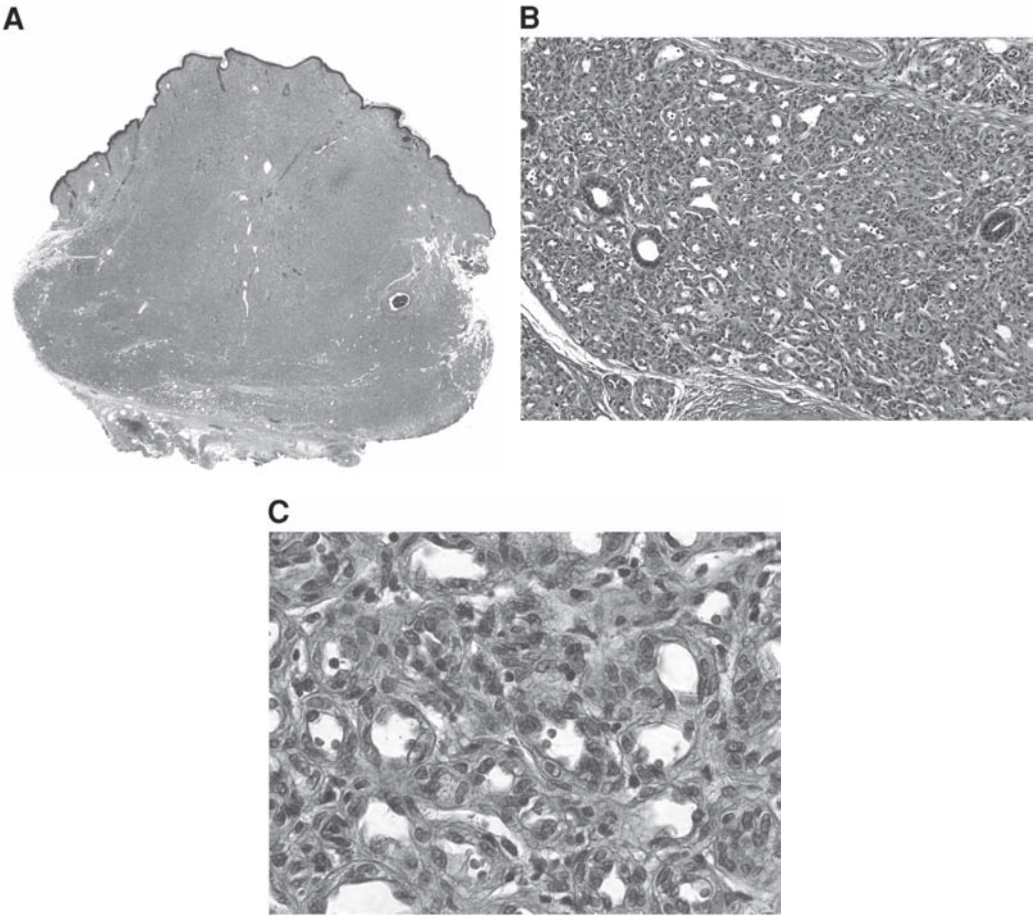

Fig. 2. Infantile hemangioma stained with hematoxylin-eosin and GLUT-1. **(A)** Scanning power view of the lesion showing a cellular proliferation involving the entire thickness of the dermis. **(B)** Higher magnification demonstrates numerous vascular channels. **(C)** Still higher magnification demonstrates 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-

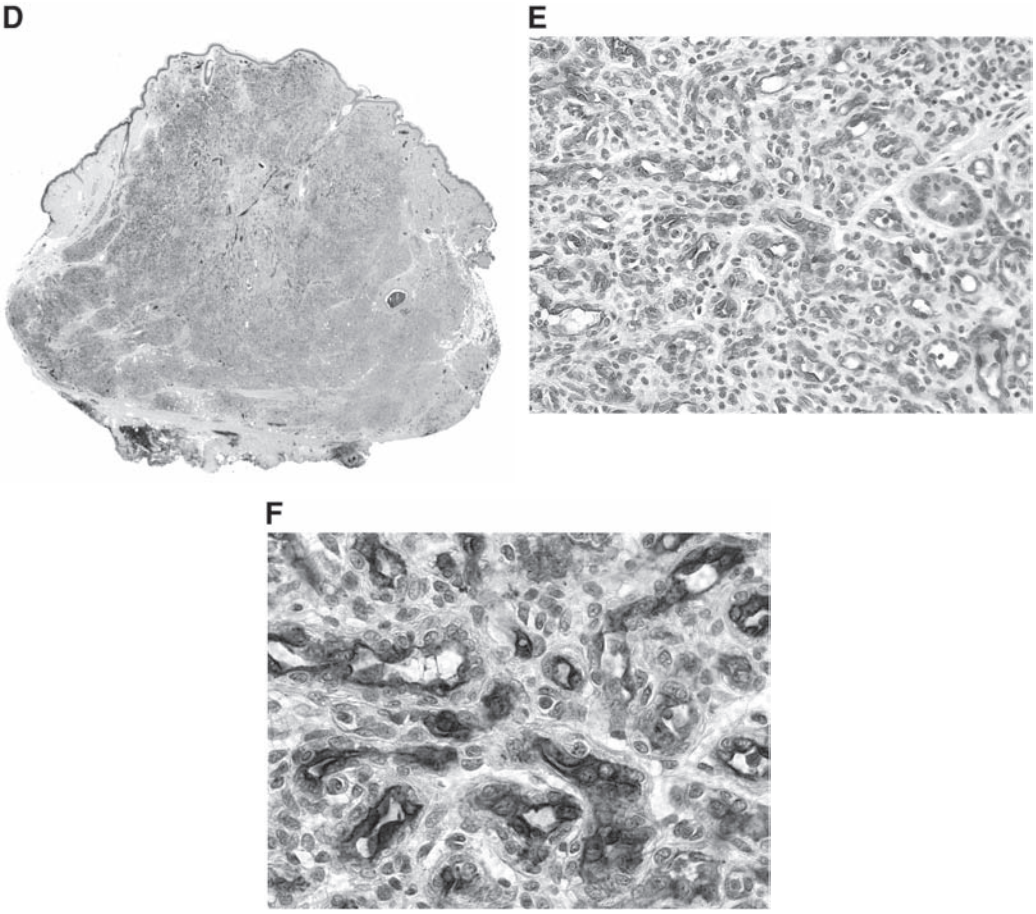

Fig. 2. Infantile hemangioma stained with hematoxylin-eosin and GLUT-1. **(D)** Scanning power view of the same lesion stained with GLUT-1. **(E)** Numerous cells in the dermis express GLUT-1 immunoreactivity. **(F)** Higher magnification shows GLUT-1 immunoreactivity in endothelial cells lining the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

References

1. Gray MH, Rosenberg AE, Dickersin GR, Bhan AK. Cytokeratin expression in epithelioid vascular neoplasms. Hum Pathol 1990;21:212–7.
2. Meis-Kindblom JM, Kindblom LG. Angiosarcoma of soft tissue: a study of 80 cases. Am J Surg Pathol 1998;22:683–97.
3. Mentzel T, Kutzner H, Wollina U. Cutaneous angiosarcoma of the face: clinicopathologic and immunohistochemical study of a case resembling rosacea clinically. J Am Acad Dermatol 1998;38:837–40.
4. Diaz-Cascajo C, de la Vega M, Rey-Lopez A. Superinfected cutaneous angiosarcoma: a highly malignant neoplasm simulating an inflammatory process. J Cutan Pathol 1997;24:56–60.
5. Burgdorf WH, Mukai K, Rosai J. Immunohistochemical identification of factor VIII-related antigen in endothelial cells of cutaneous lesions of alleged vascular nature. Am J Clin Pathol 1981;75:167–71.
6. Miettinen M, Lindenmayer AE, Chaubal A. Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens—evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. Mod Pathol 1994;7:82–90.
7. DeYoung BR, Swanson PE, Argenyi ZB, et al. CD31 immunoreactivity in mesenchymal neoplasms of the skin and subcutis: report of 145 cases and review of putative immunohistologic markers of endothelial differentiation. J Cutan Pathol 1995;22:215–22.
8. Civin CI, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hemopoiesis. III. A hematopoietic progenitor cell surface antigen defined by monoclonal antibody raised against KG-1a cells. J Immunol 1984;133:157–65.
9. Suster S, Wong TY. On the discriminatory value of anti-HPCA-1 (CD-34) in the differential diagnosis of benign and malignant cutaneous vascular proliferations. Am J Dermatopathol 1994;16:355–63.
10. Aziza JMC, Selves J, Voigt J-J, et al. Comparison of the reactivities of monoclonal QBEND10 (CD34) and BNH9 in vascular tumors. Appl Immunohistochem 1993;1:51.
11. Schlingemann RO, Rietveld F Jr, de Waal RMW, et al. Leukocyte antigen CD34 is expressed by a subset of cultured endothelial cells and on endothelial cells abluminal microprocesses in the tumor stroma. Lab Invest 1990;62:690–6.
12. Kanitakis J, Narvaez D, Claudy A. Expression of the CD34 antigen distinguishes Kaposi's sarcoma from pseudo-Kaposi's sarcoma (acroangiodermatitis). Br J Dermatol 1996;134:44–6.
13. Russell Jones R, Orchard G, Zelger B, Wilson Jones E. Immunostaining for CD31 and CD34 in Kaposi sarcoma. J Clin Pathol 1995;48:1011–6.
14. Kutzner H. Expression of the human progenitor cell antigen CD34 (HPCA-1) distinguishes dermatofibrosarcoma protuberans from fibrous histiocytoma in formalin-fixed, paraffin-embedded tissue. J Am Acad Dermatol 1993; 28:613–7.
15. Van de Rijn M, Rouse R. CD34: a review. Appl Immunohistochem 1994;2:71.
16. Westra WH, Gerald WL, Rosai J. Solitary fibrous tumor. Consistent CD34 immunoreactivity and occurrence in the orbit. Am J Surg Pathol 1994;18:992–8.
17. Weiss SW, Nickoloff BJ. CD-34 is expressed by a distinctive cell population in peripheral nerve, nerve sheath tumors, and related lesions. Am J Surg Pathol 1993;17:1039–45.

18. Burgio VL, Zupo S, Roncella S, Zocchi M, Ruco LP, Baroni CD. Characterization of EN4 monoclonal antibody: a reagent with CD31 specificity. Clin Exp Immunol 1994;96:170–6.
19. Parums DV, Cordell JL, Micklem K, et al. JC70: a new monoclonal antibody that detects vascular endothelium associated antigen on routinely processed tissue sections. J Clin Pathol 1990;43:752–7.
20. Jussila L, Valtola R, Partanen TA, et al. Lymphatic endothelium and Kaposi's sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3. Cancer Res 1998;58:1599–604.
21. Folpe AL, Veikkola T, Valtola R, Weiss SW. Vascular endothelial growth factor receptor-3 (VEGFR-3): a marker of vascular tumors with presumed lymphatic differentiation, including Kaposi's sarcoma, kaposiform and Dabska-type hemangioendotheliomas, and a subset of angiosarcomas. Mod Pathol 2000;13:180–5.
22. Lymboussaki A, Partanen TA, Olofsson B, et al. Expression of the vascular endothelial growth factor C receptor VEGFR-3 in lymphatic endothelium of the skin and in vascular tumors. Am J Pathol 1998;153:395–403.
23. North PE, Waner M, Mizeracki A, Mihm MC Jr. GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. Hum Pathol 2000;31:11–22.
24. North PE, Waner M, Mizeracki A, et al. A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. Arch Dermatol 2001;137:559–70.
25. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266:1865–9.
26. Sturzl M, Zietz C, Monini P, Ensoli B. Human herpesvirus-8 and Kaposi's sarcoma: relationship with the multistep concept of tumorigenesis. Adv Cancer Res 2001;81:125–59.
27. Kennedy MM, Cooper K, Howells DD, et al. Identification of HHV8 in early Kaposi's sarcoma: implications for Kaposi's sarcoma pathogenesis. J Clin Pathol 1998;51:14–20.
28. Schuborg C, Mertens F, Rydholm A, et al. Cytogenetic analysis of four angiosarcomas from deep and superficial soft tissue. Cancer Genet Cytogenet 1998;100:52–6.
29. Mandahl N, Jin YS, Heim S, et al. Trisomy 5 and loss of the Y chromosome as the sole cytogenetic anomalies in a cavernous hemangioma/angiosarcoma. Genes Chromosomes Cancer 1990;1:315–6.
30. Saikevych IA, Mayer M, White RL, Ho RC. Cytogenetic study of Kaposi's sarcoma associated with acquired immunodeficiency syndrome. Arch Pathol Lab Med 1988;112:825–8.