**Human Herpesvirus 8 Immunostaining**

**A Sensitive and Specific Method for Diagnosing Kaposi Sarcoma in Paraffin-Embedded Sections**

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**Key Words:** Human herpesvirus 8; Immunohistochemistry; Kaposi sarcoma; Angiosarcoma; LNA-1; Latent nuclear antigen

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**Abstract**

Human herpesvirus 8 (HHV-8) is recognized as a major causal agent of Kaposi sarcoma (KS), and it has been detected in all epidemiologic variants of KS. Until now, detection of HHV-8 in paraffin-embedded sections was done mostly by using reverse transcriptase–polymerase chain reaction.

To assess the sensitivity and specificity of an anti–HHV-8 antibody and its potential usefulness for separating KS from its mimickers, we immunostained 72 KS samples and 108 samples of potential mimickers of KS with the monoclonal antibody latent nuclear antigen-1 (LNA-1; Advanced Biotechnologies, Columbia, MD). Cases of KS included all epidemiologic variants of the disease. Non-KS lesions included 34 angiosarcomas, 4 kaposiform hemangioendotheliomas, and 70 various benign vascular lesions. Immunostaining for CD31, CD34, and/or von Willebrand factor (factor VIII) also were performed in all cases.

All but 1 case of KS (sensitivity, 99%) and none of the non-KS lesions (specificity, 100%) stained with the LNA-1 anti–HHV-8 antibody. The LNA-1 anti–HHV-8 antibody is a reliable marker of all variants of KS. Because KS mimickers are consistently negative for this marker, its use for diagnostic purposes is recommended.

Kaposi sarcoma (KS) is a vascular, often multicentric lesion characterized by 4 epidemiologic manifestations: classic, iatrogenic, endemic (ie, African), and associated with AIDS.1 The classic form of KS initially was described by Moriz Kaposi as a cutaneous sarcoma of elderly people (most patients are men and older than 50 years).2 Classic KS is observed chiefly in the Mediterranean region, Eastern Europe, and the Middle East. The iatrogenic variant is observed characteristically in a context of organ transplantation, long-term cytotoxic drug administration, and the use of corticoids or in autoimmune diseases. It generally occurs 24 months after the onset of therapy.2 In endemic (African) KS, male predominance is also a characteristic feature, but patients are usually younger (younger than 40 years). An often fatal, lymphadenopathic form occurring in children has been described in this KS form.2 Among the KS cases associated with acquired immunodeficiency states, AIDS-associated KS is the most frequent. Among HIV-infected patients, homosexuals and bisexuals are high-risk groups.3 KS is the main proliferating lesion in patients with AIDS, of whom 15% to 20% develop it.4 The clinical course and histopathologic manifestations of these 4 epidemiologic forms are roughly similar. Tumor sites include skin, mucosa (oropharynx and digestive tract), lymph nodes, and visceral organs.

In 1994, Chang et al4 identified 2 unique herpesvirus-like DNA sequences in AIDS-associated KS, sequences corresponding to human herpesvirus 8 (HHV-8), a virus of the γ subtype of the herpesvirus family. This virus is akin to herpesvirus saimiri, a virus that is responsible for lymphoproliferative lesions in new-world primates.5 HHV-8 DNA has been detected in virtually all epidemiologic forms of KS but not in the adjacent uninvolved skin of patients with KS or in...
unaffected subjects.Interestingly, whereas about 5% of the population worldwide probably is infected with HHV-8, the incidence of KS is less than 1/100,000. The presence of HHV-8 is now considered to be a major initiating event for the development of KS. It is observed in the spindle cells of KS and in endothelial cells and also has been found in tumor leukocytes by some but not all authors.

Until now, HHV-8 has been detected in KS by using polymerase chain reaction (PCR) and only seldom by using immunochemical analysis applied to paraffin-embedded tumor tissue samples.

By using PCR, HHV-8 has been detected in 95% to 100% of KS cases. This finding is important because it permits a firm diagnosis of KS in cases of exiguous or otherwise inadequate histologic material. Conflicting results have been reported in the literature about the presence of HHV-8 in angiosarcoma. By using PCR, some authors detected viral DNA in some angiosarcomas, whereas others did not. In addition to KS, HHV-8 viral DNA also has been detected in a subset of myofibroblastic inflammatory tumors, primary effusion lymphomas, and in multicentric Castleman disease. The role of HHV-8 in the histogenesis of multiple myeloma is questionable. Evidence of HHV-8 DNA also has been detected in a few non-Hodgkin lymphomas and in 1 Hodgkin lymphoma.

Materials and Methods

We retrieved 72 cases of KS and 108 of non-KS vascular lesions from the files of the Oscar Lambret Cancer Center, Lille, France; the University Institute of Pathology, Lausanne, Switzerland; the François Baclesse Cancer Center, Caen, France; and the Bergonié Institute, Bordeaux, France. We then immunostained the samples for HHV-8.

The diagnosis of KS was based on epidemiologic data, morphologic features, and reactivity for endothelial cell markers (CD34, CD31, and/or von Willebrand factor). All cases of KS for which epidemiologic data were not defined clearly were discarded from the series. Early-stage cases of cutaneous KS were diagnosed in the context of an HIV infection or in HIV-negative, elderly Mediterranean patients. These patients had multiple synchronous lesions or a history of excised KS. Distribution of KS according to epidemiologic manifestations is shown in Table 1. Non-KS lesions included 34 angiosarcomas with a spindle cell component, 4 kaposiform hemangioendotheliomas, and 70 benign vascular lesions. Fifty KS samples were fixed in 4% buffered formalin, 14 in conventional aqueous Bouin fluid, and 8 in Holland Bouin fluid. Histologic diagnoses were based on the examination of H&E-stained slides.

Additional paraffin-embedded sections of all 180 cases were evaluated immunohistochemically according to the streptavidin-biotin-peroxidase method of Hsu et al (LSAB kit, DAKO, Glostrup, Denmark). The following antibodies were used: CD34 (monoclonal, clone Qbend 10, dilution 1:200, 30-minute incubation, Dakopatts, Glostrup, Denmark), CD31 (monoclonal, clone 1C/70A, dilution 1:320, 30-minute incubation, Dakopatts), von Willebrand factor (factor VIII; polyclonal, dilution 1:800, 30-minute incubation, Dakopatts), von Willebrand factor (factor VIII; polyclonal, dilution 1:800, 30-minute incubation, Immunotech, Marseille, France), and HHV-8 (monoclonal antibody to latent nuclear antigen [LNA-1] open reading frame 73, dilution 1:1,000, 60-minute incubation, Advanced Biotechnologies, Columbia, MD).

Tissue sections were submitted to microwave oven heating (20 minutes in EDTA buffer [pH 8]). Then, the sections were immunostained using the LSAB kit (DAKO) in an automated immunostainer (TechMate Horizon, DAKO). All steps were performed at room temperature, and diaminobenzidine was used as a chromogen. Appropriate positive and negative control samples were used throughout.

Table 1
Kaposi Sarcoma (KS) Breakdown According to Manifestations

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Classic KS (n = 45)</th>
<th>AIDS-Associated KS (n = 16)</th>
<th>Endemic (African) KS (n = 8)</th>
<th>Iatrogenic KS (n = 3)</th>
<th>Total (N = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>37</td>
<td>16</td>
<td>8</td>
<td>2</td>
<td>63</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Median age (range), y</td>
<td>72 (52-92)</td>
<td>41 (31-62)</td>
<td>32 (17-47)</td>
<td>64 (49-80)</td>
<td>60 (31-92)</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower extremities (skin)</td>
<td>29 (1 patch-stage KS)</td>
<td>3 (2 patch-stage KS)</td>
<td>5</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>Upper extremities (skin)</td>
<td>10 (1 patch-stage KS)</td>
<td>3 (1 patch-stage KS)</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Head and neck</td>
<td>3</td>
<td>5 (one patch stage KS)</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Genital</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Lymph node (cervical/inguinal)</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mediastinum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Retroperitoneum</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Skin (multiple lesions)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Skin, laryngeal mucosa, tonsils.
† Scrotum, glans penis, collum glandis.
For anti–HHV-8 staining, the positive control sample consisted of a lymph node involved by Castleman disease. Labeling for HHV-8 was considered positive if the staining was observed exclusively in the tumor cell nuclei (including spindle cells and endothelial cells), had a granular appearance, and had no concomitant cytoplasmic staining. It was considered negative when no staining was observed in the tumor cell nuclei, as opposed to the positive control samples.

**Results**

Relevant data on patient and tumor characteristics are given in Table 1. Most tumors involved the skin. KS samples included surgical specimens or skin excisions (n = 63), surgical biopsy specimens (n = 2), and needle biopsy specimens (n = 7).

All but 1 case of KS showed positive nuclear staining. For every case, the staining was granular and was observed in spindle cells for nodular KS Image 1 and in endothelial cells for patch-stage Image 2, plaque-stage, and lymphangioma-like variants of KS. Staining intensity varied from one case to another. Staining was diffuse in 54 cases (75%) and focal in 18 cases (25%). In early-stage KS, the staining was very focal with generally fewer than 30% labeled endothelial cells. There were no specific variations in staining according to the epidemiologic forms of KS. The case that was negative for HHV-8 was a case of classic KS occurring in a 60-year-old man who had multiple cutaneous lesions on both legs with a typical histologic picture.

All 108 non-KS vascular lesions, including spindle cell angiosarcomas, were negative for HHV-8.

**Discussion**

In addition to the limited number of series, much disparity is observed in the literature regarding HHV-8 immunoposexpression in vascular lesions and, more specifically, in angiosarcomas and KS.11,13-15 By using the recently developed LNA-1 anti–HHV-8–associated viral antigen in daily practice

<p>| Table 21 |</p>
<table>
<thead>
<tr>
<th>Non–Kaposi Sarcoma Lesions Examined for Human Herpesvirus 8</th>
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<tbody>
<tr>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>Angiosarcoma</td>
</tr>
<tr>
<td>Soft tissues of extremities</td>
</tr>
<tr>
<td>Breast</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Skin</td>
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<tr>
<td>Spleen</td>
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<tr>
<td>Head and neck</td>
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<tr>
<td>Kaposiform hemangioendothelioma</td>
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<tr>
<td>Benign vascular lesions</td>
</tr>
<tr>
<td>Pyogenic granuloma</td>
</tr>
<tr>
<td>Cutaneous capillary hemangioma</td>
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<tr>
<td>Juvenile hemangioma</td>
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<tr>
<td>Nasal hemangioma (capillary lobular)</td>
</tr>
<tr>
<td>Liver hemangioma</td>
</tr>
<tr>
<td>Intramuscular hemangioma</td>
</tr>
<tr>
<td>Microvenular hemangioma</td>
</tr>
<tr>
<td>Hobnail hemangioma</td>
</tr>
<tr>
<td>Spindle cell hemangioma</td>
</tr>
<tr>
<td>Cellular angiolipoma</td>
</tr>
<tr>
<td>Cystic lymphangioma</td>
</tr>
<tr>
<td>Benign lymphangioendothelioma</td>
</tr>
<tr>
<td>Postradiation benign lymphangiomatous papules</td>
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<tr>
<td>Myopericytoma</td>
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</tbody>
</table>
for almost 2 years, we gradually have become convinced that
this antibody is a sensitive and specific marker for KS and that
it might represent an important addition to the immunologic
armamentarium of pathologists. To confirm our first impres-
sions, we decided to examine a large series of paraffin-
embedded vascular neoplasms for HHV-8 immunoexpression.
Our results showing 99% sensitivity and 100% specificity
strongly support our first impressions and are in keeping with
data reported in the literature.11,13-15 Until now, the detection
of HHV-8 in cell cultures or tumor cells was performed
mainly by using reverse transcriptase (RT)-PCR.6,10,11
Immunohistochemical analysis seldom was used for this
purpose,8,12,13 although it is clearly quicker, easier, more prac-
tical, and less expensive to perform than RT-PCR. Of 72 cases
of KS, 71 were positive for HHV-8 in our series. Only 1 case
was negative, certainly owing to technical problems related to
fixative conditions that were uncontrolled in the study.

Because we and others8,13 obtained similar results by
using an LNA-1 anti–HHV-8 antibody, it seems that the use
of this type of antibody could be recommended for
confirming the diagnosis of KS and, more important, to rule
out KS mimickers such as kaposiform hemangioendothe-
lioma, spindle cell hemangiomia, spindle cell angiosarcoma,
and benign lymphangioendothelioma (progressive lymphang-
gioma). Although nodular KS in the skin usually is a
straightforward diagnosis, recognizing KS in unusual or
unexpected locations (eg, mediastinum, retroperitoneum,
gastrointestinal tract) might be difficult. In this situation,
positive staining for HHV-8 is of great help.

In our series, the diagnosis of KS was based on clinical,
histologic, and immunohistochemical data and not on the
intracellular presence of HHV-8 DNA sequences. Some
might consider this a limitation of the study, but it also is
important to stress that RT-PCR, in addition to being
complex, is a very sensitive method that occasionally might
give false-positive results or erroneous interpretations.
Indeed, the presence of HHV-8 PCR products in a lesion is
by no means synonymous with KS; circulating infected cells
or contamination11 also are potential sources of positive
results. In fact, they might represent possible explanations
for the reported positive detection of HHV-8 DNA sequences
in 1 venous hemangioma25 and in angiosarcomas,14 the latter
finding not being confirmed by others.11 Thus, RT-PCR
should never be performed alone but always in combination
with adequate microscopic and immunohistochemical exami-
nation. Performing anti–HHV-8 staining is very important in
this context because it permits clear (morphologic) identifi-
cation of the cell types (tumor cells vs circulating lympho-
cytes) that are positive for this marker.

HHV-8 immunostaining is a sensitive (99%) and
specific (100%) method for diagnosing KS in paraffin-
embedded sections. It is particularly useful for identifying
unusual clinical or histologic manifestations of KS and for
separating KS from its mimickers (eg, kaposiform heman-
gioendothelioma, spindle cell hemangiomia, benign
lymphangioendothelioma).

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References


