

TECHNICAL NOTE

Detection of herpesvirus-like DNA in the bronchoalveolar lavage fluid of patients with pulmonary Kaposi's sarcoma

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ABSTRACT: Pulmonary involvement is a clinically important form of visceral Kaposi's sarcoma in immunocompromised patients. Recently, herpesvirus-like deoxyribonucleic acid (DNA) sequences, defining a new herpesvirus termed "human herpesvirus 8" (HHV8) or "Kaposi's sarcoma-associated herpesvirus" (KSHV), were detected in Kaposi's sarcoma of acquired immune deficiency syndrome (AIDS) and non-AIDS patients. We describe the successful detection of HHV8 DNA in the bronchoalveolar lavage (BAL) fluid of patients with pulmonary Kaposi's sarcoma.

Three immunocompromised patients, two HIV seropositive and one after kidney transplantation, suffered from respiratory symptoms and showed pulmonary infiltrates on chest radiography after development of biopsy proven Kaposi's sarcoma of the skin. Bronchoscopy revealed the typical Kaposi like livid endobronchial lesions. BAL fluid was analysed for the presence of HHV8 DNA using a nested polymerase chain reaction (PCR) assay.

HHV8 DNA was detected in the BAL fluid of all three patients. In addition, HHV8 DNA could be detected in the skin biopsy tissue, lymph node, and peripheral blood mononuclear cells of these patients.

Our data show that human herpesvirus 8 deoxyribonucleic acid can be detected in the bronchoalveolar lavage fluid of patients with pulmonary Kaposi's sarcoma. If further studies reveal a high specificity for human herpesvirus 8 deoxyribonucleic acid detection, this test will improve the tools for the diagnosis of pulmonary Kaposi's sarcoma without further need of biopsies.

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Kaposi's sarcoma is a common malignant skin tumour in immunocompromised patients. In acquired immune deficiency syndrome (AIDS) patients, homosexual males are far more frequently affected (47% of newly diagnosed Kaposi's sarcoma) than intravenous drug users (4%) [1]. In renal transplant recipients, the incidence has been estimated to be less than 1% [2]. Pulmonary involvement of Kaposi's sarcoma has been described in up to 25% of all AIDS patients, and in about 45% of patients developing Kaposi's sarcoma of the skin after solid organ transplantation [2, 3]. In contrast to the skin, pulmonary involvement contributes decisively to patients' morbidity and mortality [4]. Diagnosis of pulmonary Kaposi's sarcoma is based on the clinical history of cutaneous tumours and characteristic endobronchial lesions seen by bronchoscopy. Histological confirmation, however, can often not be obtained by bronchial biopsy due to the small sample size and the risk of extensive haemorrhage.

Recently, herpesvirus-like deoxyribonucleic acid (DNA) sequences, defining a new herpesvirus termed "Kaposi's sarcoma-associated herpes virus" (KSHV) or "human herpes virus 8" (HHV8), were detected in all forms of Kaposi's sarcoma in AIDS and non-AIDS patients [5–9]. We describe the first successful detection of HHV8 DNA

in the bronchoalveolar lavage (BAL) fluid of patients with pulmonary Kaposi's sarcoma.

Patients and methods

Three immunocompromised patients with biopsy proven Kaposi's sarcoma of the skin underwent bronchoscopy with BAL because of respiratory symptoms and infiltrates on chest radiography.

A 41 year old male patient (patient No. 1) had received a kidney transplant because of end-stage renal failure due to chronic glomerulonephritis. Immunosuppression after transplantation consisted of a 7 day course of monoclonal anti-T-lymphocyte antibodies (OKT3), followed by cyclosporin A, azathioprine and prednisone. The postoperative period was complicated by repeated rejection episodes, treated with steroid pulses and a second course of OKT3. After 1 yr, immunosuppression was reduced to cyclosporin A and azathioprine. Two years after transplantation, the patient developed biopsy proven Kaposi's sarcoma of the skin. Azathioprine was discontinued but, despite the reduction of the immunosuppressive therapy, dissemination to the cervical lymph nodes

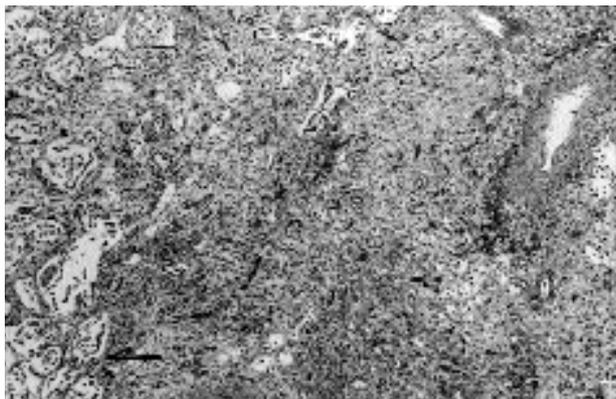


Fig. 1. — Pulmonary Kaposi's sarcoma of lung tissue obtained in the renal transplant recipient at autopsy. Extensive destruction of lung tissue (left upper corner) by the Kaposi's sarcoma. (Haematoxylin and eosin stain; internal scale bar=200 μm).

was observed. Immunosuppression was finally stopped and a transplant nephrectomy performed. The patient developed respiratory symptoms, and bronchoscopy with BAL was performed. Bronchoscopy showed extensive hypervascularized, and livid lesions in the main and lobar bronchi. Two months later, the patient died and autopsy confirmed disseminated Kaposi's sarcoma, including extensive pulmonary involvement (fig. 1).

Two other HIV seropositive male patients (aged 33 and 45 yrs) developed respiratory symptoms 7 months and 2 yrs after biopsy proven diagnosis of Kaposi's sarcoma of the skin. Whereas the Kaposi's sarcoma was the AIDS-defining disease in the older patient, the younger patient had a previous history of opportunistic infections, including repeated candida stomatitis, perianal herpes simplex, and cerebral toxoplasmosis. At bronchoscopy, typical Kaposi's sarcoma lesions were seen in trachea and bronchi and a BAL was performed in both patients.

A control group consisted of 10 immunocompromised patients, including eight AIDS patients and two renal transplant recipients (mean age 47 yrs range 28–76 yrs), without evidence of cutaneous or pulmonary Kaposi's sarcoma.

Sample analysis

To obtain BAL fluid, 200–300 mL of 0.9% NaCl was instilled to the middle lobe with a fluid recovery of 135–170 mL. Fresh or frozen cells of BAL fluid were used. DNA was extracted as described previously using slight modifications [10]. In brief, the cell pellet was digested over night in proteinase K at 37°C (200 $\mu\text{g}\cdot\text{mL}^{-1}$ proteinase K, 10 mmol Tris-HCl (pH 7.4), 25 mmol ethylene

diamine tetra-acetic acid (EDTA)) followed by phenol/chloroform-extraction and ethanol precipitation. The pellet was dissolved in sterile water. For the analysis of archival tissue, 2–4 sections, 5 μm thick, were cut from the paraffin blocks under stringent conditions to avoid cross-contamination. Sections were deparaffinized by xylene, the xylene removed by ethanol, dried in a lyophilizer and DNA extracted by proteinase K (200 $\mu\text{g}\cdot\text{mL}^{-1}$ proteinase K, 50 mmol Tris-HCl (pH 8.0), 0.5% Tween 20, 1 mmol EDTA) and phenol/chloroform as described above.

Five microlitres of DNA was applied to the nested polymerase chain reaction (PCR) using two outer primers (KS3: 3'ACAGCAACACCCAGCTAGCA and KS4: 5'-AGATCGTCAAGCACTCGCAG) for the first round and the previously published primers KS330/233 for the second round [5]. Thirty cycles were used in each round, with the following cycling conditions: denaturation at 94°C for 1 min; reannealing at 55°C for 45 s; extension at 72°C for 45 s. All PCR products were analysed on a 2% agarose gel and visualized by ethidium bromide staining. Stringent laboratory conditions and appropriate negative controls were used to avoid cross-contamination and false positive results.

Results

On chest radiography, all patients showed extensive bilateral infiltrates and bronchoscopy revealed hypervascularized lesions in the trachea and the bronchial tree. In the renal transplant recipient, the total count of cells was elevated in the BAL fluid to 332×10^6 cells $\cdot\text{L}^{-1}$ (normal $<300 \times 10^6$ cells $\cdot\text{L}^{-1}$), and the percentage of granulocytes was slightly increased (normal $<5\%$). In the two HIV positive patients, the cell count was within normal limits, whereas the proportion of lymphocytes was slightly increased ($>5\%$) (table 1). Cytomegalovirus was isolated from the BAL fluid by rapid cell culture in one AIDS patient, but no viral proteins could be detected in the cytological smear by immunohistochemistry. No additional infectious agents could be found, including mycobacteria or *Pneumocystis carinii*. Conventional cytological smears did not reveal malignant cells.

HHV8 DNA was detected in the BAL fluid of all three patients by nested PCR (fig. 2). In addition, HHV8 DNA was detected in the prior skin biopsies of the Kaposi's sarcoma in all three patients as well as in the peripheral blood mononuclear cells of the two patients tested. In the renal transplant patient, HHV8 DNA was also found in a cervical lymph node metastasis and in the tumour tissue of the lung obtained at autopsy. No

Table 1. — Total cell count and cell differentiation in the bronchoalveolar lavage fluid of patients with pulmonary Kaposi's sarcoma

Pt No	Underlying disease	Total cell count $\times 10^6\cdot\text{L}^{-1}$	Macrophages		Neutrophils		Lymphocytes	
			%	$\times 10^6\cdot\text{L}^{-1}$	%	$\times 10^6\cdot\text{L}^{-1}$	%	$\times 10^6\cdot\text{L}^{-1}$
1	Kidney transplant	332	87	281	8	26	4	13
2	HIV infection	201	84	170	5	10	11	22
3	HIV infection	257	92	236	2	5	6	15

Normal values: total cell count $<300 \times 10^6\cdot\text{L}^{-1}$; macrophages $>90\%$; neutrophilic granulocytes $<5\%$; lymphocytes $<5\%$. Pt: patient; HIV: human immunodeficiency virus.

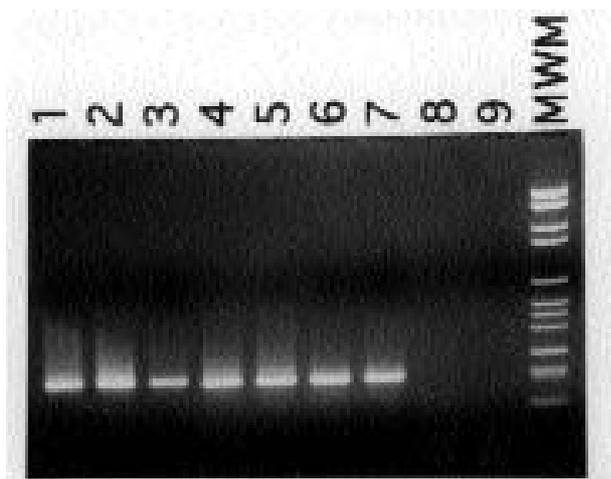


Fig. 2. – Detection of human herpesvirus 8 (HHV8) deoxyribonucleic acid (DNA) by nested polymerase chain reaction (PCR). Ethidium bromide-stained agarose gel showing the PCR products of the bronchoalveolar lavage (BAL) fluid, peripheral blood mononuclear cells and skin biopsy of the kidney transplant recipient (lane 1, 2 and 3, respectively) and a human immunodeficiency virus (HIV) positive patient (lane 4, 5 and 6, respectively). Lane 7: BAL fluid of the other HIV positive patient. Lane 8 and 9: negative controls. Lane 10: molecular weight marker (MWM).

HHV8 DNA was detected in the BAL fluid of the 10 immunocompromised patients without evidence of Kaposi's sarcoma.

Discussion

Although rare in solid organ transplant patients, pulmonary involvement of Kaposi's sarcoma has been described in up to 25% of living AIDS patients with cutaneous disease and in more than 50% of patients in autopsy studies [3, 11–13]. Various opportunistic infections have to be considered in the differential diagnosis of pulmonary infiltrates in immunocompromised patients. At bronchoscopy, extensive forms of Kaposi's sarcoma show typical, hypervascularized endobronchial lesions. However, bronchial and transbronchial biopsies to obtain tissue for the definitive histological diagnosis are usually avoided because of the risk of bleeding. Furthermore, pulmonary parenchyma is the primary site of lung Kaposi's sarcoma and minor endobronchial lesions may be missed at bronchoscopy. Definitive histological diagnosis can be obtained by open lung biopsy or video-assisted thoracoscopic surgery; however, even in obvious lesions, biopsies may not be diagnostic [14]. BAL is safe and is often performed in these critically ill patients to find opportunistic pulmonary infections. In addition, lavage recovers cells from the alveoli and can be directed to the site of primary radiological infiltrates [15]. However, the lavage fluid does not usually show tumour cells in patients with pulmonary Kaposi's sarcoma [16, 17].

The present data show that HHV8 DNA can be detected in the BAL fluid of patients with pulmonary Kaposi's sarcoma. DNA fragments of the new herpesvirus HHV8, have been detected in the various forms of Kaposi's sarcoma [5–9], and there is strong evidence for a causative role of HHV8 in the development of Kaposi's sarcoma [18]. The detection of HHV8 DNA in the BAL fluid,

therefore, strongly supports the pulmonary involvement of Kaposi's sarcoma. It was possible to detect HHV8 DNA in all three samples from patients with pulmonary Kaposi's sarcoma, indicating a high sensitivity of the test. Specificity and positive predictive value for the diagnosis of pulmonary Kaposi's sarcoma, however, remain to be determined. In the BAL fluid of 10 immunocompromised patients without clinical evidence of Kaposi's sarcoma, no HHV8 DNA was found, supporting the idea that the detection of HHV8 is strongly associated with Kaposi's sarcoma and can be applied as a diagnostic test. In addition, the presence of HHV8 DNA in patients without clinical evidence of overt Kaposi's sarcoma may indicate patients at risk of developing the tumour [18].

Our data suggest, that the detection of human herpesvirus 8 deoxyribonucleic acid in the bronchoalveolar lavage fluid can be used as a primary diagnostic tool for the confirmation of pulmonary Kaposi's sarcoma in immunocompromised patients. Larger studies will be needed to precisely define the sensitivity and specificity of the test for the diagnosis of pulmonary Kaposi's sarcoma in the different groups of immunocompromised patients.

References

1. DesJarlais DC, Marmor M, Thomas P, Chamberland M, Zolla-Pazner S, Sencer DJ. Kaposi's sarcoma among four different AIDS risk groups. *N Engl J Med* 1984; 310: 1119.
2. Harwood AR, Osoba D, Hofstader SL, *et al.* Kaposi's sarcoma in recipients of renal transplants. *Am J Med* 1979; 67: 759–765.
3. Ognibene FP, Steis RG, Marcher AM, *et al.* Kaposi's sarcoma causing pulmonary infiltrates and respiratory failure in the acquired immunodeficiency syndrome. *Ann Intern Med* 1985; 102: 471–475.
4. Rucker L, Meador J. Kaposi's sarcoma presenting as homogeneous pulmonary infiltrates in a patient with acquired immunodeficiency syndrome. *West J Med* 1985; 142: 831–833.
5. Chang Y, Cesarman E, Pessin MS, *et al.* Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994; 266: 1865–1869.
6. Dupin N, Grandadam M, Calvez V, *et al.* Herpesvirus-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. *Lancet* 1995; 345: 761–762.
7. Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and those without HIV infection. *N Engl J Med* 1995; 332: 1181–1185.
8. Ambroziak JA, Blackburn DJ, Herndier BG, *et al.* Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. *Science* 1995; 268: 582–583.
9. Su I-J, Hsu YS, Chang Y-C, Wang I-W. Herpesvirus-like DNA sequence in Kaposi's sarcoma from AIDS and non-AIDS patients in Taiwan. *Lancet* 1995; 345: 722–723.
10. Cathomas G, Morris P, Pekle K, Cunningham I, Emanuel D. Rapid diagnosis of cytomegalovirus pneumonia in marrow transplant recipients by bronchoalveolar lavage using the polymerase chain reaction, virus culture, and the direct immunostaining of alveolar cells. *Blood* 1993; 81: 1909–1914.

11. Garay SM, Belenko M, Fazzini E, Schinella R. Pulmonary manifestations of Kaposi's sarcoma. *Chest* 1987; 91: 39–43.
12. Meduri GU, Stover DE, Lee M, Myskowski PL, Caravelli JF, Zaman MB. Pulmonary Kaposi's sarcoma in the acquired immune deficiency syndrome. *Am J Med* 1986; 81: 11–18.
13. Lemlich G, Schwam L, Lebwohl M. Kaposi's sarcoma and acquired immunodeficiency syndrome: postmortem findings in twenty four cases. *J Am Acad Dermatol* 1987; 16: 319–325.
14. Nash G, Fligiel S. Kaposi's sarcoma presenting as pulmonary disease in the acquired immunodeficiency syndrome: diagnosis by lung biopsy. *Hum Pathol* 1984; 15: 999–1001.
15. Cadranet J, Gillet-Juvin K, Antoine M, *et al.* Site-directed bronchoalveolar lavage and transbronchial biopsy in HIV-infected patients with pneumonia. *Am J Respir Crit Care Med* 1995; 152: 1103–1106.
16. Fourret PJ, Touboul JL, Mayaud CM, Akoun GM, Roland J. Pulmonary Kaposi's sarcoma in patients with acquired immune deficiency syndrome: a clinicopathological study. *Thorax* 1987; 42: 262–268.
17. Miller RF, Tomlinson MC, Cottrill CP, Donald JJ, Spittle MF, Semple SJG. Bronchopulmonary Kaposi's sarcoma in patients with AIDS. *Thorax* 1992; 47: 721–725.
18. Whitby D, Howard MR, Tenant-Flowers M, *et al.* Detection of Kaposi's sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* 1995; 346: 799–802.