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Detection of KSHV in Transbronchial Biopsies in Patients With Kaposi Sarcoma

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Abstract

In transbronchial biopsies, Kaposi sarcoma (KS) is difficult to correctly diagnose by H&E staining due to the inherent vascular nature of the lung tissue, coupled with the subtle nature of the changes in early KS. Since KS-associated herpesvirus (KSHV) has been found in all clinical forms of KS, the detection of KSHV genomic DNA sequences and/or viral products may be helpful in the diagnosis of pulmonary KS. From their files during the past 10 years, the authors identified ten HIV/AIDS patients who were positive for KS in transbronchial biopsies and four HIV/AIDS patients with KS-negative transbronchial biopsies. Immunohistochemistry with antibodies against the latency-associated nuclear antigen (LANA-1 or LNA) of KSHV was performed. Nested polymerase chain reactions (PCR) with KSHV ORF-K1 or -K9 were performed in all cases, and the KSHV sequences were detected in 9/10 (90%) KS cases. Immunohistochemical analysis was able to detect 4/10 (40%) cases. One case was negative by both PCR and immunohistochemistry. Of the five KS cases that were not diagnosed definitively ("consistent with" or "suspicious for"), two were confirmed by both immunoreactivity and PCR. One of the negative control cases was positive for KSHV by PCR but not by immunohistochemistry. The patient was thereafter found to have a clinical history of pulmonary KS at another hospital. In conclusion, in transbronchial biopsies of the lung suspicious for KS, PCR is the most sensitive technique available for clinical diagnosis of KS. Immunohistochemistry analysis might be helpful in difficult pulmonary KS cases.

Keywords

transbronchial biopsy; Kaposi sarcoma-associated herpesvirus; immunohistochemistry; polymerase chain reaction; Kaposi sarcoma

With the arrival of the era of acquired immunodeficiency syndrome (AIDS) and solid organ transplantation, some rare tumors that were seldom seen in Western countries have emerged as a fatal threat to these patients, including Kaposi sarcoma (KS). Skin and lymph nodes are the most common presenting sites for KS; much less often, it presents in visceral organs. It is generally believed that patients without mucocutaneous involvement infrequently have pulmonary KS.¹ Although rarely occurring in the thorax, KS has accounted for approximately one third of respiratory episodes in patients with AIDS and KS who have required evaluation for pulmonary complaints.² The outcome of pulmonary KS is invariably fatal, with a median survival of only a few months^{3–6} if not diagnosed and treated in its early stage. However, early diagnosis can be problematic by clinical examination, radiographic tests, and even bronchoscopy. The difficulty in diagnosing pulmonary involvement in these patients is largely due to nonspecific clinical and radiologic presentations and coexistent opportunistic infections and subtle histologic findings. The vascular proliferation is usually low-grade and may closely resemble the adjacent compressed lung parenchyma. Coexistent infections with reactive changes may complicate the histologic diagnosis.

In the past 10 years, circumstantial epidemiologic evidence has suggested that KS lesions are infected by Kaposi sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus 8) using polymerase chain reaction (PCR)-based studies. However, the application of research data to the practice of anatomic pathology has been limited. Nuovo and Nuovo attempted to use reverse transcription in situ PCR (RT-PCR) to differentiate KS from its mimics, such as atypical vascular proliferation and hemosiderotic/angiomatoid/aneurysmal dermatofibromas, in AIDS patients.⁷ They concluded that RT-PCR can help in the differential diagnosis. Two separate studies showed that an immunohistochemistry method using an antibody to ORF73 product has high sensitivity and specificity in differentiating KS from its mimics and other vascular and nonvascular spindle cell lesions.^{8,9} Tamm et al have demonstrated that AIDS patients with pulmonary KS have KSHV DNA in bronchoalveolar lavage fluid using highly sensitive and specific PCR analysis, indicating that the assay may aid in pulmonary KS identification.¹⁰ No other studies using transbronchial biopsies have thus far been reported. In the present study we compare immunohistochemical examination for latency-associated nuclear antigen (LANA-1 or LNA) encoded by ORF73 with KSHV sequence-specific PCR results in transbronchial biopsies for KS.

Materials and Methods

Case Selection

A total of 14 HIV/AIDS patients who underwent transbronchial biopsy in our hospital during the past 10 years were reviewed (Table 1). All were men, with ages ranging between 28 and 49 years. Of the 14, 10 had diagnoses of “KS,” “consistent with KS,” or “suspicious for KS” in transbronchial biopsies. Some were known to have KS in other anatomic sites. The remaining four patients were known to have KS of skin and had negative transbronchial biopsies (control group). Some of the 14 patients had radiographic or computed tomography data available with nonspecific findings, not diagnostic for pulmonary KS. All cases were reviewed by three pathologists.

Immunohistochemistry

The detection of viral LANA-1 protein was performed on sections of formalin-fixed and paraffin-embedded tissue blocks, using antibody against LANA-1 (1:2,000 dilution; ABI, Columbia, MD) and the streptavidin-biotin complex method. All slides were subjected to a heat-based antigen retrieval method (pressure cooker) in 0.1 mol/L citrate pH 6.0 for 5 minutes. The presence of cells with clear and unequivocal nuclear staining was identified as positive. The intensity and proportion of positive cells were assessed in a semiquantitative manner.¹¹ A known KS skin case was used as a positive control, and negative controls of each case were run.

Polymerase Chain Reaction

The paraffin-embedded tissue sections were treated with xylene to remove paraffin and then digested with proteinase K (200 µg/mL) overnight at 56°C. The supernatant from the overnight digestion mixture was heat-inactivated and either used for PCR amplification directly or for DNA isolation by phenol-chloroform extraction and ethanol precipitation. Semi-nested PCR was run to amplify fragments of KSHV ORF-K1 and -K9, products of which are an early lytic transmembrane glycoprotein and viral interferon regulatory factor (vIRF), respectively, as previously described with minor modifications.¹² The amplified gene segments and PCR primers were as follows: K1-1 (585 bp) with primers LGH2089 (5'-GTTCTGCCAGGCATAG TC-3') and KS34 (5'-ATTTGACAGGCGAGA CGACAG-3'); K1-1a (473 bp) with primers KS33 (5'-CGGTTTGCTTTCGAGG ACTATT-3') and KS34; K9-1 (536 bp) with primers K9F1 (5'-TGGACCCAGGCCAAAGAC-3') and K9R1 (5'-TGCCAAT CTCTTGTAACCCCGCG-3'); and K9-1a (369 bp) with primers K9F1 and K9R2 (5'-GTAAGTGGTATACGGAAGCGGGTG CG-3'). PCR amplification was performed in a 25-µL final volume containing 100 ng genomic DNA for 1 cycle at 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute, and 1 cycle at 72°C for 5 minutes. DNA from BC-1 cell line was used as a positive control. Deionized water and DNA from KSHV-negative P3HR-1 cells were used as negative controls for the PCR amplification. The PCR products were analyzed with agarose gel electrophoresis. The presence of either K1 or K9 sequence or both was considered a positive case.

Results

The demographic data of the patients and the results of immunohistochemical study and PCR analysis are summarized in Table 1. Of the 10 patients with a positive diagnosis for pulmonary KS, 4 had previous diagnosis at other anatomic sites, including skin, lymph node, or liver. The remaining six patients did not have KS elsewhere, and four were definitively diagnosed as KS and two were “suspicious” or “consistent with” in the transbronchial biopsies. All four negative cases (control group) had skin KS previously. The key histologic features included spindle cell proliferation, ectatic vascular spaces, nuclear atypia, increased mitosis, and extravasated red blood cells (Figs. 1A, 1B).

Immunohistochemistry with a monoclonal antibody to LANA-1 showed a nuclear staining pattern in the spindle-shaped tumor cells in 4 of 10 KS cases. The two cases with a definitive

diagnosis for KS had strong intensity (3/3) and high proportion (5/5), whereas the two cases that had a nondefinitive diagnosis for KS had less intensity (1/3 or 2/3) and lower proportion (2/5 to 4/5). Nonspecific background staining was not observed (Figs. 1C, 1D). No staining was observed with any of the negative control cases.

PCR analysis detected KSHV sequences in 9/10 (90%) KS cases (3 were K1 positive only, 3 were K9 positive only, and 3 were positive for both K1 and K9 (Fig. 2). The case (case 5) that was PCR negative was also negative immunohistochemically. On review of recut slides, no residual KS was seen in this negative case. One of the negative control cases was positive for both K1 and K9 sequences by PCR but not by immunohistochemistry. Review of this case did not reveal KS by routine H&E staining, but the patient's clinical history was found positive for pulmonary KS subsequently at another hospital; thus, this case may be a false negative on H&E or sampling error.

Discussion

In 1872, Moritz Kaposi, a Hungarian dermatopathologist, described an aggressive “idiopathic multiple pigmented sarcoma” of the skin, later known as Kaposi sarcoma. Since that time, four clinical/epidemiologic forms of KS have been established. The histologic features of all these types are indistinguishable and all advance in the same developmental sequence from early patch to plaque and eventually nodular stage. The KS lesions are composed of a proliferation of miniature vessels surrounding larger ectatic vessels, slit-like endothelium-lined vascular spaces, and spindle-shaped cells accompanied by sparse infiltrate of lymphocytes and plasma cells. Hemosiderin pigment and extravasated red blood cells are frequently present. Intracytoplasmic and extracellular hyaline globules may be identified. The early lesions (patch and plaque stage) sometimes resemble granulation tissue and often raise diagnostic difficulty, particularly in AIDS patients. The advanced nodular lesions are composed predominantly of spindle cells that may be confused with a fibrosarcoma.

Immunohistochemically, the KS spindle cells stain for certain endothelial markers such as CD34+ and factor VIII. These markers can be very useful in certain clinical settings such as skin and soft tissue. However, for organs that are inherently vascular, such as the lungs, the endothelial markers will not be helpful. Under reactive conditions, lymph nodes can undergo nodal angiomatosis and vascular transformation of the subcapsular sinus and therefore can be difficult to differentiate from KS. More specific and sensitive markers are needed for lesions at these sites. Antibodies against KSHV viral proteins have become the center of attention and promising candidates for this purpose.

KSHV was discovered in 1994 and has been shown to be present nearly universally in KS cases at diverse anatomic sites and in all clinical forms. The data obtained so far have been based largely on molecular studies.

Sequences have also been detected in a number of lymphoproliferative disorders, such as primary effusion lymphoma and multicentric Castleman's disease, both of which are overrepresented in AIDS patients. There has also been emerging evidence that KSHV is

present in other tumors such as inflammatory myofibroblastic tumors,^{13,14} other vascular lesions such as angiosarcomas,¹⁵ plasma cell myelomas,¹⁶ and epithelial tumors of the skin in solid organ transplant patients,^{17,18} but data have been controversial. A few research laboratories, by using immunohistochemical analysis, have shown that almost all spindle cells in KS were positive for LANA-1,^{19–21} and LANA-1 is the only antigen that has been consistently demonstrated to be expressed at high level.^{19,22–24}

In the present study, immunoreactivity with a monoclonal antibody against LANA-1 was positive in only 4 of 10 KS cases with varying intensity and proportion (40% sensitive). Two of the four cases were initially diagnosed as “consistent with” and “suspicious for” KS and were confirmed by immunohistochemistry and PCR. The other six cases (two “consistent with KS” cases, one “suspicious” case, and three definitive cases) were all negative for LANA-1. One of these cases was also negative by PCR analysis for KSHV sequences (see below). Overall, of the five KS cases that were not definitively diagnosed, two (40%) were confirmed by immunoreactivity. This implies that antibody against LANA-1 may help in the diagnosis of difficult lung KS cases. Although this is a small study, it appears promising. The negative control cases were all negative immunohistochemically. PCR analysis, on the other hand, detected the KSHV sequences in 9/10 (90%) KS cases. The case that was PCR negative was also negative immunohistochemically. This patient did not have KS lesions in other anatomic sites, and histologically the specimen was characterized by slight spindle cell proliferation and extravasation of red blood cells. On review of recut slides, no residual KS was seen in this negative case. This could represent a case that was falsely positive histopathologically or there was no residual KS in the material submitted for PCR. The fact that one of the negative control cases was positive by PCR indicates possible sampling error, which is not uncommon in transbronchial biopsies for KS lesions.

It has been shown that in patch stage, LANA-1 expression is detected in fewer than 10% of the cells surrounding small ectatic vessels by immunohistochemistry. In plaque and nodular lesions, LANA-1 is detected in up to 50% and 90% of the tumor cells.¹⁹ The histopathologic features of KS involving the lung have been described by several investigators; these features include classic, telangiectatic, and inflammatory forms. Although the distinct stages depicted in the skin lesions are not widely used for lung KS, these features resemble those found in the cutaneous lesions.^{25–28} The staining pattern of lung KS for LANA-1 has not been previously described. The minute nature of transbronchial samples would conceivably make it difficult to perform this type of study. In the current study, the cellularity, an index of the stage in skin lesions, did not seem to correlate with the positivity of immunohistochemistry. However, the two KS cases that were strongly positive immunohistochemically may imply that these two cases were indeed late and nodular lesions, and the other two positive cases and the negative cases may represent early lesions. PCR amplification of KSHV-specific sequences continues to be the most sensitive measure in detecting the presence of the virus and was able to pick up false-negative case by H&E staining and immunohistochemical study. The lower sensitivity of the current immunohistochemical study than that in other studies from soft tissue and skin can be explained as follows: small sample; sampling error; early-stage versus late-stage lesion; and sensitivity of the antibody. We believe that larger studies and better sampling techniques and devices are necessary to address these issues.

In conclusion, the PCR method continues to be the most sensitive diagnostic tool in detecting pulmonary KS, while immunohistochemistry analysis might be helpful in clinching suspicious pulmonary KS cases and in confirming KS lesions that are at late stage.

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Figure 1.

A, Case #2, with a definitive KS diagnosis, shows prominent spindle cell proliferation and extravasated red blood cells. Vascular clefts are also seen. There is minimal nuclear atypia, and mitotic figures are scarce (H&E, original magnification $\times 200$). B, Case #6, with a diagnosis of “consistent with KS.” This lesion is composed of ectatic vascular spaces, extravasated red blood cells, hemosiderin-laden macrophages and minimal cytologic atypia. There is only mild spindle cell proliferation (H&E, original magnification $\times 200$). C, Immunohistochemistry with the monoclonal antibody to LANA-1 shows a diffuse nuclear staining pattern in the spindle cells (case #2, original magnification $\times 200$). D, Immunohistochemistry with the monoclonal antibody to LANA-1 only highlights scattered cells that appear to line vascular clefts with nuclear staining pattern (case #6, original magnification $\times 200$).

Figure 2.

A, Agarose gel electrophoresis detection of amplified KSHV ORF-K1 sequence. MW, molecular weight marker. Lanes 1 through 10 contain amplification products derived from the 10 patients with positive transbronchial biopsy for KS. Lanes 11 through 14 represent patients of the control group. Lanes 2, 3, 4, 7, 8, 10, and 14 were interpreted as positive. Lane 7 showed a faint band that does not project well in the photograph. Lanes 15 and 16 contain positive and negative controls, respectively. B, Agarose gel electrophoresis detection of amplified KSHV ORF-K9 sequence. Lanes 1 through 10 contain amplification products derived from the 10 patients with positive transbronchial biopsy for KS. Lanes 11 through 14 represent patients of the control group. Lanes 1, 2, 6, 8, 9, 10, and 14 were interpreted as positive. Lanes 15 and 16 contain positive and negative controls, respectively.

Table 1**Patient Profile and Summary of Results**

Patient No.	Age, Year/Sex	Diagnosis (Lung)	Diagnosis (Other Site)	IHC-HHV8 Intensity (1–3)	IHC-HHV8 Proportion (1–5)	PCR Result
1	28/M	KS	No	0/3	0/5	Positive
2	43/M	KS	No	3/3	5/5	Positive
3	49/M	Susp KS	No	0/3	0/5	Positive
4	34/M	KS	No	0/3	0/5	Positive
5	30/M	KS	No	0/3	0/5	Negative
6	35/M	c/w KS	Skin	2/3	2/5	Positive
7	40/M	c/w KS	LN, skin	0/3	0/5	Positive
8	33/M	KS	LN, liver	3/3	5/5	Positive
9	30/M	c/w KS	No	0/3	0/5	Positive
10	31/M	Susp KS	LN, skin	1/3	4/5	Positive
11	36/M	c/w ARDS	Skin only	0/3	0/5	Negative
12	33/M	BOOP	Skin only	0/3	0/5	Negative
13	40/M	Inflammation	Skin only	0/3	0/5	Negative
14	34/M	Inflammation	Skin only	0/3	0/5	Positive

KS, Kaposi sarcoma; Susp, suspicious; c/w, consistent with; ARDS, adult respiratory distress syndrome; BOOP, bronchiolitis obliterans organizing pneumonia; LN, lymph node; PCR, polymerase chain reaction; IHC-HHV8, immunohistochemistry-human herpesvirus 8.