Sensitivity and specificity of D2-40 in differentiating Kaposi sarcoma from its mimickers

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Background
Kaposi sarcoma (KS) is a low-grade malignant vascular neoplasm, whose etiology is associated with human herpes virus 8 (HHV8). Owing to its broad clinicopathological spectrum, KS mimics a variety of non-KS vascular lesions both clinically and histologically, thus posing a diagnostic challenge. Immunohistochemical markers are needed to confirm diagnosis.
Objective
To study the sensitivity and specificity of D2-40 in comparison with HHV8-latent nuclear antigen 1 to identify KS among other cutaneous vascular proliferative lesions.
Patients and methods
The present study was carried out on 50 cases with cutaneous vascular proliferation. Examination of hematoxylin and eosin-stained sections was done for all cases to verify the diagnosis. Additional sections of all cases were subjected to immunohistochemical stains for the following primary antibodies: HHV8 and D2-40 monoclonal antibody.
Results
The study included 19 KS cases and 31 non-KS. Among KS lesions 15.8% were patch stage, 26.3% plaque stage, 36.84% nodular stage, 10.5% pyogenic granuloma-like KS, and 10.5% intravascular KS. All KS cases stained positive for HHV8-latent nuclear antigen 1, whereas none of other vascular lesions showed positive staining. Immunohistochemistry using D2-40 showed positive staining in 70% (n=35) and negative staining in 30% (n=15) of the total cases. All KS lesions expressed D2-40 in both endothelial lining and spindle cells. A total of 16 cases of non-KS vascular lesions expressed D2-40 in endothelial lining only. D2-40 positivity of both endothelial lining and spindle cells was found to be equally sensitive and specific to HHV8 for diagnosis of KS.
Conclusion
D2-40 positivity of both vascular lining and spindle cells can be considered both sensitive and specific stain to differentiate KS from other vascular lesions.

Keywords:
D2-40, HHV8, Kaposi sarcoma

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Introduction

Cutaneous vascular proliferations are vast and complex. Classification of vascular proliferations is still unsatisfactory owing to overlapping features and lack of full understanding of the pathogenesis of some of them [1]. They are generally classified into reactive, benign neoplasms, low-grade malignant neoplasms, and malignant neoplasms. According to some authors, benign tumors may include reactive vascular proliferations, malformations, and ectasias [2]. Nature of some of the vascular proliferative lesions still remains controversial whether reactive or neoplastic [3].

Owing to the overlap in the clinical and pathologic appearance, detection of markers using immunohistochemistry can offer a better understanding of vascular lesions and help to arrive at an accurate diagnosis [4,5].

Kaposi sarcoma (KS) is a low-grade angioproliferative tumor with a broad clinicopathologic spectrum. The differential diagnoses of KS are extensive, including both vascular and nonvascular lesions [6,7]. Human herpes virus 8 (HHV8) is an oncogenic virus that plays an important role in cellular proliferation and tumor formation [8,9]. A number of malignancies are associated with HHV8, including multicentric Castleman's disease and primary effusion lymphoma. Almost all KS are positive for HHV8 [10,11]. Identification of HHV8-latent nuclear antigen 1 (LNA-1) is considered the gold standard in the diagnosis of KS [12,13].

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KS expresses both the lymphatic and the blood vascular endothelial markers. It has been found that CD34 is a more reliable marker than CD31 for labeling KS. Spindle cells are less consistently stained with CD31 [14]. Both CD31 and CD34 markers do not offer great help to distinguish KS from its mimickers [12,14,15]. Lymphatic endothelium highly expresses podoplanin, which does not exist in the blood vasculature. D2-40 is a unique monoclonal antibody that reacts with podoplanin, a fixation-resistant epitope in lymphatic endothelium [16]. It is useful to detect lymphatic invasion in malignant tumors [17,18].

Delay in the diagnosis or wrong diagnosis of cutaneous vascular proliferations may have serious consequences [19]. The use of immunohistochemical techniques is important to arrive at a definitive diagnosis [4]. Vascular endothelial markers can be divided into pan-endothelial markers, lymphatic endothelial markers, and blood vessel endothelial markers. Pan-endothelial markers include CD34, CD31, and ETS-related genes, which are expressed in both lymphatic and blood vessel endothelial cells [20,21]. Immunohistochemistry for lymphatic endothelium markers can be used to distinguish between cells derived from lymphatic or vascular endothelium [17,22].

The aim of the present work was to study the sensitivity and specificity of D2-40 in comparison with HHV8-LNA-1 to identify KS among other cutaneous vascular proliferative lesions.

Patients and methods

This case–control study was conducted on 50 cases diagnosed with cutaneous vascular lesions. All types of cutaneous vascular lesions were included in the study. A total of 25 cases were recruited from the outpatient clinic of Dermatology, Venereology, and Andrology Department, Alexandria Main University Hospital. Consent was taken from each case before participation in the study. Another 25 cases of cutaneous vascular lesions were retrospectively retrieved from the archives of Dermatology and pathology Departments, Faculty of Medicine, University of Alexandria. Cases whose laboratory reports could not be retrieved from the archives or tissue blocks had insufficient tissues for evaluation were excluded. The study was approved by the Alexandria Faculty of Medicine Research Ethics Committee. All new cases were subjected to history taking, including history of the present vascular lesions, local dermatological examination, and photography of vascular lesions. Scalpel incisional/excisional biopsies were taken from the lesions. The specimens were fixed in 10% neutral formalin for 24 h to ensure ideal fixation. Regarding 25 retrospective cases, all information was obtained from medical records, and blocks were retrieved. For each case, 4-μm-thick sections were cut from the formalin-fixed, paraffin-embedded blocks of the tumor. Sections were stained with conventional hematoxylin and eosin (H&E) stain and examined under light microscopy for provisional histologic diagnosis of the vascular tumors. Additional sections of all cases were subjected to immunohistochemical stains for the following primary antibodies:

(1) HHV8 monoclonal antibody: the staining was done with a validated Rat IgG2c/k monoclonal antibody [Medaysis, Livermore, California, USA; Anti-HHV8 (LN53)].
(2) D2-40 monoclonal antibody: the staining was done with a validated mouse IgG1 monoclonal antibody (Thermo Scientific, Cheshire, WA7 1TA, UK).

Immunohistochemistry staining protocol entailed the following

The 5-μm-thick sections, mounted on positively charged, coated slides (Polysine, Bio Optica, Milano, Spain), were deparaffinized in xylene (two changes, 10 min each). The sections were then rehydrated by washing in descending grades of alcohol (two changes, 5 min each) and finally brought to water for another 5 min. Immunohistochemistry was performed manually using a labeled streptavidin-biotin antigen-detection kit (Lab Vision UltraVision Detection System: anti-Polyvalent, TP-015-HD; Thermo Fisher Scientific, Fremont, California, USA) according to manufacturer's instructions. Endogenous peroxidase activity was first blocked by applying hydrogen peroxide (3%) to each slide for 10 min then the Ultra V block was applied to reduce nonspecific background staining. Epitope retrieval was done thermally by immersing tissue sections in 10 mM citrate buffer solution (pH 6.0) for 15 min in a microwave oven at 60°C. Each of the primary antibodies used was applied in a dilution of 1 : 100, followed by the kit Biotinylated Goat anti-Polyvalent, streptavidin peroxidase, diaminobenzidine plus substrate, and diaminobenzidine plus chromogen. Counterstaining was done using Harris hematoxylin.

Evaluation of the immunostained slides was performed by two blinded pathologists; slides were examined under low power examination (×100) for assessment

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of staining (positive/negative) for each antibody. Positive staining for HHV8 was nuclear and that of D2-40 was cytoplasmic. Positive D2-40 immunostaining was further divided according to the type of stained cells whether endothelial only or endothelial and spindle-shaped cells.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0. (IBM Corp., Armonk, New York, USA). Qualitative data were described using number and percent. The Kolmogorov–Smirnov test was used to verify the normality of distribution quantitative data were described using range (minimum and maximum), mean, SD, median, and interquartile range. Significance of the obtained results was judged at the 5% level. Sensitivity and specificity were calculated as follows:

(1) Sensitivity=(true positives)/(true positives+false negatives).
(2) Specificity=(true negatives)/(true negatives +false positives).
(3) Positive predictive value=(true positives)/(true positives+false positives).
(4) Negative predictive value=(true negatives)/(true negatives+false negatives).
(5) Accuracy=(true positive+true negative)/total number.

Results

Of the 50 cases included in the present study, 35 (70.0%) were males and 15 (30.0%) were females. A total of 13 (26%) cases presented with solitary lesion and 37 (74.0%) cases presented with multiple lesions. The H&E diagnoses were as follows: KS in 19 (38%) cases and non-KS in 31 (62%) cases (Table 1). Males represented 18 (94.7%) of 19 of KS cases, whereas females represented one (5.3%) of 19. The patients' age ranged from 28 to 81 years old. The mean age was 62.32±51.14 years. Duration of KS lesions ranged from one month up to 10 months. Of 19 of KS cases, 18 (94.7%) presented with multiple lesions, and only one KS case presented with solitary lesion (5.3%).

The histopathological types of KS seen were three (15.8%) cases of patch stage KS, five (26.3%) cases of plaque stage KS, seven (36.84%) cases of nodular stage KS, two (10.5%) cases of pyogenic granuloma-like KS, and two (10.5%) cases of intravascular KS variant.

Table 1 Histopathological diagnosis (hematoxylin and eosin) of the studied case (*N*=50)

Diagnosis	H&E [*n* (%)]
KAPOSI sarcoma	19 (38.0)
Non-KAPOSI	31 (62.0)
Acroangiodermatitis	5 (16.1)
ALHE	3 (9.7)
Cirsoid aneurysm	2 (6.5)
Pyogenic granuloma	6 (19.4)
Angiolipoma	2 (6.5)
Lymphangioma	4 (12.9)
Infantile hemangioma	1 (3.2)
Angiokeratoma	1 (3.2)
Bacillary angiomatosis	1 (3.2)
Verrucous hemangioma	1 (3.2)
Glomangioma	1 (3.2)
Hobnail hemangioma	1 (3.2)
Lymphangiohemangioma	3 (9.7)

ALHE, angiolymphoid hyperplasia with eosinophilia; H&E, hematoxylin and eosin.

Immunohistochemistry using HHV8-LNA-1 showed positive staining in 19 (38%) cases and negative staining in 31 (62%) cases. All KS cases (*n*=19) displayed focal positive nuclear staining for HHV8-LNA-1 with granular pattern, whereas non-KS cases were negative (Table 2).

Immunohistochemistry using D2-40 revealed positive cytoplasmic staining in 35 (70%) cases. Positive cases were further subdivided according to the type of stained cells, whether D2-40 stains endothelial lining only or endothelial lining and spindle-shaped cells. All KS 19 (100%) cases displayed D2-40-positive reactivity in both endothelial lining and spindle cells, whereas 16 (51.6%) of 31 non-KS cases showed positive staining of endothelial lining only. Vascular lesions that did not show positive staining for D2-40 were cirsoid aneurysm, angiolipoma, infantile hemangioma, verrucous hemangioma, cavernous hemangioma, angiokeratoma, and glomangioma (Tables 3 and 4, Fig. 1).

Sensitivity and specificity of D2-40

Sensitivity and specificity were estimated for HHV8 as a differentiating marker between KS and other vascular lesions. Then sensitivity and specificity were estimated for D2-40 as a differentiating marker between KS and its mimics. Then both sensitivities and specificities were compared together (Figs 2 and 3).

The positivity of D2-40 of both endothelial lining and spindle cells was found to be equally sensitive and specific to HHV8 for diagnosis of KS (Table 5).

Table 2 Relation between hematoxylin and eosin diagnosis and human herpes virus 8-latent nuclear antigen 1 immunostaining (*N*=50)

	HHV8-LNA-1			
Histopathological diagnosis (H&E)	Positive [*n* (%)]	Negative [*n* (%)]	χ^2	*P*
Kaposi sarcoma (*n*=19)	19 (100.0)	0		
Non-Kaposi sarcoma (*n*=31)	0	31 (100)	50.0*	<0.001*

χ^2, χ^2 test; H&E, hematoxylin and eosin; HHV8, human herpes virus 8; LNA-1, latent nuclear antigen 1. *P*: *P* value for association between different categories. *Statistically significant at *P* value less than or equal to 0.05.

Table 3 Relation between histopathological diagnosis and D2-40 immunostaining (*N*=50)

	D2-40 [*n* (%)]			
	Vascular lining only		Vascular lining and spindle cells	
Histopathological (H&E) diagnosis	Positive	Negative	Positive	Negative
KAPOSI sarcoma (*n*=19)	0.0	19 (100.0)	19 (100.0)	0
Non-KAPOSI (*n*=31)	16 (51.6)	15 (48.4)	0	31 (100.0)

H&E, hematoxylin and eosin.

Table 4 Distribution of non-Kaposi sarcoma regarding D2-40 staining (*N*=31)

	Total cases	D2-40	
H&E diagnosis	*N*	Positive	Negative
Acroangiodermatitis	5	4	1
ALHE	3	2	1
Pyogenic granuloma	6	2	4
Lymphangiohemangioma	3	3	0
Lymphangioma	4	4	0
Bacillary angiomatosis	1	1	0
Cirsoid aneurysm	2	0	2
Angiolipoma	2	0	2
Infantile hemangioma	1	0	1
Angiokeratoma	1	0	1
Verrucous hemangioma	1	0	1
Glomangioma	1	0	1
Hobnail hemangioma	1	0	0

ALHE, angiolymphoid hyperplasia with eosinophilia; H&E, hematoxylin and eosin.

Discussion

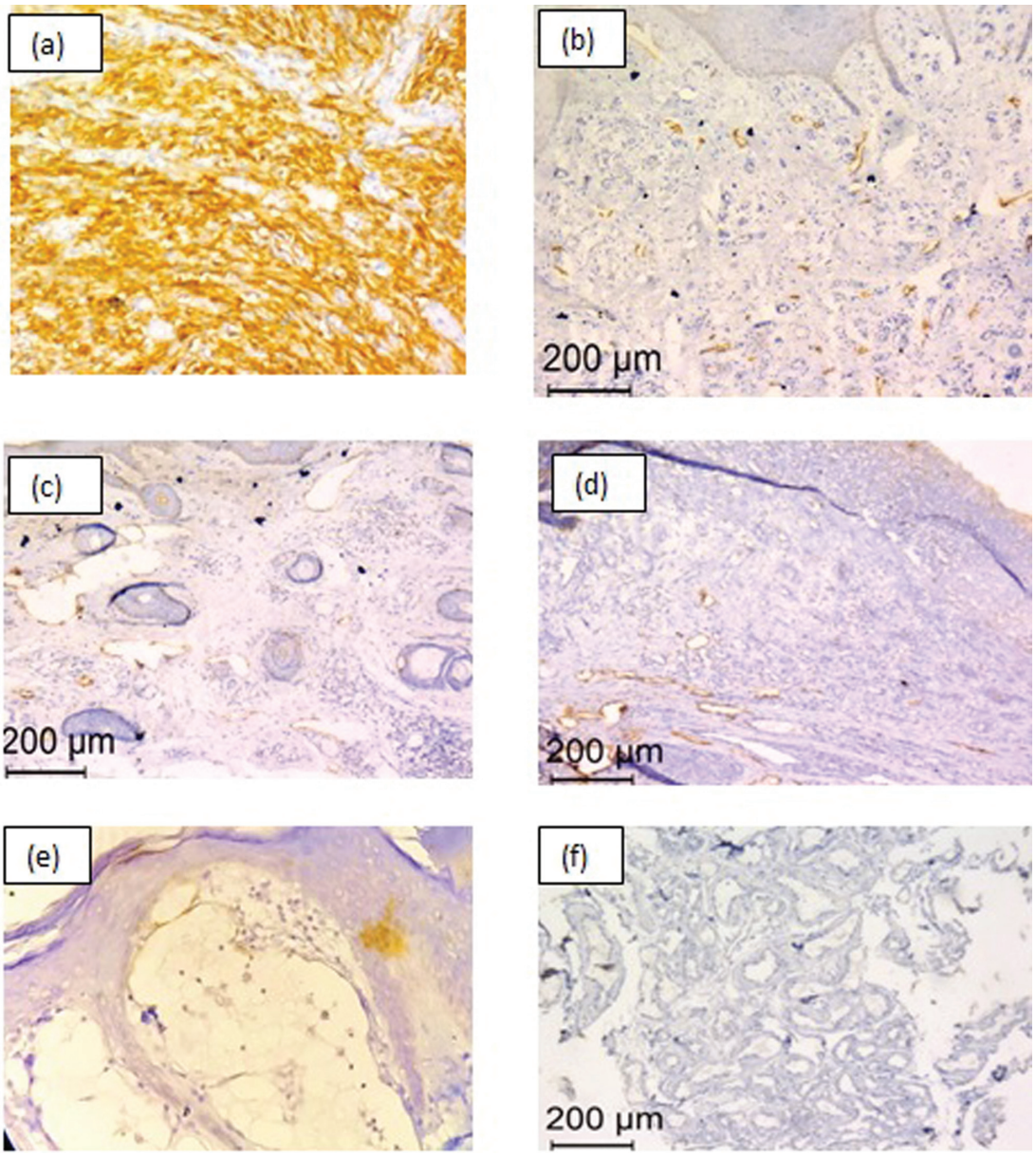
KS has a variety of mimics both clinically and histologically, thus posing a diagnostic challenge [6,7]. Regardless the clinical forms, all KS forms have characteristic histopathology that varies according to the stage of disease. KS shows evidence of angiogenesis, inflammation, and spindle cell proliferation. As KS progresses from patch to plaque, then further more to tumor stage, the density of spindle cell gradually increases from just few cells between collagen bundles to fascicle formation [23–25]. The origin of the spindle cells in KS is still debated, as these cells express markers of both blood and lymphatic vessel endothelium. HHV8-LNA-1 is specifically expressed in KS cells and not in nontumor endothelial cells. HHV8 is believed to induce transformation of endothelial cells so that they acquire spindle cell morphology. There is a concept that this disease originates from a cell type capable of undergoing lymphatic differentiation [26–28]. On the contrary, D2-40 is a selective monoclonal antibody to lymphatic endothelium. D2-40 can be used immediately on formalin-fixed paraffin-embedded tissue sections without the requirement of epitope retrieval, and the staining is very stable. It is a very sensitive and specific marker of lymphatic endothelium in normal tissues [16,17].

In the present study, almost all patients with KS presented with multiple lesions. Lesions were located on the lower limbs in more than half of the cases. This is in parallel to several previous studies which reported that most KS cases presented with multiple lesions, with the lower limbs as the most common site [29–32]. According to the present study, the main cutaneous vascular lesions that mimicked KS were acroangiodermatitis lesions. Other studies reported that several lesions can mimic KS [7,33–35]. Acroangiodermatitis is one of the lesions that need to be differentiated from KS as both have also common site of occurrence, namely, the lower limbs.

In the present study, all KS cases, regardless the histological stage, stained positive for HHV8-LNA-1, showing focal nuclear staining with granular pattern, whereas none of other vascular lesions showed positive staining for HHV8-LNA-1. This finding is consistent with other studies [11,13,36–38]. Several previous studies reported positive stain in spindle cells and cells that lined the slit-like or ectatic thin-walled vascular spaces, whereas endothelial cells surrounding normal blood vessels were negative. Several studies confirmed the importance of HHV8

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Figure 1



D2-40 staining of different vascular proliferations. (a) Kaposi sarcoma case showed diffuse staining in both endothelial lining and spindle cells (D2-40 stain, original magnification ×400). (b–e) Expression of D2-40 was found only in the endothelial lining the lymphatic channels of acroangiodermatitis, ALHE, pyogenic granuloma, and lymphangioma, respectively (D2-40 stain, original magnification ×400). (f) Cirsoid aneurysm showed negative staining (D2-40 stain, original magnification ×400). ALHE, angiolymphoid hyperplasia with eosinophilia.

in distinguishing KS from other cutaneous vascular lesions [13,24,28,37,39]. However, unfortunately, it is not always available in many laboratories, and according to the present study, the stain is frequently focal and can be difficult to interpret.

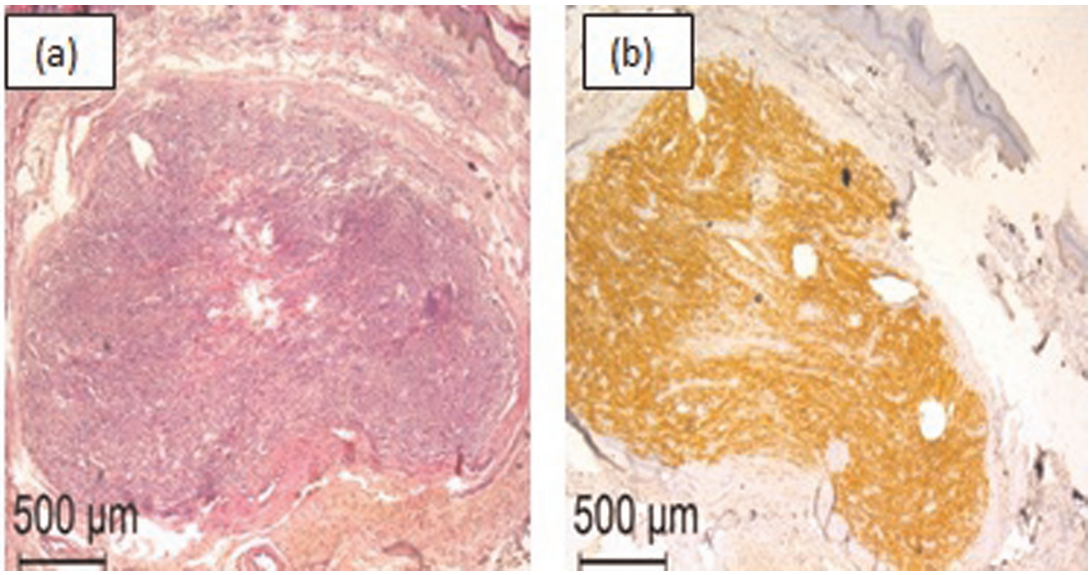
In the present study, immunostaining using D2-40 showed positive staining in almost two-thirds of cases, including both KS and non-KS vascular proliferations. D2-40 staining was expressed in both endothelial lining and spindle cells in all KS cases. Non-KS cases expressed D2-40 in endothelial lining. Some of these lesions are lymphatic in origin like lymphangiomas, whereas others might have reactive lymphatic proliferation or dilatation, such as angiolymphoid hyperplasia with eosinophilia (ALHE), pyogenic granuloma, and acroangiodermatitis. On the contrary, it was also found that vascular proliferation formed of clearly identifiable arteries, and veins were negative for D2-40, including cases of cirsoid aneurysm, infantile hemangioma, verrucous hemangioma, glomangioma angiokeratoma, and angiolipoma.

Apart from normal tissues, several studies found D2-40-positive reactions in vascular proliferations with lymphatic endothelial origin other than KS, whereas

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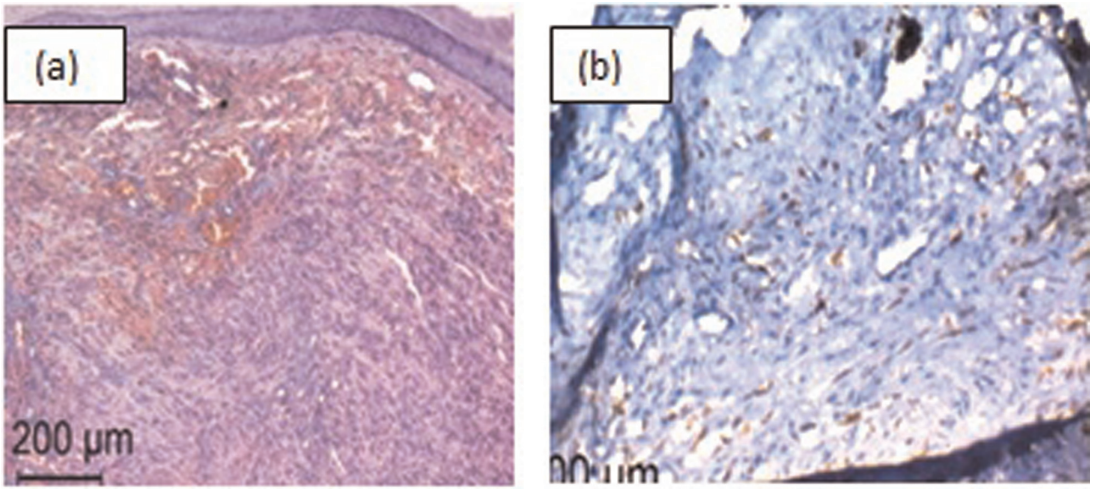
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Figure 2



(a) Intravascular KS (hematoxylin–eosin, original magnification ×100). (b) D2-40 stain shows diffuse positive staining of the endothelial lined spaces and spindle cells (D2-40 stain, original magnification ×100). KS, Kaposi sarcoma.

Figure 3



(a) Nodular stage KS (hematoxylin–eosin, original magnification ×400). (b) Spindle cells show focal nuclear granular positivity for HHV8 (HHV8-LNA-1 stain, original magnification ×400). HHV8, human herpes virus 8; KS, Kaposi sarcoma; LNA-1, latent nuclear antigen 1.

Table 5 Sensitivity and specificity of D2-40

	HHV8 [*n* (%)]						
	Non- KAPOSI (*N*=31)	KAPOSI (*N*=19)	Sensitivity	Specificity	PPV	NPV	Accuracy
D2-40 (endothelial lining)							
Negative	15 (48.4)	19 (100.0)	0.0	48.4	0.0	44.1	30.0
Positive	16 (51.6)	0					
D2-40 (endothelial lining and spindle cells)							
Negative	31 (100.0)	0	100.0	100.0	100.0	100.0	100.0
Positive	0	19 (100.0)					

HHV8, human herpes virus 8; NPV, negative predictive value; PPV, positive predictive value.

negative staining in vascular proliferations of pure blood vessels origin [18,40,41] Previous studies showed variable results of D2-40 staining in non-KS vascular proliferations, including pyogenic granuloma, acroangiodermatitis, and angiolymphoid hyperplasia with eosinophilia. The common finding is that

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non-KS lesions that showed positive staining were restricted to the endothelial lining of the lymphatic spaces without positively stained spindle-shaped cells [16,18,36,40,41].

D2-40 stained all cases of cutaneous KS at different stages of progression, including patch, plaque, and nodular stages. Results of the present study were in accordance with previous studies, where almost all cases of KS of regardless the stage stained positive for D2-40 [16,18,41–43]. It has been previously reported that there is a significant relationship between the intensity not the extent of D2-40 stain and different stages of KS [41]. This is expected from the fact that as KS progresses to the tumor stage, more spindle cell proliferation is seen and so intensity of stain is expected to increase. D2-40 positivity in both endothelial cells lining the irregular vascular spaces and spindle cells in all KS lesions supporting the lymphatic origin or differentiation in KS had been previously reported in other studies [42,44].

According to the results of the present study, using D2-40 positivity against both endothelial lining and spindle cells was found to be highly sensitive and specific to differentiate KS from non-KS vascular lesions. In all non-KS that showed positive reaction to D2-40, the stain was observed in thin-walled lymphatic endothelium. This finding is specifically useful to differentiate KS for acroangiodermatitis. Acroangiodermatitis may have similar clinical and histological features as KS [7,33,45]. Both conditions commonly occur on lower limbs, and both are characterized by vascular proliferation, extravasation of red blood cells, and hemosiderin deposits. Acroangiodermatitis usually shows dermal fibrosis with proliferation of fibroblasts that look spindled, which might be confused with spindle cell proliferation of KS. Requena and Sangueza [46] reported that histopathological differential diagnosis between acroangiodermatitis and patch stage KS could be done by H&E, keeping in mind that acroangiodermatitis is characterized by lobular arrangement of thick-walled blood vessels in the upper dermis. However, in the current study, it was found that despite the histological differences, the distinction between KS and acroangiodermatitis is often challenging, and immunostaining is needed to confirm diagnosis. D2-40 can clearly help to differentiate, as spindle cells in KS, which are transformed endothelial cells under effect of HHV8, stained positive with D2-40. On the contrary, spindle cells in acroangiodermatitis are fibroblast, so did not take up the D2-40 stain. So, whenever HHV8 stain is unavailable, D2-40 can offer an equal sensitive and specific stain to differ KS from acroangiodermatitis.

Therefore, it is important during interpretation of D2-40 staining in vascular lesions to comment on pattern of staining and not as positive or negative staining, as all vascular proliferation of lymphatic lineage will take D2-40 stain but will be confined to the lining of lymphatic vessels, whereas only in KS in addition to endothelial lining, the spindle-shaped cells also stained positive.

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Conflicts of interest
There is no conflicts of interest.

References

1 Goh SGN, Calonje E. Cutaneous vascular tumours: an update. Histopathology 2008; 52:661–673.

2 Calonje E, Brenn T, Lazar A, Billings SD. McKee's pathology of the skin: with clinical correlations. 5th ed., 2020; 1822–1826.

3 Wassef M, Blei F, Adams D, Alomari A, Baselga E, Berenstein A, *et al.* Vascular anomalies classification: recommendations from the international society for the study of vascular anomalies. Pediatrics 2015; 136: e203–e214.

4 Fisher C. Immunohistochemistry in diagnosis of soft tissue tumours. Histopathology 2011; 58:1001–1012.

5 Compton LA, Murphy GF, Lian CG. Diagnostic immunohistochemistry in cutaneous neoplasia: an update. Dermatopathology 2015; 2:15–42.

6 McClain CM, Haws AL, Galfione SK, Rapini RP, Hafeez Diwan A. Pyogenic granuloma-like Kaposi's sarcoma. J Cutan Pathol 2016; 43:549–551.

7 Marušić Z, Billings SD. Histopathology of spindle cell vascular tumors. Surg Pathol Clin 2017; 10:345–366.

8 Edelman DC. Human herpesvirus 8-a novel human pathogen. Virol J 2005; 2:1–32.

9 Akula SM, Pramod NP, Wang FZ, Chandran B. Integrin α3β1 (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. Cell 2002; 108:407–419.

10 Said J. Kaposi's Sarcoma-Associated Herpesvirus (KSHV): a new viral pathogen associated with Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. West J Med 1997; 167:37–38.

11 Patel RM, Goldblum JR, Hsi ED. Immunohistochemical detection of human herpes virus-8 latent nuclear antigen-1 is useful in the diagnosis of Kaposi sarcoma. Mod Pathol 2004; 17:456–460.

12 Pantanowitz L, Otis CN, Dezube BJ. Immunohistochemistry in Kaposi's sarcoma. Clin Exp Dermatol 2010; 35:68–72.

13 Cheuk W, Wong KOY, Wong CSC, Dinkel JE, Ben-Dor D, Chan JKC. Immunostaining for human herpesvirus 8 latent nuclear antigen-1 helps distinguish kaposi sarcoma from its mimickers. Am J Clin Pathol 2004; 121:335–342.

14 Pyakurel P, Pak F, Mwakigonja AR, Kaaya E, Heiden T, Biberfeld P. Lymphatic and vascular origin of Kaposi's sarcoma spindle cells during tumor development. Int J Cancer 2006; 119:1262–1267.

15 Russell Jones R, Orchard G, Zelger B, Wilson Jones E. Immunostaining for CD31 and CD34 in Kaposi sarcoma. J Clin Pathol 1995; 48:1011–1016.

16 Fukunaga M. Expression of D2-40 in lymphatic endothelium of normal tissues and in vascular tumours. Histopathology 2005; 46:396–402.

17 Kong L-L, Yang N-Z, Shi L-H, Zhao G-H, Zhou W, Ding Q, *et al.* The optimum marker for the detection of lymphatic vessels. Mol Clin Oncol 2017; 7:515–520.

18 Kahn HJ, Bailey D, Marks A. Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcomas. Mod Pathol 2002; 15:434–440.

19 Wildgruber M, Sadick M, Müller-Wille R, Wohlgemuth WA. Vascular tumors in infants and adolescents. Insights Imaging 2019; 10:6–8.

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20 Ordonez NGMD. Immunohistochemical endothelial markers: a review. Adv Anat Pathol 2012; 19:281–295.

21 Naeem N, Mushtaq S, Akhter N, Hussain M, Hassan U. Effectiveness of vascular markers (immunohistochemical stains) in soft tissue sarcomas. J Coll Phys Surg Pak 2018; 28:352–356.

22 Ordóñez NG. Podoplanin: a novel diagnostic immunohistochemical marker. Adv Anat Pathol 2006; 13:83–88.

23 Radu O, Pantanowitz L. Kaposi sarcoma pathology. Arch Pathol Lab Med 2013; 137:289–294.

24 Buonaguro FM, Tornesello ML, Buonaguro L, Satriano RA, Ruocco E, Castello G, *et al.* Kaposi's sarcoma: aetiopathogenesis, histology and clinical features. J Eur Acad Dermatol Venereol 2003; 17:138–154.

25 Grayson W, Pantanowitz L. Histological variants of cutaneous Kaposi sarcoma. Diagn Pathol 2008; 3:1–11.

26 Gramolelli S, Schulz TF. The role of Kaposi sarcoma-associated herpesvirus in the pathogenesis of Kaposi sarcoma. J Pathol 2015; 235:368–380.

27 Masood R, Cesarman E, Smith DL, Gill PS, Flore O. Human herpesvirus-8-transformed endothelial cells have functionally activated vascular endothelial growth factor/vascular endothelial growth factor receptor. Am J Pathol 2002; 160:23–29.

28 Katano H, Sato Y, Kurata T, Mori S, Sata T. High expression of HHV-8-encoded ORF73 protein in spindle-shaped cells of Kaposi's sarcoma. Am J Pathol 1999; 155:47–52.

29 Mohanna S, Maco V, Bravo F, Gotuzzo E. Epidemiology and clinical characteristics of classic Kaposi's sarcoma, seroprevalence, and variants of human herpesvirus 8 in South America: a critical review of an old disease. Int J Infect Dis 2005; 9:239–250.

30 Polat AK, Karaali MG, Aksu AEK, Leblebici C, Gürel MS. Evaluation of clinical course, histopathological and treatment characteristics of patients with Kaposi's sarcoma. Turk Arch Dermatol Venereol 2018; 52:131–136.

31 Vangipuram R, Tyring SK. Epidemiology of Kaposi sarcoma: review and description of the nonepidemic variant. Int J Dermatol 2019; 58:538–542.

32 Stratigos JD, Potouridou I, Katoulis AC, Hatziolou E, Christofidou E, Stratigos A, *et al.* Classic Kaposi' s sarcoma in Greece: a clinico-epidemiological profile. Int J Dermatol 1997; 36:735–740.

33 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; 134:44–46.

34 Urquhart JL, Uzieblo A, Kohler S. Detection of HHV-8 in pyogenic granuloma-like kaposi sarcoma. Am J Dermatopathol 2006; 28:317–321.

35 Forrestel AK, Naujokas A, Martin JN, Maurer TA, McCalmont TH, Laker-Opwonya MO, *et al.* Bacillary angiomatosis masquerading as Kaposi's sarcoma in East Africa. J Int Assoc Provid AIDS Care. 2015; 14:21–25.

36 Bun K, Hye L, Lee S, Eun H, So L, Park Y, *et al.* Immunohistochemical characteristics of Kaposi sarcoma and its mimicries. Korean J Pathol 2006; 40:361–367.

37 Robin YM, Guillou L, Michels JJ, Coindre JM. Human herpesvirus 8 immunostaining: a sensitive and specific method for diagnosing Kaposi sarcoma in paraffin-embedded sections. Am J Clin Pathol 2004; 121:330–334.

38 Hong A, Davies S, Lee CS. Immunohistochemical detection of the human herpes virus 8 (HHV8) latent nuclear antigen-1 in Kaposi's sarcoma. Pathology 2003; 35:448–450.

39 Dupin N, Fisher C, Kellam P, Ariad S, Tulliez M, Franck N, *et al.* Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci U S A 1999; 96:4546–4551.

40 Miteva M, Galimberti ML, Ricotti C, Breza T, Kirsner R, Romanelli P. D2-40 highlights lymphatic vessel proliferation of angiolymphoid hyperplasia with eosinophilia. J Cutan Pathol 2009; 36:1316–1322.

41 Kandemir NO, Barut F, Gun BD, Keser SH, Karadayi N, Gun M, *et al.* Lymphatic differentiation in classic Kaposi's sarcoma: patterns of D2-40 immunoexpression in the course of tumor progression. Pathol Oncol Res 2011; 17:843–851.

42 Dubina M, Goldenberg G. Positive staining of tumor-stage Kaposi sarcoma with lymphatic marker D2-40. J Am Acad Dermatol 2009; 61:276–280.

43 Rosado FG, Itani DM, Coffin CM, Cates JM. Utility of immunohistochemical staining with FLI1, D2-40, CD31, and CD34 in the diagnosis of acquired immunodeficiency syndrome-related and non-acquired immunodeficiency syndrome-related Kaposi sarcoma. Arch Pathol Lab Med 2012; 136:301–304.

44 Cancian L, Hansen A, Boshoff C. Cellular origin of Kaposi's sarcoma and Kaposi's sarcoma-associated herpesvirus-induced cell reprogramming. Trends Cell Biol 2013; 23:421–432.

45 Chor PJ, Santa Cruz DJ. Kaposi's sarcoma. A clinicopathologic review and differential diagnosis. J Cutan Pathol 1992; 19:6–20.

46 Requena L, Sangueza OP. Cutaneous vascular proliferation. Part II. Hyperplasias and benign neoplasms. J Am Acad Dermatol 1997; 37:887–922.