

# Absence of human herpesvirus-8 in pulmonary inflammatory myofibroblastic tumor: immunohistochemical and molecular analysis of 20 cases

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**Inflammatory myofibroblastic tumors are uncommon lesions composed of spindled myofibroblasts within a variable background of collagen and inflammatory cells. Although the true nature of these lesions is not fully elucidated, identification of consistent cytogenetic alterations in the anaplastic lymphoma kinase (ALK) gene suggests that they may be neoplastic. A small number of inflammatory myofibroblastic tumors have been reported to harbor human herpesvirus-8 (HHV-8), implicating the virus in its pathogenesis. In this study, 20 cases of pulmonary inflammatory myofibroblastic tumor were analyzed for the presence of HHV-8 with immunohistochemical and molecular methods. In all cases, antibodies to the latent nuclear antigen of the virus were applied. Four open reading frames (ORFs), including ORFs K2, 16, 26, and 72, were targeted utilizing real-time polymerase chain reaction (PCR). The cohort included 9 men and 11 women with a mean age of 37 years (range, 1–81). Microscopically, the tumors were composed of cytologically bland spindle cells with myofibroblastic differentiation. On immunohistochemical studies, 20% of cases (4/20) demonstrated diffuse cytoplasmic positivity with ALK. Immunohistochemical staining for the latent nuclear protein of the virus was negative in all cases (0/20). All tumors (100%, 20/20) tested with real-time PCR were negative for all four ORFs, whereas 100% (10/10) of positive control Kaposi sarcoma cases were positive. *Her2* gene expression was present in all (20/20) inflammatory myofibroblastic tumors confirming the presence of amplifiable deoxyribonucleic acid in the tissue lysate. This study documents the absence of HHV-8 in pulmonary inflammatory myofibroblastic tumors, suggesting that further investigation is required to clarify the pathogenesis of this lesion.**

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Inflammatory myofibroblastic tumor of the lung occurs in all age groups without gender bias but, most commonly, in individuals under 40 years of age.<sup>1,2</sup> Microscopically, inflammatory myofibroblastic tumor is composed of a proliferation of spindled myofibroblasts within a variable background of

collagen and inflammatory cells. Although the pathogenesis of these lesions is not fully elucidated, recent studies suggest they may be neoplastic proliferations with potential to invade the mediastinum, hilar lymph nodes, and diaphragm.<sup>3</sup> The neoplasia concept is supported by the presence of cytogenetic alterations in the anaplastic lymphoma kinase (ALK) gene, including *TPM3-ALK*, *TPM4-ALK*, and *CLTC-ALK* fusions, and ALK protein expression.<sup>4,5</sup> Clonal aberrations in the 2p23 and *ALK* gene regions have been demonstrated in both pulmonary and extrapulmonary inflammatory myofibroblastic tumors.<sup>3,6</sup>

Viral infections, in particular human herpesvirus-8 (HHV-8), have been implicated in inflammatory myofibroblastic tumor pathogenesis. HHV-8 infects a wide range of normal cells including endothelial

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cells, lymphoid cells, dendritic cells, and fibroblasts. This parallels the spectrum of the neoplasms with a well-established role for HHV-8 in their pathogenesis, including Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease. HHV-8 infection of human fibroblasts is well documented in experimental studies. It is known that the virus enters cells utilizing receptor-mediated endocytosis<sup>7</sup> and displays activation of lytic and persistence of latent genes.<sup>7,8</sup> In light of these findings, inflammatory myofibroblast tumor appears to be a reasonable choice for HHV-8 testing. Gómez-Román *et al*,<sup>9</sup> reported HHV-8 deoxyribonucleic acid (DNA) sequences in seven cases of inflammatory myofibroblast tumor, and also identified viral transcripts from several open reading frames (ORF).<sup>10</sup> In the English language literature, only three additional cases of pulmonary inflammatory myofibroblastic tumor have been studied for HHV-8 by immunohistochemical and molecular methods.<sup>11</sup> Thus, in the largest study to date, we investigated the presence of HHV-8 in pulmonary inflammatory myofibroblast tumor.

## Materials and methods

### Case Selection

Seventy-five cases diagnosed as inflammatory pseudotumor or inflammatory myofibroblastic tumor between 1988 and 2004 were retrieved from the registry of the Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology. The cases were re-reviewed to exclude inflammatory/reactive and frankly sarcomatoid lesions. Availability of tissue blocks and/or unstained slides served as another exclusion criterion. Following such selection, 20 cases comprised the current study. Clinical and demographic findings were compiled from the charts.

### Immunohistochemistry

Tissue blocks were routinely processed and serial sections of 3 μm thick and mounted on positive-charged microscope slides, incubated in a heat bath, deparaffinized, and rehydrated. Citrate buffer (pH 6.0, at 100°C for 30 min) was used for antigen retrieval. Antibodies were used according to the manufacturer's instructions. Slides were counterstained with hematoxylin and covered by nonaqueous medium. Antibodies against HHV-8 latent nuclear antigen (clone 13B10, 1:40, Vector Laboratories, Burlingame, CA, USA) and ALK (clone ALK-1, 1:100, Dako, Carpinteria, CA, USA) were applied in all 20 cases.

### Real-Time Polymerase Chain Reaction

Two 7 μm unstained slides from formalin-fixed, paraffin-embedded tissue for each case were

deparaffinized and DNA was obtained through proteinase K digestion, phenol/chloroform/isoamyl alcohol extraction, and ethanol precipitation. DNA was stored at -20°C before use. Primers and probes for real-time polymerase chain reaction (PCR) were designed based on the published sequence in the GenBank database (U75698) available via the National Center for Biotechnology Information. Four sets of primers and probes, as shown in Table 1, aimed to detect four ORFs of HHV-8, were synthesized by Integrated DNA Technologies Inc. The real-time PCR was performed in an ABI 7500 Real-Time PCR System. PCR cycles were 2 min at 50°C, followed by 10 min at 95°C and 40 cycles of 15 s at 95°C and 1 min at 60°C. *Her2* gene was used to test for the presence of amplifiable DNA in the tissue lysate. BC-1, a lymphoma cell line containing HHV-8 genome from ATCC (ATCC no. CRL-2230), and 10 cases of Kaposi sarcoma were used as positive controls.

## Results

The cohort included 9 men and 11 women with a mean age of 37.3 years (range, 1–81) (Table 2). Eighty percent (16/20) of cases presented as well-circumscribed parenchymal masses, 15%

**Table 1** Targeted sequences and probes

Targeted ORG	Size of PCR product	Sequences of primers and probes
ORF K2 (viral IL-6 homolog)	98 base pairs	5'-ATTTGGGTGGACTGTAGTGCG TCT-3' (forward primer) 5'-TGAGCTTCTCTGTATGTCCCA TCCT-3' (FAM/TAMRA) (probe) 5'-AAACGTTGGACGTCATGGAGCT TCT-3' (reverse primer)
ORF 16 (viral Bcl-2 homolog)	100 base pairs	5'-GGTTAGCGGCGATGGAAACAT GAA-3' (forward primer) 5'-AGCCCTGGCTATACTGACCTTTG GCAGTTT-3' (FAM/TAMRA) (probe) 5'-TCGCAGGTGAGGTTTCGTTGGA TAA-3' (reverse primer)
ORF 72 (cyclin D homolog)	86 base pairs	5'-TGCGCAGATCAAAGTCCGAAA CAG-3' (forward primer) 5'-ATCTGTTCCACTGCCGCTGTA GAA-3' (FAM/TAMRA) (probe) 5'-GGCAAGCATATGGGATGCCA TGT-3' (reverse primer)
ORF26 (minor capsid protein)	234 base pairs	5'-AGCCGAAAGGATTCCACCAT-3' (forward primer) 5'-CGCTATTCTGCAGCAGCTGTGG TGTACCA-3' (FAM/TAMRA) (probe) 5'-TCCGTGTTGTCTACGTCCAG-3' (reverse primer)
Her2-control	143 base pairs	5'-ATGCAGATTGCCAAGGTATGC-3' (forward primer) 5'-CCGGAGCAAACCCCTATGTCCA CAA-3' (VIC/TAMRA) (probe) 5'-GGAAGCACCCATGTAGACCT TCT-3' (reverse primer)

ORF, open reading frame; PCR, polymerase chain reaction.

**Table 2** Clinicopathological, immunohistochemical, and molecular findings

Case number	Age (years)/gender	Location/size (cm)	ALK-1 IHC	HHV-8 IHC	HHV-8 PCR
1	1/M	3.5/LLL	—	—	—
2	20/M	0.7 <sup>a</sup> /RML	+	—	—
3	42/F	4.5/RLL	—	—	—
4	40/M	2.4/LUL	—	—	—
5	51/F	3.1/RML	—	—	—
6	63/M	7.5/LUL	—	—	—
7	26/F	2/RUL	—	—	—
8	81/M	4.5/RUL	—	—	—
9	11/M	2.2 <sup>a</sup> /RUL	—	—	—
10	18/F	1.9 <sup>a</sup> /RLL	—	—	—
11	47/F	2.4 <sup>a</sup> /LUL	—	—	—
12	26/M	2.2/EB	+	—	—
13	63/F	2.1/RLL	—	—	—
14	16/F	0.9 <sup>a</sup> /EB	—	—	—
15	32/F	9/LLL	—	—	—
16	54/M	1.5/RLL	—	—	—
17	26/M	3/LLL	+	—	—
18	37/F	2.9 <sup>a</sup> /RML	+	—	—
19	36/F	2.3/LLL	—	—	—
20	56/F	6/RLL	—	—	—

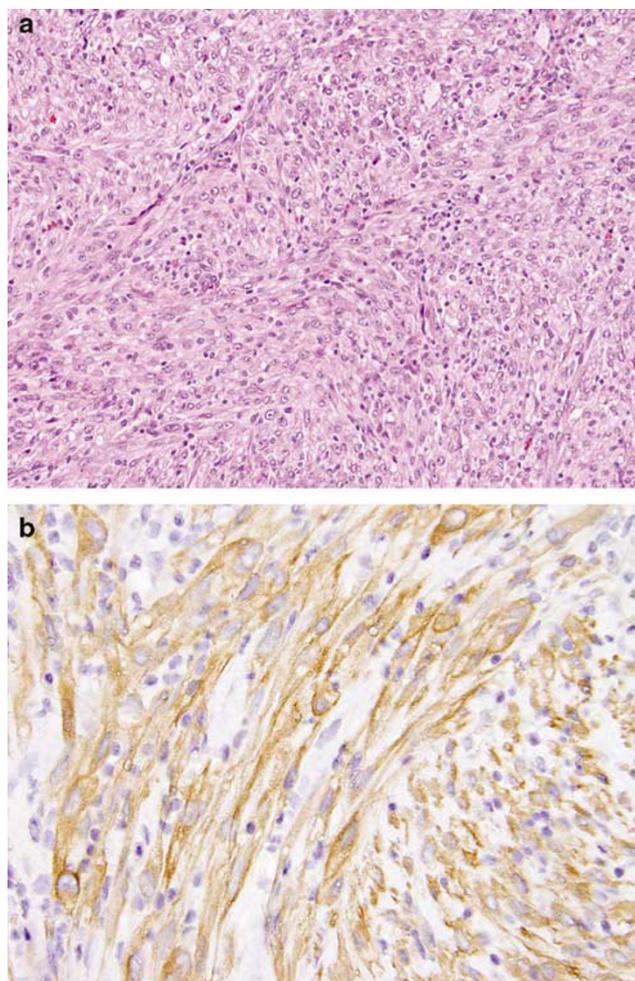
ALK, anaplastic lymphoma kinase; EB, endobronchial; F, female; HHV, human herpesvirus; IHC, immunohistochemistry; LLL, left lower lobe; LUL, left upper lobe; M, male; RLL, right lower lobe; RML, right middle lobe; RUL, right upper lobe.

<sup>a</sup>Size estimated from glass slides.

(3/20) were central involving bronchi, and one case (5%) presented as a 7.5 cm mass involving the chest wall. The average tumor size was 3.8 cm (range, 1.5–7.5). Grossly, tumors were firm, white to gray masses without necrosis. Histologically, tumors were comprised of spindle cells with fibroblastic and myofibroblastic differentiation arranged in fascicles and/or storiform pattern (Figure 1a). The spindle cells had abundant, bipolar, pale eosinophilic cytoplasm, and contained oval nuclei with fine nuclear chromatin and inconspicuous nucleoli. Variable inflammatory infiltrates of plasma cells, lymphocytes, foamy macrophages, and histiocytes were admixed. Cytologic atypia was minimal and mitoses rare. On immunohistochemical studies, 20% (4/20) of cases showed diffuse cytoplasmic staining with ALK (Figure 1b) and all cases were negative (20/20) with HHV-8. All tumors (20/20) were negative for the four ORFs of HHV-8 in real-time PCR, whereas 100% (10/10) of Kaposi sarcomas, serving as positive control, were positive. *Her2* gene expression in all of the inflammatory myofibroblastic tumors confirmed the presence of amplifiable DNA in the tissue lysate.

## Discussion

There is a strong association between HHV-8 and Kaposi sarcoma, multicentric Castleman disease, and primary effusion lymphoma. HHV-8 has also been reported to be present in pemphigus vulgaris,



**Figure 1** Histological and immunohistochemical features. (a) The tumor is composed of spindled myofibroblasts admixed with clusters of plasma cells, lymphocytes and macrophages (H&E,  $\times 200$ ). (b) ALK expression (immunohistochemistry,  $\times 400$ ).

extranodal marginal zone lymphoma of salivary gland, hemophagocytic syndrome, multiple myeloma, Kikuchi lymphadenitis, and sarcoidosis.<sup>12–17</sup> However, the latter list of diseases is controversial as viral detection varies among studies.<sup>18–21</sup> Two publications by Gómez-Román *et al*,<sup>9,10</sup> documenting HHV-8 in lesional myofibroblasts of inflammatory myofibroblastic tumor, drew attention to the possible role of the virus in the pathogenesis of this tumor. In the largest series to date, twenty cases of pulmonary inflammatory myofibroblastic tumor were studied for the presence of HHV-8. Whereas the demographic, clinical, and histological features in all cases were typical for inflammatory myofibroblastic tumor, no viral DNA or latent nuclear antigen was identified utilizing immunohistochemical or molecular studies.

The observations reported by Gómez-Román *et al*,<sup>10</sup> were based on five cases of ALK-negative pulmonary inflammatory myofibroblastic tumor and raised the question of whether the presence of virus is restricted to the lesions lacking ALK expression

**Table 3** Reported cases of pulmonary inflammatory myofibroblastic tumor evaluated for HHV-8

Study	Cases	Age (years)/sex	ALK-1 IHC	HHV-8 IHC	HHV-8 PCR
Gómez-Román <i>et al</i> <sup>9,10a</sup>	1	60/M	—	NA	+
	2	39/F	—	NA	+
	3	75/M	—	NA	+
	4	49/F	—	NA	+
	5	79/M	<sup>b</sup>	NA	+
Yamamoto <i>et al</i> <sup>22</sup>	6	4/M	+	—	—
	7	22/F	+	—	—
	8	23/M	—	—	—

ALK, anaplastic lymphoma kinase; HHV, human herpesvirus; IHC, immunohistochemistry; NA, data not available.

<sup>a</sup>Data compiled from two studies.

<sup>b</sup>One of the cases lacked material for immunohistochemistry.

and/or to lesions of the lung (Table 3). In a subsequent report, Yamamoto *et al*<sup>22</sup> addressed this issue utilizing 21 ALK-positive and ALK-negative tumors from multiple sites including lung. All 21 cases were negative for HHV-8, leading the authors to suggest the virus does not play a role in the pathogenesis of inflammatory myofibroblastic tumor. However, the Yamamoto *et al* study<sup>22</sup> had a limited number of pulmonary inflammatory myofibroblastic tumors, only three cases, and targeted only one ORF (ORF26). Similar to the study by Gómez-Román *et al*,<sup>10</sup> we focused on pulmonary lesions and expanded the number of cases to 20, including 4 ALK-positive tumors; all 20 were negative for HHV-8. As we found no viral DNA, it was not surprising that all our cases were negative for latent nuclear antigen, corroborating the immunohistochemical findings on inflammatory myofibroblastic tumor from varying sites reported by Yamamoto *et al*<sup>22</sup> and pediatric cases reported by Mergan *et al*.<sup>23</sup>

Molecular identification of HHV-8 is known to produce both false-positive and false-negative results.<sup>24–26</sup> The latter is thought, in part, to be related to HHV-8 sequence variation, which can range up to 35% in certain regions of the viral genome, as in ORFK1.<sup>26</sup> The use of a single primer set based on a sequence with a high level of sequence variation can lower the PCR sensitivity and, therefore, the rate of HHV-8 detection. Another potential source of false-negative results is targeting large fragments of viral DNA. Based on our experience and data in the literature, 250 base pairs are often beyond the upper size limit of what can readily be amplified by PCR from formalin-fixed, paraffin-embedded tissue.<sup>24</sup> False-positive results are thought to be due to utilization of high cycle protocols and/or complimentary primer sets, as in nested PCR. Through a set of validation tests, Pan *et al*,<sup>25</sup> demonstrated that nested PCR is readily contaminated, even in strictly controlled environments, and use of standard PCR with more than one set of primers is recommended. In their study, 6 of 16 negative controls tested

**Table 4** HHV-8 PCR target sequences employed in recent studies

Study	Target sequences	Positive sequences
Gomez-Roman <i>et al</i> <sup>9,10</sup>	ORFK2, ORFK7, ORFK13, ORFK16,	ORFK2, ORFK13
	ORF21, ORF25,	ORF16
	ORF26, ORF72,	ORF72
	ORF73, ORF74,	
	ORF75	
Ding <i>et al</i> <sup>27</sup>	ORF26	ORF26
Yamamoto <i>et al</i> <sup>22</sup>	ORF26	None
This study	ORFK2	None
	ORF16	
	ORF26	
	ORF72	

HHV, human herpesvirus; ORF, open reading frame; PCR, polymerase chain reaction.

positive for HHV-8 sequences in nested PCR using primers to ORF26, but none were positive utilizing standard PCR. The contamination by nucleic acids, particularly from previously amplified material (carry-over), is likely the main source of false-positive results.

To achieve reliable results, we employed a detection system addressing the issues surrounding the occurrence of false-positive and -negative results described in the literature. First, our detection system used four ORFs of the viral genome (Table 4), including two primer pairs that enabled detection of HHV-8 in the Gómez-Román *et al* study,<sup>10</sup> namely ORFK2 (viral interleukin-6 homolog) and ORF72 (cyclin D homolog), a primer pair used in the Ding *et al*<sup>27</sup> study ORF26 (minor capsid protein), and another employed in several Kaposi sarcoma studies, that is, ORF16 (viral Bcl-2 homolog). Second, to increase efficiency of PCR amplification, the targeted sequences did not exceed 234 base pairs. Third, to overcome the problem of carry-over contamination, a closed-tube detection system, real-time PCR, was used. Nevertheless, application of this more stringent and sensitive detection system revealed no evidence of the viral genome in a large set of cases. These data suggest that: (1) HHV-8 plays no role in pathogenesis of pulmonary inflammatory myofibroblastic tumor and (2) further investigation is needed to elucidate other potential causative factors for this lesion.

## References

- Gal AA, Koss MN, McCarthy WF, *et al*. Prognostic factors in pulmonary fibrohistiocytic lesions. *Cancer* 1994;73:1817–1824.
- Soud AK, Ziemba MC, Dubansky AS, *et al*. Inflammatory myofibroblastic tumor in children. *Cancer* 1993;72:2042–2048.
- Yousem SA, Shaw H, Cieply K. Involvement of 2p23 in pulmonary inflammatory pseudotumors. *Hum Pathol* 2001;32:428–433.

- 4 Griffin CA, Hawkins AL, Dvorak C, *et al*. Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. *Cancer Res* 1999;59:2776–2780.
- 5 Lawrence B, Perez-Atayde A, Hibbard MK, *et al*. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. *Am J Pathol* 2000;157:377–384.
- 6 Coffin CM, Patel A, Perkins S, *et al*. ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor. *Mod Pathol* 2001;14:569–576.
- 7 Akula SM, Naranatt PP, Walia NS, *et al*. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) infection of human fibroblast cells occurs through endocytosis. *J Virol* 2003;77:7978–7990.
- 8 Krishnan HH, Naranatt PP, Smith MS, *et al*. Concurrent expression of latent and a limited number of lytic genes with immune modulation and antiapoptotic function by Kaposi's sarcoma-associated herpesvirus early during infection of primary endothelial and fibroblast cells and subsequent decline of lytic gene expression. *J Virol* 2004;78:3601–3620.
- 9 Gomez-Roman JJ, Oejo-Vinyals G, Sanchez-Velasco P, *et al*. Presence of human herpesvirus-8 DNA sequences and overexpression of human IL-6 and cyclin D1 in inflammatory myofibroblastic tumor (inflammatory pseudotumor). *Lab Invest* 2000;80:1121–1126.
- 10 Gomez-Roman JJ, Sanchez-Velasco P, Oejo-Vinyals G, *et al*. Human herpesvirus-8 genes are expressed in pulmonary inflammatory myofibroblastic tumor (inflammatory pseudotumor). *Am J Surg Pathol* 2001;25:624–629.
- 11 Yamamoto H, Kohashi K, Oda Y, *et al*. Absence of human herpesvirus-8 and Epstein-Barr virus in inflammatory myofibroblastic tumor with anaplastic large cell lymphoma kinase fusion gene. *Pathol Int* 2006;56:584–590.
- 12 Smith KJ, Skelton HG. Human herpesvirus 8 DNA sequences in pemphigus: the role of the virus in oncogenic and autoimmune manifestations. *Arch Dermatol* 1998;134:751.
- 13 Yamasaki S, Iino T, Nakamura M, *et al*. Detection of human herpesvirus-8 in peripheral blood mononuclear cells from adult Japanese patients with multicentric Castleman's disease. *Br J Haematol* 2003;120:471–477.
- 14 Fredricks DN, Martin TM, Edwards AO, *et al*. Human herpesvirus 8 and sarcoidosis. *Clin Infect Dis* 2002;34:559–560.
- 15 Marionni G, Marchese-Ragona R, Marino F, *et al*. MALT-type lymphoma and Warthin's tumour presenting in the same parotid gland. *Acta Otolaryngol* 2004;124:318–323.
- 16 Li CF, Ye H, Liu H, *et al*. Fatal HHV-8-associated hemophagocytic syndrome in an HIV-negative immunocompetent patient with plasmablastic variant of multicentric Castleman disease (plasmablastic microlymphoma). *Am J Surg Pathol* 2006;30:123–127.
- 17 Tedeschi R, Luostarinen T, De PP, *et al*. Joint Nordic prospective study on human herpesvirus 8 and multiple myeloma risk. *Br J Cancer* 2005;93:834–837.
- 18 Cathomas G, Stalder A, Regamey N, *et al*. No evidence of HHV-8 infection in patients with pemphigus vulgaris/foliaceus. *Arch Dermatol* 1998;134:1162.
- 19 Knoell KA, Hendrix Jr JD, Stoler MH, *et al*. Absence of human herpesvirus 8 in sarcoidosis and crohn disease granulomas. *Arch Dermatol* 2005;141:909–910.
- 20 Folk GS, Abbondanzo SL, Childers EL, *et al*. Plasmablastic lymphoma: a clinicopathologic correlation. *Ann Diagn Pathol* 2006;10:8–12.
- 21 Malnati MS, Dagna L, Ponzone M, *et al*. Human herpesvirus 8 (HHV-8/KSHV) and hematologic malignancies. *Rev Clin Exp Hematol* 2003;7:375–405.
- 22 Yamamoto H, Oda Y, Saito T, *et al*. p53 Mutation and MDM2 amplification in inflammatory myofibroblastic tumours. *Histopathology* 2003;42:431–439.
- 23 Mergan F, Jaubert F, Sauvat F, *et al*. Inflammatory myofibroblastic tumor in children: clinical review with anaplastic lymphoma kinase, Epstein-Barr virus, and human herpesvirus 8 detection analysis. *J Pediatr Surg* 2005;40:1581–1586.
- 24 Krafft AE, Duncan BW, Bijwaard KE, *et al*. Optimization of the isolation and amplification of RNA from formalin-fixed, paraffin-embedded tissue: the armed forces institute of pathology experience and literature review. *Mol Diagn* 1997;2:217–230.
- 25 Pan L, Milligan L, Michaeli J, *et al*. Polymerase chain reaction detection of Kaposi's sarcoma-associated herpesvirus-optimized protocols and their application to myeloma. *J Mol Diagn* 2001;3:32–38.
- 26 Zong JC, Ciuffo DM, Alcendor DJ, *et al*. High-level variability in the ORF-K1 membrane protein gene at the left end of the Kaposi's sarcoma-associated herpesvirus genome defines four major virus subtypes and multiple variants or clades in different human populations. *J Virol* 1999;73:4156–4170.
- 27 Ding Y, Saylor RL, Brown H, *et al*. Pulmonary inflammatory pseudotumor with HHV-8. *Am J Surg Pathol* 2002;26:1089–1091.