

## CASE REPORT

# Human herpesvirus 8 (HHV-8) detected in two patients with Kaposi's sarcoma-like pyogenic granuloma

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**Aims:** To report the finding of human herpesvirus 8 (HHV-8) in two patients with Kaposi's sarcoma (KS)-like pyogenic granuloma. This form of pyogenic granuloma closely resembles KS histologically and it has been reported that immunohistochemistry in such lesions may be positive for smooth muscle actin and factor VIII related antigen, which are typically negative in KS. In both patients the lesions were positive for CD31, CD34, smooth muscle actin, and factor VIII related antigen, a profile typical of KS-like pyogenic granuloma. The lesions were tested for the presence of HHV-8 DNA, which to date has been consistently found in all types of KS.

**Methods:** The lesions were tested for the presence of HHV-8 DNA using the polymerase chain reaction (PCR). A known HHV-8 positive KS specimen was used as the positive control. Six samples of non-KS vascular skin lesions were used as negative controls for the PCR reaction.

**Results:** Both lesions were positive on PCR for HHV-8 and the specificity of product was confirmed by direct sequencing. None of the six control vascular skin lesions was positive for HHV-8. These results strongly indicate KS as the true diagnosis and are supported by the reported clinical course in both cases.

**Conclusions:** Techniques targeting HHV-8 DNA for detection to confirm a diagnosis of KS are both sensitive and specific. In cases where the differential diagnosis includes KS-like pyogenic granuloma, caution should be taken not to diagnose solely on the basis of immunohistochemistry phenotype. In such cases, PCR targeting HHV-8 DNA sequences is a better diagnostic tool.

The increase in incidence of Kaposi's sarcoma (KS), previously uncommon in Europe outside of the Mediterranean, has mirrored the rise in the incidence of human immunodeficiency virus (HIV) infection. The recent description of DNA sequences of a new herpes virus in KS lesions in patients with AIDS<sup>1</sup> led to the demonstration of the virus in all forms of KS.<sup>2-7</sup> KS herpesvirus (KSHV) or human herpesvirus 8 (HHV-8) also plays a role in the pathogenesis of body cavity based/primary effusion lymphoma<sup>8</sup> and multicentric Castleman's disease.<sup>9</sup> HHV-8 is a  $\gamma$ -herpesvirus, which has been localised to vascular endothelial cells and perivascular spindle shaped cells,<sup>10,11</sup> and which replicates in peripheral blood mononuclear cells.<sup>12,13</sup> Previous studies have shown that polymerase chain reaction (PCR) based detection of HHV-8 DNA or RNA in suspected skin lesions is indicative of KS and is rare in other skin lesions.<sup>14-16</sup> Here, we report two cases of multiple hyperpigmented lesions which on haematoxylin and eosin stain closely resembled KS (fig 1). Subsequent immunohistochemical examination displayed cells within both lesions that stained positive for smooth muscle actin and the endothelial cell markers factor VIII, CD31, and CD34. This phenotype suggests a diagnosis of KS-like pyogenic granu-

loma according to a previously reported study,<sup>17</sup> where it was stated that KS is typically negative for smooth muscle actin positive pericytes and factor VIII related antigen.

We used a PCR system to test the lesions for the presence of HHV-8 DNA, which to date has been consistently found in all types of KS. PCR analysis was performed targeting the open reading frame 26 (ORF26) gene of HHV-8. Formalin fixation results in widespread crosslinkage between nucleic acids and proteins, with the result that DNA extracted from formalin fixed tissues is fragmented into sequences of variable size.<sup>18</sup> It has been shown that because the DNA is fragmented it is increasingly difficult and unreliable to amplify longer sequences in formalin fixed, paraffin wax embedded tissue.<sup>19</sup> Thus, the primers used, chosen by searching the published literature, amplified a sequence of < 250 bp. The viral load in KS lesions has been reported as low,<sup>20</sup> and DNA in formalin fixed, paraffin wax embedded histological samples is subject to degradation over time. Therefore, to increase the sensitivity of the system two rounds of 40 and then 30 cycles of PCR were completed. The specificity of the PCR system was examined with six samples of unrelated, similarly fixed cutaneous vascular lesions, and the specificity of the product amplified was confirmed by direct DNA sequencing. Both test cases were positive and all of the negative control samples were negative for HHV-8. In the light of the subsequent clinical behaviour of the lesions, which was similar in both cases, the possible diagnostic implications are discussed.

"Previous studies have shown that PCR based detection of HHV-8 DNA or RNA in suspected skin lesions is indicative of Kaposi's sarcoma and is rare in other skin lesions"

## CASE HISTORIES

### Case 1

South Infirmery: in 1994 a 72 year old man presented with a painful pigmented nodule on his left ankle. Initially, the biopsy was reported as KS and treatment was with intralesional vinblastine and cryotherapy. He returned in 1996 with two similar lesions behind his left knee, which were also reported as KS, and were also treated with intralesional vinblastine and cryotherapy. The following year he developed several small lesions on his left leg, which were successfully treated with electron beam radiotherapy, but recurred six months later, requiring more radiotherapy, after which the patient failed to attend follow up. HIV testing was negative before surgery for a rectal tumour in 1998.

### Case 2

Cork University Hospital: in 2000 a 75 year old man presented with a well circumscribed, darkly pigmented, 0.6 cm diameter

**Abbreviations:** APC, adenomatous polyposis coli; HHV-8, human herpesvirus 8; KS, Kaposi's sarcoma; ORF, open reading frame; PCR, polymerase chain reaction

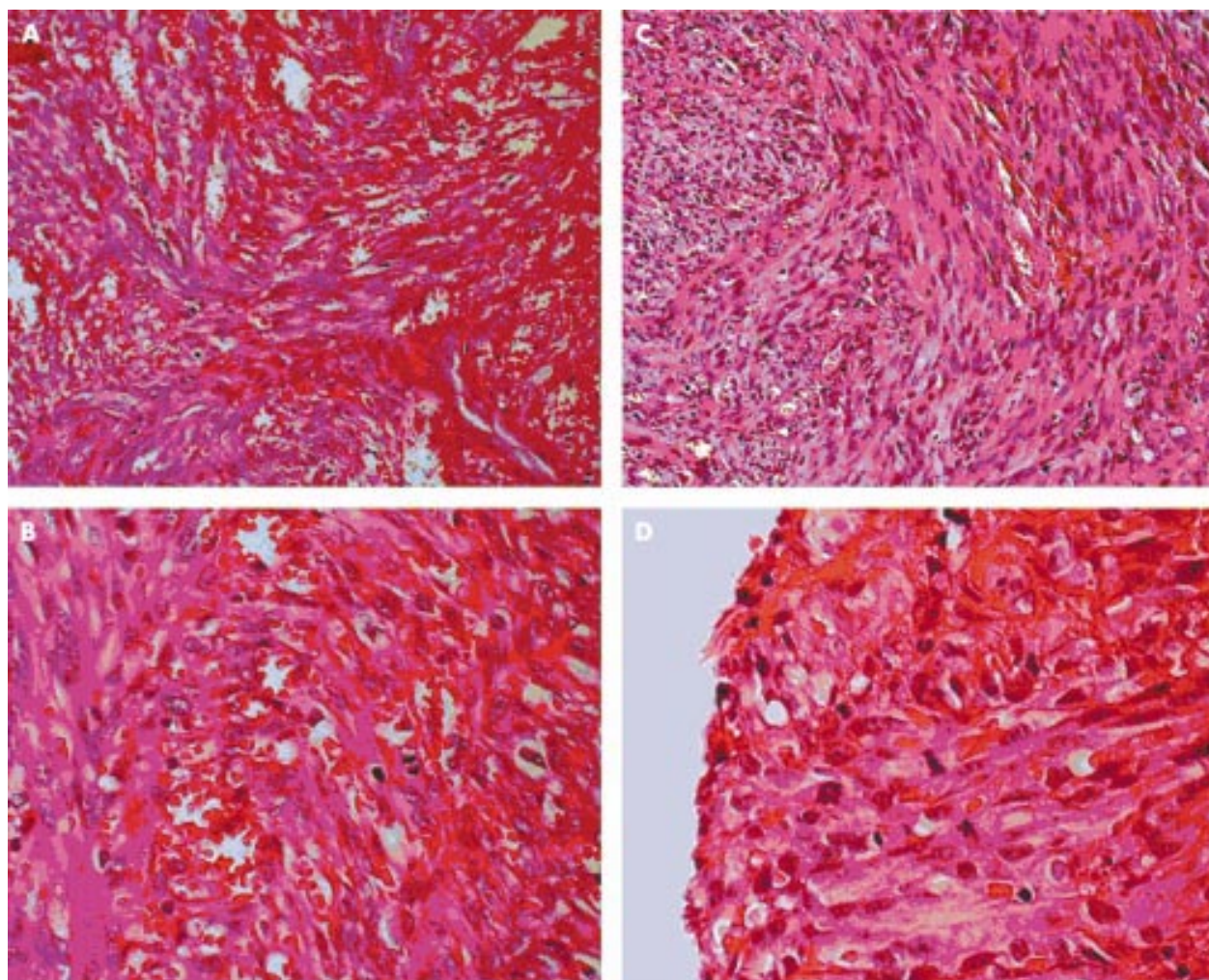


Figure 1 Kaposi's sarcoma-like pyogenic granuloma, haematoxylin and eosin stain. (A,B) Case 1 and (C,D) case 2. Low and high power views showing histological features typical of Kaposi's sarcoma-like pyogenic granuloma, namely: exophytic growth, well circumscribed, lobular nodules made up of fascicular arrangements of spindle cells and slit-like vessels.

**Table 1** Results of PCR of 186 bp HHV-8 DNA fragment in positive control Kaposi's sarcoma, negative control cutaneous vascular lesions, and two cases of Kaposi's sarcoma-like pyogenic granuloma

Sample	Histological diagnosis	HHV-8 positive PCR
Positive control	HIV associated Kaposi's sarcoma	4/4
109-94	Case 1: Kaposi's sarcoma-like pyogenic granuloma	4/4
6634-00	Case 2: Kaposi's sarcoma-like pyogenic granuloma	4/4
5289-00	Epithelioid haemangioma	0/4
6974-00	Benign venous haemangioma	0/4
5278-98	Epithelioid angiosarcoma	0/4
7086-00	Capillary haemangioma	0/4
5344-00	Benign dermatofibroma	0/4
5278-00	Pyogenic granuloma	0/4

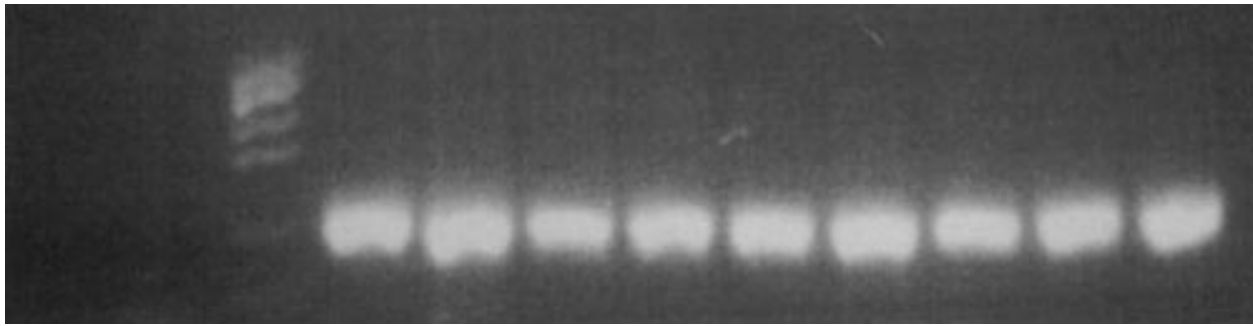
HHV-8, human herpesvirus 8; HIV, human immunodeficiency virus.

nodules in the first web space of the left foot. On examination, similar smaller lesions were present on the fourth toe of the left foot and two were seen on the left thigh. At surgery he underwent excision biopsy of all four lesions. Initially, the histopathology report was one of KS, but with the subsequent immunohistochemistry report this was modified to KS-like pyogenic granuloma. The patient has gone on to develop several similar lesions on both forearms and right foot and these have been treated with electron beam radiotherapy.

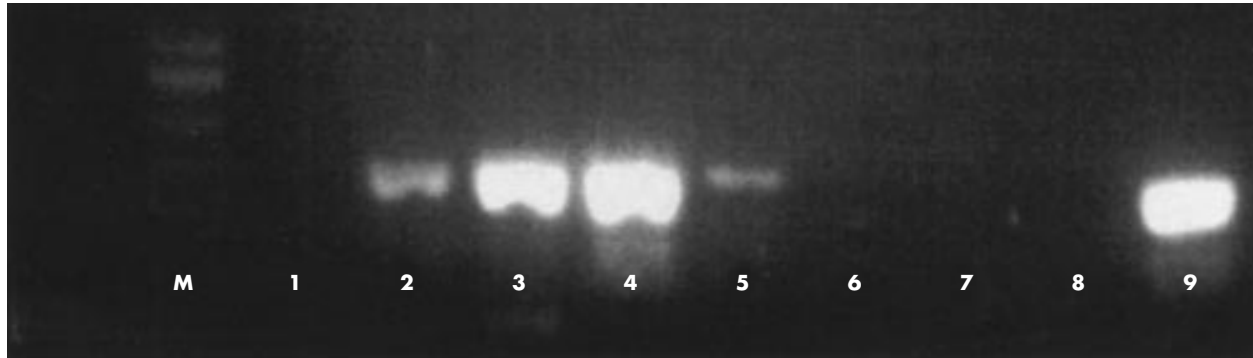
## METHODS

### Samples

Archival, formalin fixed, paraffin wax embedded histological samples from the two cases in question were obtained from the department of histopathology, Mercy Hospital, Cork, Ireland. Positive controls were from a known KS biopsy (kindly supplied by Dr J Coyne, Manchester, UK). Negative control tissue samples were from six similarly treated samples of non-KS cutaneous vascular lesions (table 1). Sections of



**Figure 2** Uniform amplification of a 133 bp fragment of the adenomatous polyposis coli (APC) gene in all nine samples (one from each histology specimen) confirming amplifiable DNA.



**Figure 3** Detection of 186 bp human herpesvirus 8 (HHV-8) DNA fragment in both test cases; M, marker; lane 1, no DNA negative control; lanes 2 and 3, case 1; lanes 4 and 5, case 2; lanes 6–8, negative control cutaneous vascular lesions; lane 9, Kaposi's sarcoma positive control.

5  $\mu$ m thickness were cut and duplicate sections were examined from each block.

#### DNA isolation

DNA was obtained from all samples using DNeasy® tissue kits (Qiagen Ltd, Crawley, UK) according to the manufacturer's instructions, resulting in a total volume of 100  $\mu$ l/sample.

#### PCR

All reactions were carried out on an Eppendorf Mastercycler® gradient thermocycler (Eppendorf, Hamburg, Germany), and using HotStart Taq Master Mix® kit reagents (Qiagen). All PCR products were run on a 1  $\times$  Tris acetate EDTA, 2% agarose gel using SYBR® Green 1 nucleic acid stain (Amresco® Inc, Ohio, USA), according to the manufacturer's instructions, and visualised at 302 nm with a transilluminator. Confirmation of the effective isolation of DNA from the tissue sections was achieved by PCR amplification of a normal gene, namely a 133 bp fragment of the adenomatous polyposis coli (APC) gene. The primer pair was 5'-GGA CTA CAG GCC ATT GCA GAA-3' and 5'-GGC TAC ATC TCC AAA AGT CAA-3'. The reaction mix contained 1  $\mu$ l primer mix, 15  $\mu$ l Taq mix, 9  $\mu$ l sterile distilled H<sub>2</sub>O, and 5  $\mu$ l of DNA isolate. The reaction conditions were 95°C for 15 minutes, followed by 40 cycles of 94°C for one minute, 55°C for one minute, 72°C for one minute, and finally two minutes at 72°C.

Amplification of HHV-8 was achieved using a PCR reaction amplifying a 186 bp sequence of the ORF26 gene of the HHV-8 genome. The primer pair used was 5'-CTC GAA TCC AAC GGA TTT GA-3' and 5'-ATA TGT GCG CCC CAT AAA TG-3', as published previously.<sup>21</sup> Each reaction mix contained 1  $\mu$ mol of primer mix, 15  $\mu$ l Taq mix, 4  $\mu$ l sterile distilled H<sub>2</sub>O, and 10  $\mu$ l of DNA isolate. The reaction conditions were 95°C for 15 minutes, followed by 40 cycles of 94°C for one minute, 60°C for one minute, 72°C for one minute, and finally five minutes at 72°C. A second round of PCR was then performed under the same

conditions using 1  $\mu$ l of the first PCR reaction solution in a reaction mix containing 1  $\mu$ mol of primer mix, 15  $\mu$ l Taq mix and 13  $\mu$ l sterile distilled H<sub>2</sub>O.

#### Methods used to counteract contamination

The PCR reagents were mixed in a separate area from that where the DNA was added. Before the addition of DNA the reaction mix was first placed in UV light of wavelength 254 nm and intensity 30 000  $\mu$ J/cm<sup>2</sup> for 2  $\times$  2 minutes to crosslink any contaminating DNA. Negative controls containing no DNA were used in every PCR reaction. Filter tips were used throughout the entire process.

#### DNA sequencing

Where a PCR product was detected on a gel its specificity was confirmed by direct sequencing by standard methods. Briefly, PCR products were purified on centrifuge columns (Qiagen) and then dried by alcohol precipitation. The dried PCR product was then sent together with a sample of each primer for direct sequencing to MWG AG Biotech, Ebersberg, Germany according to their instructions.

#### RESULTS

Two 5  $\mu$ m tissue sections were cut from each tissue sample examined, DNA was isolated from each section, and each DNA isolate was subjected to PCR analysis twice to give a total of four results for each histology sample. Amplification of the 133 bp fragment of the APC gene was successful in all samples (fig 2). The 186 bp HHV-8 DNA fragment was repeatedly detected in the positive control KS sample and both test cases, but not in the other cutaneous vascular lesions (fig 3). Thus, the specificity of the reaction was not affected by the high cycle number, as reflected in the lack of amplification of viral DNA in the negative control samples. The results of the DNA sequencing as reported by MWG AG Biotech were identical to

**Take home messages**

- When the differential diagnosis includes Kaposi's sarcoma-like pyogenic granuloma, diagnosis should not be based on the immunohistochemical phenotype alone
- In such cases, polymerase chain reaction techniques for the detection of human herpesvirus 8 DNA appear to be the better diagnostic tool

the predicted HHV-8 target sequence in product amplified from both test samples and the positive control.

**DISCUSSION**

The association between HHV-8 and all forms of KS is now well established and the detection of HHV-8 in suspected KS lesions has been proposed as a strong diagnostic indicator.<sup>15 16</sup> In cases where diagnostic doubt exists, such as with cases of KS-like pyogenic granuloma, a recent report suggested immunohistochemical phenotyping as a useful tool in distinguishing KS from KS-like pyogenic granuloma, the former being negative and the latter positive for smooth muscle actin and factor VIII related antigen.<sup>17</sup>

Here, we report two cases of multiple KS-like pyogenic granulomas where the differential diagnosis includes KS. Although they are uncommon, similar cases have been reported previously.<sup>17 22</sup> The clinical picture seen on follow up of both cases was typical of classic KS, which often runs a benign, indolent course for 10 years or more with gradual development of additional lesions. However the reported immunohistochemical profile supported a diagnosis of KS-like pyogenic granuloma, with lesions from both cases being positive for smooth muscle actin and factor VIII related antigen.

"The implications of this finding are to highlight the value of PCR as a diagnostic tool where diagnostic doubt exists in suspected cases of Kaposi's sarcoma-like pyogenic granuloma"

We used PCR to show the presence of HHV-8 in both cases. The PCR system used was more sensitive and more specific than a nested system, which we also used to amplify first a 233 bp and then a 186 bp internal fragment of HHV-8 (data not shown). This is in agreement with previously published data that the degree of false positivity is increased with the nested PCR system.<sup>23</sup> The specificity of product amplified was confirmed by direct sequencing.

The implications of this finding are to highlight the value of PCR as a diagnostic tool where diagnostic doubt exists in suspected cases of KS-like pyogenic granuloma. A pathological diagnosis of KS has important clinical consequences for patients presenting with hyperpigmented skin lesions, and in particular for those who have not been tested for HIV, although the clinical presentation of KS in the context of HIV infection is different to that seen in classic KS.

Given the benign clinical course, and that both patients in this report were immunocompetent, we imagine that the same lesions would behave more aggressively in the immunocompromised patient. It is already known that infection with the virus alone is not sufficient for the development of KS and that additional cofactors are required.<sup>24</sup> The hypothesis now gaining acceptance is that all forms of KS (classic, African, immunosuppressive treatment related, and epidemic) have a common aetiology in HHV-8 and that the virus does not just opportunistically colonise lesions.

In conclusion, for cases where the differential diagnosis includes KS-like pyogenic granuloma caution should be taken not to base diagnosis solely on the immunohistochemical phenotype. In such cases, PCR techniques targeting HHV-8 DNA would appear to represent the better diagnostic tool.

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