2. Studies of Cancer in Humans

Because KSHV/HHV8 was discovered only recently, few analytical data are available on its possible association with cancer in humans. Most of the available information derives from case series and case–control and cohort studies. This field of research is rapidly evolving, and the information reported below will be up-to-date for only a limited time.

2.1 Kaposi's sarcoma

A recent monograph on the evaluation of carcinogenic risks to humans dealt with Kaposi's sarcoma and human immunodeficiency viruses in detail (IARC, 1996). For completeness, the sections on the descriptive epidemiology on Kaposi's sarcoma from

that monograph have been incorporated in modified and shortened versions in the present monograph.

2.1.1 *Pathology and clinical disease*

2.1.1.1 *Epidemiological and clinical presentation*

Epidemiologically, Kaposi's sarcoma has been classified into sporadic (classic), endemic (African), epidemic (AIDS-related) and immunosuppression-associated (usually in transplant recipients) types; however, the histopathology of all of these types of Kaposi's sarcoma is identical (Templeton, 1981; Cockerell, 1991). In 1872, Dr Moriz Kaposi, a Hungarian dermatologist, first described an idiopathic, multiple pigmented sarcoma, now called 'classic' or sporadic Kaposi's sarcoma (Kaposi, 1872; Breimer, 1994). For many years, Kaposi's sarcoma was thought to be a lesion that affected predominantly elderly men of Mediterranean and eastern European origin (Dörffel, 1932; Landman *et al.*, 1984; Franceschi & Geddes, 1995). The presence of Kaposi's sarcoma was first noted in Africa in the 1920s (Williams, 1992). In the 1960s, it was reported to comprise up to 8% of malignancies, with endemic foci in parts of Africa (Oettlé, 1962; MacLean, 1963; Hutt & Burkitt, 1965; Williams, 1975). 'Endemic' Kaposi's sarcoma, like the 'classic' type, predominates in men but also occasionally affects children (Hutt & Birkitt, 1965; Williams, 1975; Ziegler & Katongole-Mbidde, 1996). The geographic distribution of endemic Kaposi's sarcoma in Africa prior to the AIDS epidemic was reported to be similar but not identical to that of Burkitt's lymphoma. In the early 1980s, a fourth variant of Kaposi's sarcoma, the 'epidemic' type, heralded the onset of the AIDS epidemic (Hymes *et al.*, 1981). Today, Kaposi's sarcoma is an AIDS-defining condition in HIV-infected individuals.

'Classic' or endemic Kaposi's sarcoma affects predominantly the skin of the lower limbs; internal organs are rarely involved. The disease typically follows an indolent course, patients surviving for an average of 10–15 years (Tappero *et al.*, 1993). Young children tend to have more severe disease than adults, the lesions often affecting the lymphatic system and internal organs rather than the skin, and shorter survival (Oettlé, 1962; Ziegler & Katongole-Mbidde, 1996). Kaposi's sarcoma in immunocompromised individuals — mainly transplant recipients and long-term users of steroids and cytotoxic drugs — often involves internal organs, lymph nodes and the face, mimicking the 'epidemic' type (Tappero *et al.*, 1993). In transplant recipients, Kaposi's sarcoma appears before most other tumours and may regress completely when immunosuppressive therapy is terminated (Penn, 1988a,b). In the epidemic form, the lesions are usually multiple, progress rapidly and may affect any area of the skin as well as internal organs. The tumours frequently begin as dusky-red or violet macules, progressing over weeks or months to plaques and raised, usually painless, firm nodules and plaques. Although the tumour may affect the legs, as seen with 'classic' Kaposi's sarcoma, lesions of the trunk, arms, genitalia and face are also common (Smith & Spittle, 1987). Lymph nodes and the oral cavity, most notably the palate, may be extensively involved. Oral Kaposi's sarcoma is often associated with involvement elsewhere in the gastrointestinal tract (Levine, 1993; Regezi *et al.*, 1993). Pulmonary Kaposi's sarcoma generally presents with short-

ness of breath and cough and is clinically difficult to distinguish from other pulmonary complications of AIDS (Levine, 1993). Median survival following a diagnosis of epidemic Kaposi's sarcoma is 14–18 months (Jacobson *et al.*, 1993; Lundgren *et al.*, 1994, 1995; Luo *et al.*, 1995).

2.1.1.2 *Histology*

The early patch-stage macular lesions contain abnormally shaped, dilated vessels surrounded by a mononuclear-cell infiltrate containing plasma cells; nuclear atypia and mitoses are rarely seen. In the plaque-stage lesions, there is proliferation of spindle-shaped cells in the superficial-to-deep dermis, with rare proliferation of spindle-shaped cells, nuclear atypia and mitoses. Spindle cells, which often surround slit-like vascular spaces, are characteristic of more advanced nodular lesions. The presence of KSHV/-HHV8 in spindle and endothelial cells and the expression of individual viral genes is discussed in section 1.1.6.1.

2.1.2 *Epidemiology*

2.1.2.1 *Incidence and geographical distribution*

The epidemiology of Kaposi's sarcoma was drastically influenced by the onset of the AIDS epidemic in the late 1970s and early 1980s. From being an exceedingly rare condition outside sub-Saharan Africa, its incidence suddenly increased dramatically among certain populations, such as homo- and bisexual men. Throughout the world, the incidence of Kaposi's sarcoma today reflects the burden of the AIDS epidemic, and as such varies considerably. Whereas the incidence appears to have reached a plateau or even a decline in parts of Europe and the United States (Dal Maso *et al.*, 1995), it is apparently rising in some African countries, such as Uganda (Wabinga *et al.*, 1993; Basset *et al.*, 1995; see Table 2).

As mentioned above, Kaposi's sarcoma represented up to 8% of all tumours in some parts of sub-Saharan Africa before the appearance of HIV infection in the 1980s. Relatively high incidence rates were reported from Israel (1970–79, 1.5/100 000 in people of each sex combined; Landman *et al.*, 1984), from Italy (1976–84, 1.05/100 000 in men, 0.27/100 000 in women; Geddes *et al.*, 1994), particularly in the south, and from Sardinia (1977–82, 1.6/100 000 in people of each sex combined; Cottoni *et al.*, 1996). Recently, high rates have also been described in two other island societies, those of Iceland and the Faeroe Islands in the North Atlantic (Hjalgrim *et al.*, 1998). Much lower age-adjusted rates are reported in Australia (1972–82, 0.065/100 000 in men, 0.029/100 000 in women; Kaldor *et al.*, 1994), England and Wales (0.014/100 000 in both men and women) and the United States (1973–79, 0.297/100 000 in men, 0.07/100 000 in women; Biggar *et al.*, 1984). On the basis of data in the Nordic cancer registries, the incidence rose among men from 0.05/100 000 in 1953–57 to 0.18/100 000 in 1978–79; in Nordic women, the corresponding rates were 0.02/100 000 and 0.08/100 000, respectively (Hjalgrim *et al.*, 1996a). Thus, in some countries, modest increases in the incidence of Kaposi's sarcoma were already occurring before the onset of the AIDS epidemic (Dictor & Attewell, 1988; Hjalgrim *et al.*, 1996a).

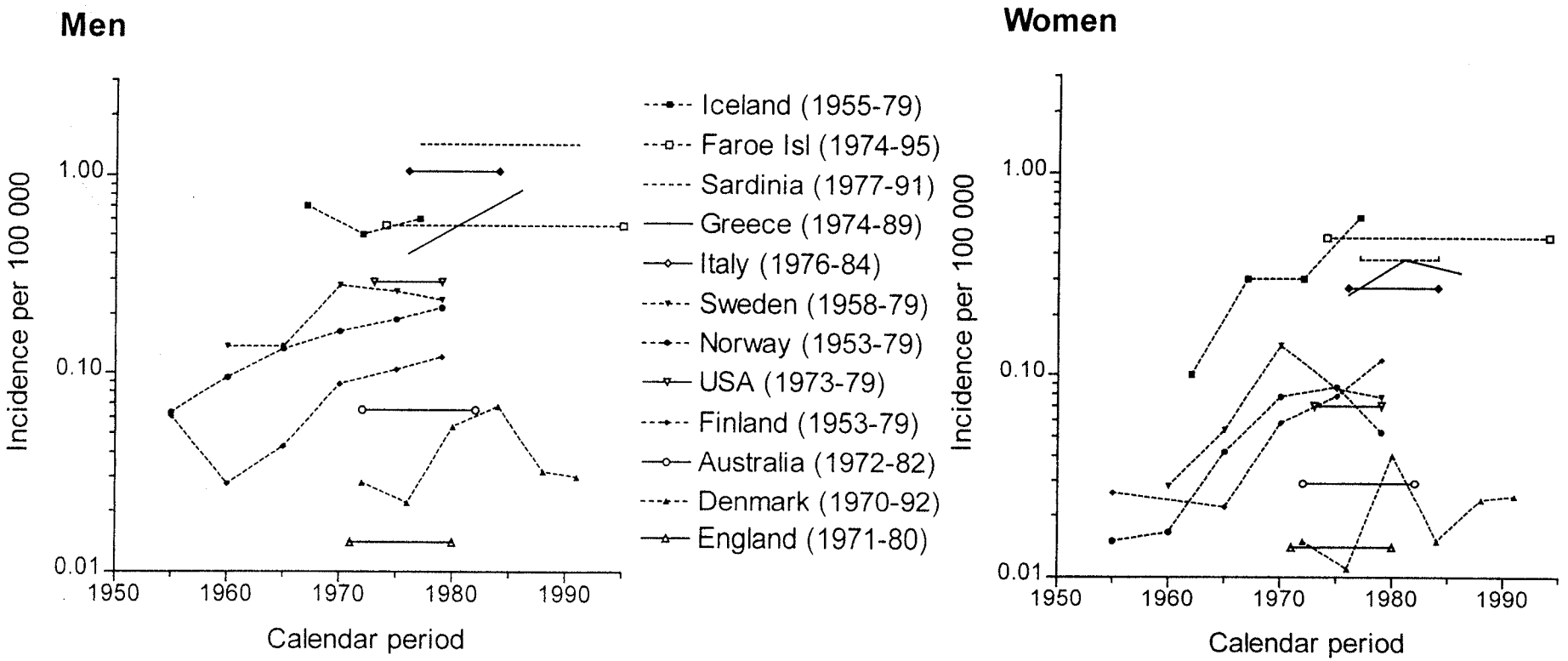
Table 2. Frequency of Kaposi's sarcoma in relation to all cancers in various areas of Africa

Reference	Location	Year(s) of study or report	Percentage of all cancers		
			Men	Women	Both
Oettlé (1962)	Former French Equatorial Africa	1953	–	–	5
	Former French West Africa	1954	–	–	1
	Ghana	1956	–	–	1
	Kenya	1948–61	–	–	2–4
	Mozambique	1958	–	–	2
	Nigeria	1934–44	–	–	2
	South Africa	1951 and 1960	–	–	1–3
	South Africa (Natal)	1957	–	–	1
	United Republic of Tanzania	1960	–	–	3
	Tunisia	1960	–	–	< 1
	Zaire	1956–57	–	–	9–13
	Zambia and Zimbabwe	1949	–	–	1
Hutt & Burkitt (1965)	Uganda	1964	–	–	4
Bayley (1984)	Zaire	1983	–	–	9
Otu (1986)	Nigeria	1986	–	–	15–20
Melbye *et al.* (1987)	Zaire	1984	16	–	–
Ngendahayo *et al.* (1989)	Rwanda	1979–86	–	–	6
Wabinga *et al.* (1993)	Uganda (registry)	1989–91	49	18	–
	Zambia				
Patil *et al.* (1992)	Children	1980–89	–	–	8.8
Patil *et al.* (1995)	Adults	1980–89	–	–	7.0
Bassett *et al.* (1995)	Zimbabwe (registry)	1990–92	23	10	–
Newton *et al.* (1996)	Rwanda (registry)	1991–93	10	3	–
Sitas *et al.* (1996)	South Africa (registry)				
	Black	1990–91	0.54	0.14	0.3
	White	1990–91	0.12	0.03	0.1

–, not reported

Studies in Australia, Denmark, the United Kingdom and the United States have shown an increased risk for Kaposi's sarcoma among persons of certain ethnicities from Central and East Africa, eastern Europe and Mediterranean countries and people of Jewish descent (Laor & Schwartz, 1979; DiGiovanna & Safai, 1981; Friedman-Birnbaum *et al.*, 1990; Grulich *et al.*, 1992; Kaldor *et al.*, 1994; Hjalgrim *et al.*, 1996b; Figure 4).

Figure 4. Reported incidence rates of 'classic' Kaposi's sarcoma



From Hjalgrim *et al.* (1998)

Dotted lines indicate world-standardized rates and solid lines the rates standardized to local populations; calendar period reflects period of observations.

2.1.2.2 *Demographic variations*

Formerly a tumour affecting predominantly the elderly (Oettlé, 1962; Templeton, 1981; Hutt, 1984, Geddes *et al.*, 1994; Hjalgrim *et al.*, 1996a), Kaposi's sarcoma has shown a substantial alteration in age distribution in recent years, in both developed and developing countries. Whereas the median age in developed countries before the AIDS epidemic was over 70 years, it is now in the late thirties.

In Europe and the United States, childhood Kaposi's sarcoma is very rare, even since the advent of the AIDS epidemic. In the early 1990s, the age-specific incidence rates in African countries such as Uganda and Zimbabwe showed a modest peak for children aged zero to four years, a decline until age 15 years and then the main peak at age 35–39 years in men and 25–29 years in women (Wabinga *et al.*, 1993; Basset *et al.*, 1995).

Studies based on registry data have found a male:female ratio of 'classic' Kaposi's sarcoma of 2–3:1 (Biggar *et al.*, 1984; Franceschi & Geddes, 1995; Hjalgrim *et al.*, 1996a). In a study based on data from the Nordic cancer registries, the male excess was primarily restricted to men over 60 years of age (Hjalgrim *et al.*, 1996a). In Africa, male:female ratios greater than 10 reported in early studies (Wahman *et al.*, 1991) have since declined to about 3:1 (Wabinga *et al.*, 1993; Basset *et al.*, 1995; Newton *et al.*, 1996).

2.1.2.3 *Behavioural factors*

Case reports suggest that Kaposi's sarcoma may occur more frequently than expected in HIV-uninfected homo- and bisexual men (Friedman-Kien *et al.*, 1990; Peterman *et al.*, 1991) and at a rate equivalent to the total number of cases diagnosed among all men under 50 years of age per year before the AIDS epidemic (Biggar *et al.*, 1984). [The Working Group noted that surveillance bias could entirely explain this observation.] Furthermore, in an analysis of 'classic' Kaposi's sarcoma in Denmark, men who had never married (used as a rough surrogate for homosexuality) were 19 times more at risk for the disease than men who had married (Hjalgrim *et al.*, 1996b); a similar analysis of data in the United States, however, showed no such difference (Biggar & Melbye, 1996).

The risk for Kaposi's sarcoma varies greatly among the different groups at risk for HIV transmission, being particularly high in homo- and bisexual men (IARC, 1996). This elevated risk is seen even among men aged 13–24 and suggests a rapid increase in risk after homosexual contact. Beral *et al.* (1990) found that 13 616 of 88 739 (15%) AIDS patients in the United States developed Kaposi's sarcoma, the proportion varying from 21% of homo- or bisexual men to 3% of heterosexuals, 2% of intravenous drug users, 3% of transfusion recipients, 1% of haemophiliacs and 1% of children infected by perinatal transmission. Furthermore, women with AIDS who were sexual partners of bisexual men were more likely to have Kaposi's sarcoma than women who were partners of intravenous drug users (Peterman *et al.*, 1993; Serraino *et al.*, 1995). Even among homo- and bisexual men, the risk for Kaposi's sarcoma is not uniform: Schechter *et al.* (1991) conducted an analysis of all AIDS-associated cases of Kaposi's sarcoma among homo- and bisexual men in Canada between 1980 and 1989 and found that the pro-

portion of cases among AIDS patients had a strong geographical association with the original centres of the AIDS epidemic in Canada. Furthermore, homosexual men born between 1945 and 1954 were more likely to present with Kaposi's sarcoma, consistent with the hypothesis of an environmental cofactor with higher levels of exposure. In another study from the same group, Archibald *et al.* (1990) found that 56% of Canadian homosexual men with AIDS who developed Kaposi's sarcoma and only 21% of those who developed AIDS but not Kaposi's sarcoma reported that they had had more than 20 sexual partners from large cities in the United States (odds ratio, 4.6; 95% confidence interval [CI], 1.6–13). Similarly, homosexual men with AIDS in the United Kingdom were more likely to have Kaposi's sarcoma if they had had sexual contact with an American (31%) or African (26%) man than if they had not (19%) ($p < 0.05$) (Beral *et al.*, 1991). Furthermore, Peterman et *al.* (1993) found that Kaposi's sarcoma was more frequently part of the AIDS definition in homosexual men from California and New York than in homosexual men from the rest of the United States.

Most analyses of the number of sexual partners of homo- and bisexual men with Kaposi's sarcoma and of those with other manifestations of AIDS (Haverkos *et al.*, 1985; Goedert *et al.*, 1987; Archibald *et al.*, 1990; Armenian *et al.*, 1993), but not all (Lifson *et al.*, 1990a,b), found that patients with Kaposi's sarcoma had had a larger number of sexual partners. Patients with this cancer have also been reported to be more likely to have a history of sexually transmitted disease (Goedert *et al.*, 1987; Armenian *et al.*, 1993). In a case–control study in New York City, United States, Kaposi's sarcoma was found to be significantly associated with receptive anal intercourse (Marmor *et al.*, 1982; Jaffe *et al.*, 1983). Several authors have subsequently reported an increased risk for Kaposi's sarcoma among HIV-positive men whose sexual practices involve faecal contact (Beral *et al.*, 1992; Darrow *et al.*, 1992). The possible association between insertive oral–anal contact and the risk for Kaposi's sarcoma remains controversial, some studies showing a possible association (Archibald *et al.*, 1990; Beral *et al.*, 1992; Darrow *et al.*, 1992) and others not (Lifson *et al.*, 1990b; Elford *et al.*, 1992; Page-Bodkin *et al.*, 1992; Armenian *et al.*, 1993; Kaldor et al., 1993). Casabona *et al.* (1991) noted that the fraction of AIDS cases with Kaposi's sarcoma was similar in southern Europe, with a relatively high incidence of 'classic' Kaposi's sarcoma, and in northern Europe, with a relatively low incidence. In Uganda, increased risk for Kaposi's sarcoma was seen in HIV-seropositive adults of each sex who had one rather than several spouses or a history of sexually transmitted diseases, and especially those who were relatively affluent, well-educated, had travelled and had spent increasing time in contact with water (Ziegler *et al.*, 1997).

In conclusion, men who develop Kaposi's sarcoma tend to be more sexually active and to have more sexual partners from epicentres of the AIDS epidemic. In conjunction with the much higher risk for Kaposi's sarcoma among homosexual men than among other HIV transmission groups, these data indicate that an infectious sexually transmitted agent (independent of HIV) is associated with Kaposi's sarcoma. Transmission of such an agent via the blood is apparently less common, since Kaposi's sarcoma occurs in only 3% of people who acquire HIV through a blood transfusion.

2.1.2.4 *Second primary malignancies after Kaposi's sarcoma*

An association between Kaposi's sarcoma and lymphomas has been suspected for many years. Both tumours occur in association with immunosuppression and can occur in the same individual. In a hospital-based cohort of 72 patients with 'classic' Kaposi's sarcoma in New York, United States, Safai *et al.* (1980) counted a total of nine lymphoid malignancies, including four non-Hodgkin's lymphomas, during 581 person–years of follow-up. This corresponds to a significantly (20-fold) increased risk for these malignancies over that of the background population. Few studies have addressed 'classic' Kaposi's sarcoma because of its infrequency. Three deaths from non-Hodgkin's lymphoma were reported among 68 patients with Kaposi's sarcoma in the United Kingdom (Grulich *et al.*, 1992). In contrast, two larger population-based studies of 492 American and 204 Italian subjects with 'classic' Kaposi's sarcoma did not confirm the suspected association (Biggar *et al.*, 1994; Franceschi *et al.*, 1996).

2.1.3 *Case series and case–control studies*

2.1.3.1 *Detection of KSHV/HHV8 DNA in tumour tissue*

Published case series and case–control studies on the detection of KSHV/HHV8 DNA in Kaposi's sarcoma tissue are summarized in Table 3. Many of these studies are small and/or included heterogeneous controls.

KSHV/HHV8 DNA is found in nearly all Kaposi's sarcoma tissues, despite differences in detection methods and in the quality or preservation of tumour material. In 28 studies in which the detection of KHSV/HHV8 DNA was described in Kaposi's sarcoma tissues (Table 3), KSHV/HHV8 was identified in 735 of 794 (91%) Kaposi's sarcoma analysed. The rates reported in one of the studies (Noel *et al.*, 1996) were very different from those in the other studies; when these results were excluded, the percentage positivity rose to 96% (686/716). The detection rate was similar whether the patients were HIV-infected (391/417; 94%) or uninfected (335/368; 91%). Furthermore, KSHV/HHV8 was found in all four epidemiological forms of Kaposi's sarcoma, with no indication of significant differences in the detection rate in the four types. With the exception of a few studies (Rady *et al.*, 1995; Gyulai *et al.*, 1996a,b), little or no evidence of KSHV/HHV8 DNA has been found in tumours other than Kaposi's sarcoma. The exceptions include primary effusion lymphomas and Castleman's disease. The recent identification of KSHV/HHV8 in bone-marrow dendritic cells of myeloma patients awaits confirmation (Rettig *et al.*, 1997; see section 2.2.3). These and other conditions potentially associated with KSHV/HHV8 are discussed in subsequent sections (see also Table 3).

A major difficulty in assessing associations with disease on the basis of detection of DNA is in selecting appropriate control tissues in order to identify differences in infection rate. In the initial description of the virus, Chang *et al.* (1994) found that 25 of 27 AIDS-associated Kaposi's sarcomas contained KSHV/HHV8 DNA in comparison with three of 27 lymphomas from AIDS patients [odds ratio, 100, $p < 10^{-7}$] (some of whom may have had Kaposi's sarcoma as a secondary malignancy), none of 29 lymphomas from non-AIDS patients and none of 49 consecutive surgical biopsy samples ($p < 10^{-5}$).

Table 3. Presence of KSHV/HHV8 DNA in Kaposi's sarcoma (KS) tissue, other tissues from Kaposi's sarcoma patients and tissues from subjects without Kaposi's sarcoma, detected by polymerase chain reaction (PCR)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Chang *et al.* (1994)	AIDS-KS	25/27			AIDS		Fresh frozen
					Lymphoma	3/27	
					Lymph node	3/12	
					Total	6/39	
					Non-AIDS		
					Lymphoma	0/29	
					Lymph node	0/7	
					Vascular tumour	0/5	
					Opportunistic infections	0/13	
					Surgical biopsy	0/49	
					Total	0/103	
Su *et al.* (1995)	AIDS-KS	4/4	None		AIDS lymph node	0/5	
	Non-AIDS KS	2/3			Benign hyperplasia	0/10	
	Total	6/7			B-Cell lymphoma	0/12	
					T-Cell lymphoma	0/10	
					Total	0/37	
Dupin *et al.* (1995a)	Classic KS	5/5	Skin, classic	3/3	Various tissues, HIV-negative	0/6	Snap-frozen
	AIDS-KS	4/4	Skin, AIDS	2/3			
	Total	9/9	Total	5/6			
Boshoff *et al.* (1995a)	Classic KS	16/17	None		Angioma/angiosarcoma	0/4	Fresh-frozen or
	Transplant KS	8/8			Skin naevi	0/3	paraffin-embedded
	AIDS-KS	14/14			Granulomatous tissue	0/4	(nested PCR)
	HIV-negative homosexual man	1/1			Total	0/11	
	Total	39/40					

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Ambroziak *et al.* (1995)	AIDS-KS, homosexual men	12/12	None		None		
	HIV-negative homosexual man	1/1					
	Total	13/13					
Moore & Chang (1995)	AIDS-KS	10/11	Skin, AIDS	1/7	Skin from healthy subjects	1/11	Fresh-frozen
	Classic KS	6/6	Skin, classic	1/5	PBMC from healthy subjects	0/10	
	HIV-negative homosexual men	4/4	Skin, HIV-negative	1/2	Total	1/21	
	Total	20/21					
Lebbé *et al.* (1995)	Immunosuppressed KS	1/1	Skin, HIV-negative	3/9	None		
	Classic KS	10/10					
	African KS	3/3					
	AIDS-KS	2/2					
	Total	16/16					
Schalling *et al.* (1995)	AIDS-KS	25/25	Pyothorax-related B-cell lymphoma-KS, HIV-positive	0/3	HIV-positive		
	African KS	18/18			Pyothorax-related B-cell lymphoma (PBMC)	0/13	
	Classic KS	3/3			Lymph node	0/8	
	Total	46/46			Total	0/21	
			Skin, HIV-negative	0/2	HIV-negative		
					Pyothorax-related B-cell lymphoma (PBMC)	0/12	
					Skin, non-KS patient	0/1	
					Haemangioma	0/1	
					Pyogenic granuloma	0/1	
					Total	0/15	

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Chang *et al.* (1996b)	AIDS-KS African KS Total	22/24 17/20 39/44	None		HIV-positive HIV-negative Total	1/7 2/15 3/22	Paraffin-embedded Negatives retested by nested PCR
Chuck *et al.* (1996)	African KS HIV-negative homosexual men Total	4/4 1/2 5/6	None		None		Fresh-frozen (endemic) or paraffin-embedded (HIV-negative homosexual men)
O'Neill *et al.* (1996)	AIDS-KS	7/7			HIV-negative	0/1	Nested PCR Fresh-frozen or paraffin-embedded
Buonaguro *et al.* (1996)	African KS Classic KS Immunosuppressed KS AIDS-KS Total	12/12 28/28 2/2 19/19 61/61	Skin, HIV-negative	9/13	Reduction mammoplasty Penile carcinoma biopsies Xeroderma pigmentosum skin cancer Xeroderma pigmentosum autologous normal skin PBMC of HIV-positive patients Total	0/3 0/4 0/5 0/5 0/15 0/32	Snap-frozen
Cathomas *et al.* (1996)	AIDS-KS Classic KS Transplant KS Total	9/9 12/12 1/1 22/22	None		Other skin lesions, HIV-positive Other skin lesions, HIV-negative Total	0/4 0/10 0/14	Paraffin-embedded Nested PCR

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Gaidano *et al.* (1996b)	AIDS-KS	35/35	AIDS, skin (PCR; only 3/6 by Southern blot)	6/6	Hodgkin's disease Primary effusion lymphoma Other non-Hodgkin's lymphoma Persistent generalized lymph-adenopathy Anogenital neoplasia Total	0/3 3/3 0/28 0/15 0/14 3/63	Fresh-frozen (a few paraffin-embedded)
Jin *et al.* (1996a)	AIDS-KS Classic KS Total	5/5 12/12 17/17	None		Haemangiosarcoma Haemangioma Lymphangioma Lymphangiomatosis Pyogenic granuloma Haemangiopericytoma Kimura's disease Lymphangiomyomatosis Total	0/15 0/75 0/15 0/2 0/25 0/3 0/2 0/1 0/138	Paraffin-embedded
Dictor *et al.* (1996)	Classic KS AIDS-KS Total	35/40 14/14 49/54	None		Endothelial lesions	0/86	Paraffin-embedded
Marchioli *et al.* (1996)	AIDS-KS Classic KS African KS HIV-negative homosexual men Total	28/28 7/8 7/10 2/2 44/48	HIV-positive Serum Plasma	 3/28 0/13	Normal PBMC Normal skin Paediatric lymphomas Adult lymphomas Carcinomas Total	0/163 0/10 0/8 0/37 0/12 0/230	Fresh-frozen and paraffin embedded

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Luppi *et al.* (1996a)	Classic KS AIDS-KS Total	15/22 3/4 18/26	None		Normal PBMC Normal salivary glands Normal saliva samples Hyperplastic tonsils Total	0/13 0/9 0/6 2/11 2/39	Paraffin-embedded
McDonagh *et al.* (1996)	KS	9/9	None		Angiosarcoma Haemangioma Haemangiopericytoma Total	7/24 1/20 0/6 8/50	Fresh-frozen and paraffin-embedded
Corbellino *et al.* (1996a,b)	AIDS-KS	7/7	HIV-positive Lymphoid tissue Prostate glands Uninvolved skin Bone marrow Paravertebral sensory lumbar ganglion	 7/7 5/5 3/5 2/3 7/7	AIDS patients HIV-positive Paravertebral sensory lumbar ganglion HIV-negative Paravertebral sensory lumbar ganglion	0/6 0/4 0/3	Snap-frozen
Lebbé *et al.* (1997a)	Classic KS African KS Castleman's disease HIV-negative homosexual men Immunosuppressed/ transplant KS Total	16/16 3/3 1/1 3/3 2/2 25/25	Skin, classic Skin, African Skin, HIV-negative homosexual men Skin, induced Total	10/13 2/3 1/3 1/1 14/20	HIV-negative Dermatology biopsies Reduction mammaplasties Total	 0/10 0/5 0/15	Fresh-frozen[a] Nested PCR
Huang *et al.* (1997)	AIDS-KS HIV-negative	12/12 2/2	HIV-positive Normal skin HIV-negative Normal skin	 5/12 1/2	HIV-positive Intravenous drug users (PBMC) HIV-negative Healthy (PBMC)	 0/5 0/5	Fresh frozen

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Albini *et al.* (1996a)	AIDS-KS African KS Classic KS Transplant KS	59/59 21/21 32/33 6/7	Skin, HIV-positive Skin, HIV-negative	0/3 3/9	HIV-positive Skin Other tissue Lymphoma HIV-negative Lymphoma	 0/4 0/2 2/10 1/34	Fresh or paraffin embedded
Uthman *et al.* (1996)	AIDS-KS Classic KS	23/23 5/5	None		HIV-positive Skin lesions HIV-negative Leiomyoma Melanoma Basal-cell carcinoma Pityriasis rosea Molluscum contagiosum Psoriasis vulgaris Viral warts Pseudolymphoma Total	 0/28 0/3 0/8 0/11 0/4 0/6 0/6 0/8 0/7 0/53	Nested PCR
Decker *et al.* (1996)	AIDS-KS	5/5	None		HIV-negative Allograft (PBMC)[b] Healthy (PBMC)[b]	 4/5 3/5	Fresh tissue

Table 3 (contd)

Reference	KS tissue	KSHV/ HHV8 (positive/ total)	Other tissue from KS patients	KSHV/ HHV8 (positive/ total)	Tissue from subjects without KS	KSHV/ HHV8 (positive/ total)	Comments
Li *et al.* (1996)	AIDS-KS Classic KS	6/6 3/3	None		HIV-negative Skin Verrucea vulgaris Total	 0/3 0/2 0/5	Fresh tissue
Noel *et al.* (1996)	AIDS-KS HIV-negative	41/61 8/17	Cutaneous and others HIV-negative	1/19	HIV-negative Cutaneous and others	 1/26	Paraffin-embedded

Modified from Olsen and Moore (1997)
PBMC, peripheral blood mononuclear cells; HIV, human immunodeficiency virus; PCR, polymerase chain reaction
[a] Positivity rate dependent on amount of added template DNA
[b] Testing of multiple samples from same individual

Similarly, Boshoff *et al.* (1995a) found KSHV/HHV8 in 39 of 40 Kaposi's sarcoma lesions of all types but in none of 11 pathologically similar tissues (angioma/angiosarcoma, skin naevi and granulomatous tissues). In a case–control analysis of Kaposi's sarcoma tissues from HIV-positive and HIV-negative persons and skin and PBMC from HIV-negative persons, Moore and Chang (1995), who were unaware of the case or control status of the subjects, found viral DNA in 20 of 21 Kaposi's sarcoma lesions and in only one of 21 control tissues (odds ratio, 400; 95% CI, 19–17 000). Jin *et al.* (1996a) and Dictor *et al.* (1996) compared Kaposi's sarcomas from HIV-positive and HIV-negative persons with a wide variety of tissues resembling Kaposi's sarcoma, including those of endothelial origin and angiogenic and skin tumours. They found viral DNA in 88–100% of 71 Kaposi's sarcoma lesions and none of 224 control tissues. Table 3 also gives a partial list of studies in which control tissues from nearly all organ systems were examined by PCR. Overall, 34 of 1128 (3%) tissues not from Kaposi's sarcomas contained KSHV/HHV8 DNA. Several of these samples were primary effusion lymphomas (described in section 2.2.1), which are also associated with KSHV/HHV8 (Chang *et al.*, 1994; Gaidano *et al.*, 1996b), and the results in two studies (Decker *et al.*, 1996; McDonagh *et al.*, 1996) accounted for nearly half of all the positive findings.

The amount of viral DNA detected by Southern blot in Kaposi's sarcoma lesions averages from undetectable to an estimated 10–20 viral genome copies per cell equivalent. Similarly, KSHV/HHV8 DNA is readily detectable by PCR in DNA extracted from fresh tissue, whereas nested PCR is often required to obtain positive results from fixed, paraffin-embedded tissue. These conditions probably play a role with respect to the differences in positivity rate observed by different investigators.

Several groups reported a higher detection rate of KSHV/HHV8 by PCR in late plaque or nodular stages than in early or patch-stage Kaposi's sarcoma (Luppi *et al.*, 1996a; Noel *et al.*, 1996).

2.1.3.2 *Detection of KSHV/HHV8 DNA in peripheral blood mononuclear cells*

The rate of detection of HHV8 in PBMC from Kaposi's sarcoma patients varies widely (Table 4); however, most of the larger studies suggest that about 50% of PBMC samples from Kaposi's sarcoma patients give positive results when tested by nested PCR under standard conditions, e.g. using 100–500 ng of PBMC DNA (Whitby *et al.*, 1995; Bigoni *et al.*, 1996; Lefrère *et al.*, 1996; Moore *et al.*, 1996c; Lebbé *et al.*, 1997a). When assaying for the presence of KSHV/HHV8 DNA in PBMC, it is important to use sufficient DNA to detect a low copy number of viral DNA (Decker *et al.*, 1996; Blackbourn *et al.*, 1997).

As shown in Table 4, 42% (161/386) of HIV-positive patients and 53% (47/89) of HIV-negative patients with Kaposi's sarcoma had detectable KSHV/HHV8 DNA in their PBMC. Albini *et al.* (1996a) reported an exceptionally low positivity rate (3/54) for KSHV/HHV8 in PBMC from HIV-infected Kaposi's sarcoma patients. If these exceptional results are excluded, the positivity rate among HIV-infected patients in the remaining studies was 48% (158/332). In a study of Mediterranean Kaposi's sarcoma patients, Brambilla *et al.* (1996) reported a particularly high concordance for patients with

Table 4. Detection of KSHV/HHV8 DNA in peripheral blood mononuclear cells (PBMC)

Reference	KS type	KSHV/HHV8 (positive/ total)	Control PBMCs	KSHV/HHV8 (positive/ total)	Odds ratio	95% CI	Comments
Collandre *et al.* (1995)	AIDS-KS	2/10	HIV-positive, no KS	0/9			PCR Southern blot
Ambroziak *et al.* (1995)	AIDS-KS	7/7	HIV-positive, no KS	0/6			
	HIV-negative KS	3/3	HIV-negative, no KS	0/14			
	Total	10/10	Total	0/20			
Whitby *et al.* (1995)	AIDS-KS	24/46	HIV-positive, no KS	11/143			Nested PCR
			Oncology patients	0/26			
			Blood donors	0/134			
			Total	11/303			
Moore *et al.* (1996c) and correction by Parry & Moore (1997)	AIDS-KS	11/21	Homo- or bisexual AIDS patients, no KS	3/23	7.3	1.4–47.9	Nested PCR
			Haemophiliac AIDS patients, no KS	0/19	21.8	2.4–978	
			Total	3/42			
Marchioli *et al.* (1996)	AIDS-KS	46/99	HIV-positive	0/64			PCR Southern blot
	HIV-negative homosexual men	0/2	HIV-negative	0/163			
			Total	0/227			
	Total	46/101					
Humphrey *et al.* (1996)	AIDS-KS	34/98	HIV-positive, no KS	12/64	2.3	1.0–5.3	PCR Southern blot
			HIV-negative, no KS	0/11			
			Total	12/75			
Decker *et al.* (1996)	AIDS-KS	8/9	Allograft patients	4/5			Multiple samples tested to obtain positive results in controls
			Healthy donors	3/5			
			Total	7/10			

Table 4 (contd)

Reference	KS type	KSHV/HHV8 (positive/ total)	Control PBMCs	KSHV/HHV8 (positive/ total)	Odds ratio	95% CI	Comments
Lebbé *et al.* (1997a)	Classic KS	9/18	Blood donors	0/20			PCR Southern blot Nested PCR (32% of KS subjects positive with unnested PCR)
	African KS	2/3					
	Castleman's disease	0/1					
	HIV-negative homosexual men	1/4					
	Immunosuppressed or transplant KS	0/2					
	Total	12/28					
Albini *et al.* (1996a)	AIDS-KS	3/54	Healthy donors	0/4			
	Classic KS	1/6					
	Transplant	1/2					
	Total	5/62					
Brambilla *et al.* (1996)	Classic KS		None				
	Stage I	9/16					
	Stage II	1/5					
	Stage III	6/11					
	Stage IV	8/8					
	Total	24/40					
Heredia *et al.* (1996)	AIDS-KS	2/2	HIV-positive	0/2			
	HIV-negative KS	6/8	HIV-negative	0/8			
	Total	8/10	Total	0/10			
Howard *et al.* (1995)	AIDS-KS	11/17	AIDS, no KS	0/6			Nested PCR
Huang *et al.* (1997)	AIDS-KS	3/12	None				
Lefrère *et al.* (1996)	AIDS-KS	10/11	AIDS, no KS	1/14			
			HIV-positive (asymptomatic)	1/45			
			HIV-negative	0/20			

HIV, human immunodeficiency virus; PCR, polymerase chain reaction

disseminated Kaposi's sarcoma, 100% of whom had detectable KSHV/HHV8 DNA in their PBMC.

Under the same conditions, KSHV/HHV8 is only rarely detected in PBMC from blood donors or other subjects without Kaposi's sarcoma (Table 4; Ambroziak *et al.*, 1995; Whitby *et al.*, 1995; Bigoni *et al.*, 1996; Lefrère *et al.*, 1996; Moore *et al.*, 1996c; Blackbourn *et al.*, 1997).

2.1.3.3 *Detection of KSHV/HHV8 DNA in other tissues*

Similarly high rates of KSHV/HHV8 positivity have been observed in uninvolved skin of Kaposi's sarcoma patients (Moore & Chang, 1995; Albini *et al.*, 1996a; Buonaguro *et al.*, 1996; Corbellino *et al.*, 1996b; Huang *et al.*, 1997), with no significant difference between those who are HIV-infected and uninfected (Table 3). In contrast, skin biopsy samples from non-Kaposi's sarcoma patients very rarely have detectable KSHV/HHV8.

KSHV/HHV8 is also detected to varying degrees in other tissues (e.g. semen, serum, prostate glands, bone marrow) from Kaposi's sarcoma patients (Table 5; see also section 1.1.6). Corbellino *et al.* (1996a) detected KSHV/HHV8 DNA by nested PCR in all of seven paravertebral sensory lumbar ganglia from Kaposi's sarcoma patients and in none of similar materials from patients without this tumour. Howard *et al.* (1995) detected KSHV/HHV8 by nested PCR in bronchoalveolar lavage fluid from 11 of 14 HIV-positive men with both cutaneous and pulmonary Kaposi's sarcoma, but in none of six men with only cutaneous manifestations and in one of 19 HIV-positive men with no evidence of either cutaneous or pulmonary Kaposi's sarcoma; the last case presented three months later with pulmonary manifestations of Kaposi's sarcoma.

2.1.3.4 *Serology*

Serological assays have been developed to detect antibodies to either a LANA (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996) and/or a defined (Miller *et al.*, 1996; Simpson *et al.*, 1996) or undefined (Lennette *et al.*, 1996; Ablashi *et al.*, 1997) structural ('lytic') antigen of KSHV/HHV8 (Rickinson, 1996). The exact prevalence of KSHV/HHV8 infection in northern Europe and the United States, as measured by these assays, is still controversial and ranges from 0–20%. The available evidence indicates considerable geographical variation. Infection with KSHV/HHV8 seems to be widespread in several African countries (50–70%) and more common in some Mediterranean countries than in northern Europe or the United States (for a more detailed discussion of currently available serological assays and KSHV/HHV8 prevalence, see section 1.2.2).

Studies in which the association between Kaposi's sarcoma and KSHV/HHV8 antibodies was analysed are summarized in Table 6. Irrespective of differences in the assays used, most of the rates reported were more than 80% seropositivity in all epidemiological types of Kaposi's sarcoma.

Most studies in which antibodies to LANA were measured by immunofluorescence or western blotting (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996) are consistent in detecting antibodies in 80–90% of Kaposi's sarcoma patients but in only

Table 5. Detection of KSHV/HHV8 in semen and prostate tissue

Reference (country or region)	KS type	Positive/ total	Control/other populations	Positive/ total	Method
Semen					
Ambroziak *et al.* (1995) (USA)	AIDS-KS	0/4	–	–	PCR hybridization detection of amplicons
Monini *et al.* (1996a) (Italy)	–	–	Semen donors	5/10[a] 30/33[b]	Nested PCR
Corbellino *et al.* (1996c) (Italy)	–	–	Semen donors	0/20	Nested PCR
Monini *et al.* (1996b) (Italy)	AIDS-KS	1/5	AIDS without KS Semen donors	0/10 3/13	Nested PCR PCR
Marchioli *et al.* (1996) (USA)	AIDS-KS Classic KS	4/31 0/2	–	–	PCR[a]
Gupta *et al.* (1996) (USA)	AIDS-KS	2/14	AIDS without KS	0/10	Nested PCR; sampling at two times
Howard *et al.* (1997) (United Kingdom)	AIDS-KS	3/15	AIDS without KS Semen donors	3/9 0/115	Nested PCR
Huang *et al.* (1997) (USA)	AIDS-KS Classic KS	3/12 0/2	AIDS without KS HIV-positive intravenous drug users HIV-negative	0/4 0/5 0/7	PCR hybridization and in-situ PCR detection of amplicons
Viviano *et al.* (1997) (Sicily)	AIDS-KS	1/1	AIDS without KS HIV-negative	1/10 6/45	Nested PCR

Table 5 (contd)

Reference (country or region)	KS type	Positive/ total	Control/other populations	Positive/ total	Method
Prostate tissue					
Monini *et al.* (1996a) (Italy)	–	–	Benign hyperplasia and carcinoma	7/16	Nested PCR
Corbellino *et al.* (1996c) (Italy)	AIDS-KS	5/5	–	–	
Corbellino *et al.* (1996c) (Italy)	–	–	AIDS without KS HIV-negative	0/20 0/8	Nested PCR
Tasaka *et al.* (1996, 1997) (Italy and USA)	–	–	Prostate biopsy	0/52	Nested PCR
Monini *et al.* (1996b) (Italy)	–	–	Hyperplastic prostate biopsy	2/7	Nested PCR
Lebbé *et al.* (1997b) (France)	–	–	Benign hyperplasia and carcinoma	0/19	Nested PCR
Staskus *et al.* (1997) (USA)	–	–	Prostate biopsy	12/16	In-situ hybridization
Rubin *et al.* (1997) (USA)	–	–	Prostate biopsy	0/45	PCR[a]

Modified from Blackbourn & Levy (1997)
[a]Blinded analysis
[b]Unblinded analysis

Table 6. Serological studies of KSHV/HHV8 infection

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Gao *et al.* (1996a,b)	BC-1 LANA Western blot	USA		AIDS without KS, US homosexual men	7/40	ND
		AIDS (homosexuals)	32/40	HIV-positive US haemophiliacs	0/20	ND
		Italy		Ugandan cancer patients		
		AIDS	11/14	HIV-positive	25/35	ND
		Classic	11/11	HIV-negative	29/47	ND
		Uganda		Blood donors		
		AIDS	16/18	USA	0/122	ND
		Endemic	1/1	Italy	4/107	ND
	BCP-1 LANA IFA	USA		US EBV-positive	0/69	ND
		AIDS	35/40	AIDS without KS, US homosexual men	12/40	ND
		Italy		HIV-positive US haemophiliacs	0/20	ND
		AIDS	10/14	Ugandan cancer patients		
		Classic	11/11	HIV-positive	18/35	ND
		Uganda		HIV-negative	24/47	ND
		AIDS	14/18	Blood donors		
		Endemic	1/1	USA	0/122	ND
				Italy	4/107	ND
				US EBV-positive	0/69	ND
Kedes *et al.* (1996)	BCBL-1 LANA IFA	AIDS	37/45	HIV-positive US haemophiliacs	9/300	ND
		Classic	1/1	HIV-positive US transfusion recipients	2/44	ND
				Sexually transmitted disease clinic attendees		
				HIV-positive (bi/homosexual)	13/37	ND
				HIV-positive (heterosexual)	0/9	ND
				HIV-negative	10/130	ND
				US blood donors		
				HIV-positive	41/138	ND
				HIV-negative	2/141	ND
Kedes *et al.* (1997)	BCBL-1 LANA IFA	AIDS (women)	2/2	HIV-positive women	12/302	ND
				HIV-negative women	1/84	ND

Table 6 (contd)

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Lennette *et al.* (1996)	BCBL-1 LANA IFA	US (AIDS and a few classic)	47/91	HIV-positive, US homosexual men	19/94	87/94
				HIV-positive male intravenous drug users	0/13	3/13
		African endemic	28/28	HIV-positive women	0/33	7/33
	BCBL-1 TPA induced lytic IFA	US (AIDS and a few classic)	87/91	Children < 16 years	0/263	10/263
				Adults > 16 years	0/174	33/174
		African endemic	28/28	US blood donors	0/44	9/44
				US women	0/54	15/54
				Haemophiliacs	0/83	10/83
				Various tumours (Dominican Republic, Sweden, Malaysia and Netherlands)	0/147	19/147
				EBV-positive patients	0/40	8/40
				Rheumatoid arthritis	0/20	5/20
				Zimbabwe	4/37	12/37
				Nigeria	3/52	29/52
				Zaire	4/16	13/16
				Uganda	9/82	63/82
				The Gambia	11/45	38/45
				Ivory Coast	4/7	7/7
				Haiti	0/52	15/52
				Dominican Republic	0/40	5/40
				Guatemala	0/20	2/20

Table 6 (contd)

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Simpson *et al.* (1996)	ORF 65 ELISA	AIDS (USA, UK)	46/57	HIV-positive homosexual men	10/33	
		AIDS (Uganda)	14/17	HIV-positive women with sexually transmitted diseases	3/15	
		Classic (Greece)	17/18	Haemophiliacs		
				HIV-positive	0/26	
				HIV-negative	ND	
	BCP-1 LANA IFA	AIDS (US, UK)	84/103	Intravenous drug users		
		Classic (Greece)	17/18	HIV-positive	0/38	2/38
				HIV-negative	0/25	0/25
				HIV-negative homosexual men with sexually transmitted diseases	8/65	ND
				Heterosexual men	4/75	ND
				Heterosexual women	2/26	ND
				Children with rash or fever	0/24	ND
				Blood donors		
				UK	4/150	3/174
				USA	0/117	6 /117
				Greek age-/sex-matched controls	3/26	3/26
				Ugandan controls		
				HIV-positive	18/34	16/34
				HIV-negative	9/17	7/54
Miller *et al.* (1996)	Sodium butyrate-induced BC-1 western blot	AIDS	32/48	HIV-positive US homosexual men	ND	7/54
	Sodium butyrate-induced BC-1 IFA	AIDS	31/48	HIV-positive US homosexual men	ND	7/54
Davis *et al.* (1997)	ORF 26 peptide ELISA	AIDS	21/35	HIV-negative blood donors	ND	6/30
				HIV-positive homosexual men	ND	2/8
				HIV-positive haemophiliacs	ND	6/24

Table 6 (contd)

Reference	Assay	KS type	No. positive/ total	Control population	Antibodies to LANA (No. positive/total)	Antibodies to lytic antigen (No. positive/total)
Lin *et al.* (1997)	Recombinant whole ORF 65 western blot	AIDS	42/47	HIV-positive US adults	ND	11/54
				HIV-positive children	ND	0/12
				HIV-negative children	ND	0/10
				Haemophiliacs	ND	0/25
				Autoimmune patients	ND	0/25
				Children with acute illness	ND	0/25
				Healthy adults	ND	3/28
				Nasopharyngeal cancer patients (China)	ND	0/25
Smith *et al.* (1997)	BCBL-1 TPA-induced lytic IFA with Evans blue counterstain	AIDS	7/7	HIV-positive	ND	6/18
				US blood donors	ND	0/52

Modified from Olsen & Moore (1997)
ND, not determined; LANA, latency-associated nuclear antigen; IFA, immunofluorescent assay; ELISA, enzyme-linked immunosorbent assay; TPA, 12-*O*-tetradecanoylphorbol 13-acetate

30% of HIV-positive homosexual or bisexual men and less than 2% of HIV-positive patients with haemophilia or blood donors in the United Kingdom or the United States. Simpson *et al.* (1996) also found no LANA-reactive sera among 38 HIV-positive intravenous drug users. Gao *et al.* (1996a,b), in a nested case–control study within the cohort of a multicentre study on AIDS, compared 40 AIDS patients with Kaposi's sarcoma and 40 randomly selected AIDS patients without Kaposi's sarcoma, matched for $CD4^+$ count. The odds ratio for an association between LANA positivity and Kaposi's sarcoma was 16. A similar comparison of 45 AIDS patients with Kaposi's sarcoma and 37 HIV-positive homosexual and bisexual men showed an odds ratio for LANA positivity of 8.5 (Kedes *et al.*, 1996). Lennette *et al.* (1996), who found a lower LANA antibody detection rate among Kaposi's sarcoma patients (52%) than in other studies, obtained an odds ratio of 4.2 for a similar comparison.

In view of the rarity of Kaposi's sarcoma among HIV-infected haemophilia patients, the virtual absence of LANA antibodies in this group (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996) is an important finding. In these studies, a total of 346 HIV-positive persons with haemophilia from three defined cohorts were investigated.

The association between antibodies to KSHV/HHV8 structural proteins and Kaposi's sarcoma has also been examined. Miller *et al.* (1996) compared antibodies to a 40-kDa structural antigen by western blot in AIDS patients with Kaposi's sarcoma (67%) and in HIV-positive homosexual men (13%), giving an odds ratio of 13. For a recombinant capsid-related protein, vp19/ORF 65, Simpson *et al.* (1996) found 81% antibody reactivity among AIDS patients with Kaposi's sarcoma and 31% in HIV-positive homosexual men (odds ratio, 9.2). In contrast, Lennette *et al.* (1996), using an immunofluorescence assay for low titres of antibodies to undefined structural proteins, found positive results in 96% of AIDS patients with Kaposi's sarcoma and in 93% of HIV-positive homosexual men in the United States (odds ratio, 1.8). A comparison of antibody positivity for both LANA and structural antigens in cases of AIDS plus Kaposi's sarcoma and in blood donors and other groups at risk for HIV infection (haemophilia patients and intravenous drug users) yielded much higher odds ratios.

2.1.4 *Temporal associations*

The long-term consequences of infection with KSHV/HHV8 have been examined in a limited number of studies of few exposed individuals. These studies have addressed only the association between Kaposi's sarcoma and prior exposure to KSHV/HHV8, determined by either PCR or serology.

In a cohort study, Whitby *et al.* (1995) followed (for a median of 30 months) 143 HIV-positive patients who did not have Kaposi's sarcoma at the time their first or only blood sample was taken. Of the 11 men who initially had detectable KSHV/HHV8 in their PBMC by nested PCR, six (54%) developed Kaposi's sarcoma, whereas only 12 of 132 (9%) men who were KSHV/HHV8-negative developed the disease (odds ratio, 7.0; 95% CI, 2.8–13).

In a nested case–control study, Moore *et al.* (1996c) and Parry and Moore (1997) compared the detection of KSHV/HHV8 by PCR in paired samples of PBMC drawn

from 21 HIV-infected patients before and after a diagnosis of Kaposi's sarcoma with that in paired samples from 23 high-risk, HIV-infected homosexual men who later developed AIDS. Nine of the 21 Kaposi's sarcoma patients and one of the 23 homosexual controls were KSHV/HHV8-positive at the time the initial sample was taken (odds ratio, 17; 95% CI, 1.8–755). Overall, 11/21 patients and 3/23 controls (at any sample) had evidence of KSHV/HHV8 before the onset of Kaposi's sarcoma (odds ratio, 7.3; 95% CI, 1.4–48).

Lenette *et al.* (1996) analysed 13 pairs of sera collected before and after diagnosis of Kaposi's sarcoma. No clear association between seroconversion to KSHV/HHV8 (latent or lytic antibodies) and the development of Kaposi's sarcoma was found after a median interval of about 12 months.

In a longitudinal study of 40 patients who developed AIDS-associated Kaposi's sarcoma over a period of 13–103 months, 11 patients (28%) showed positive results at all visits, and 21 seroconverted to KSHV/HHV8 6–75 months before a diagnosis of Kaposi's sarcoma (Gao *et al.*, 1996b). The median duration of positivity for antibodies to LANA before the diagnosis was 33 months (Figure 5). The LANA antibody titres remained constant between seroconversion and Kaposi's sarcoma development, which the authors suggested was inconsistent with seroconversion to LANA reflecting reactivation of a pre-existing KSHV/HHV8 infection (Gao *et al.*, 1996a; Figure 6). In a subsequent study based on an indirect immunofluorescence assay for LANA on the EBV-negative KSHV/HHV8-infected cell line BCP-1, similar results were obtained (Gao *et al.*, 1996a).

2.2 Lymphoproliferative disorders

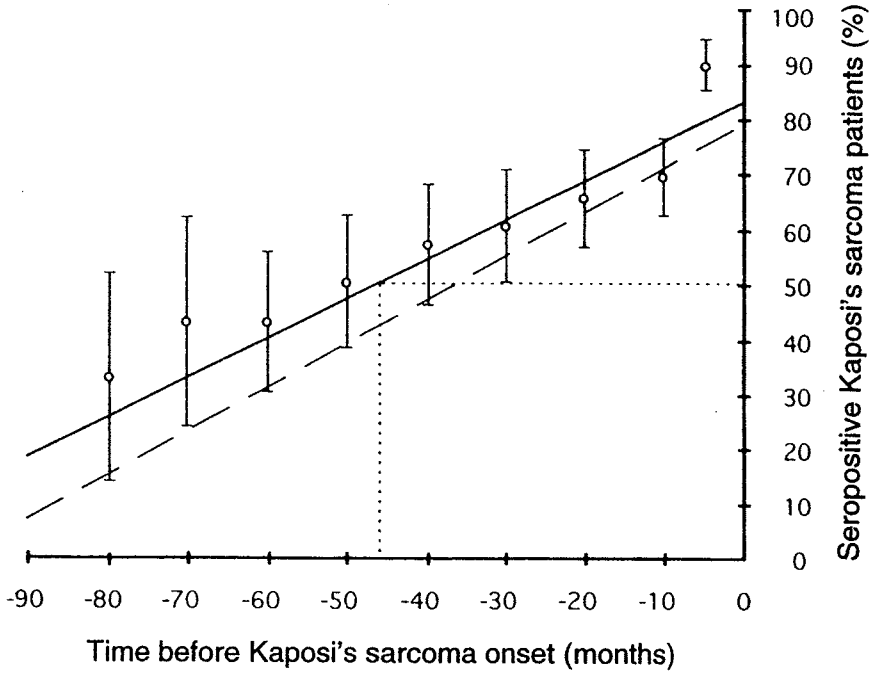
2.2.1 *Primary effusion lymphomas*

2.2.1.1 *Pathology and clinical presentation*

Another neoplastic condition associated with KSHV/HHV8 is primary effusion lymphoma. AIDS-related lymphomas presenting as primary malignant lymphomatous effusions in body cavities were first recognized in the late 1980s (Knowles *et al.*, 1989; Walts *et al.*, 1990; Karcher *et al.*, 1992). This lymphoma is a rare, distinct subtype of non-Hodgkin's lymphoma that has morphological features shared by large-cell immunoblastic lymphomas and anaplastic large-cell lymphoma (Ansari *et al.*, 1996; Carbone *et al.*, 1996a; Cesarman *et al.*, 1996b). Primary effusion lymphoma is defined by distinctive clinical, immunophenotypic and molecular genetic features (Cesarman *et al.*, 1995a). It presents predominantly as malignant effusions in the pleural, pericardial or peritoneal cavities, usually without significant tumour mass or lymphadenopathy; however, lymphomatous infiltration of serosal surfaces adjacent to the site of the primary malignant effusion is sometimes seen (Komanduri *et al.*, 1996).

Morphologically, the cells bridge the features of large-cell immunoblastic and anaplastic large-cell lymphomas. They are usually large and irregularly shaped, with abundant cytoplasm and variably chromatic and pleomorphic nuclei. One or more prominent nucleoli are usually present, and mitotic features are abundant (Ansari *et al.*, 1996).

Figure 5. Prevalence of seropositivity for BCP-1 immunofluorescence and for latent nuclear antigen in 39 homosexual AIDS patients before onset of Kaposi's sarcoma



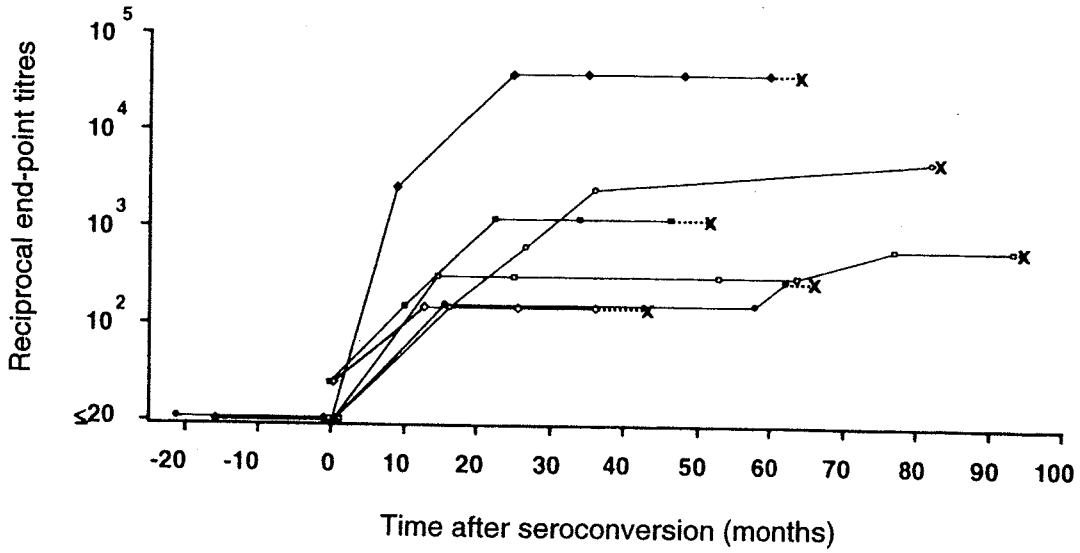
From Gao *et al.* (1996a,b)

Date of seroconversion was estimated to be the mid-point between last negative and first positive serological test. For comparison, seropositivity for KSHV/HHV8 by immunoassay for latent nuclear antigen (dashed line) is plotted against seropositivity by BCP-1 immunofluorescence (solid line). Fifty percent of the Kaposi's sarcoma patients were seropositive 46 months before onset of the disease by BCP-1 immunofluorescence assay. Error bars are standard errors of the mean calculated from a binomial distribution.

Under the electron microscope, the cells are large, with lobulated nuclei containing marginated heterochromatin and prominent rope-like nucleolonemas. The cytoplasm is moderate in amount and exhibits short, blunt, surface projections. KSHV/HHV8 particles are not identified in the cytoplasm, but nuclear particles measuring 110 nm have been observed (Renne *et al.*, 1996a; Said *et al.*, 1996a,b).

Primary effusion lymphoma cells have indeterminate (null) immunophenotypes, lacking expression of any lineage-associated B- or T-lymphocyte antigens (Table 7), but usually express the common leukocyte antigen CD45. A B-cell lineage is indicated by the presence of clonal immunoglobulin gene rearrangement (Knowles *et al.*, 1989; Cesarman *et al.*, 1995b; Komanduri *et al.*, 1996). The B-cell derivation is also supported by the monoclonal nature of primary effusion lymphoma, as demonstrated by a consistent rearrangement of the immunoglobulin genes and by expression of monotypic κ or λ mRNA in the cell cytoplasm (Nador *et al.*, 1996). Primary effusion lymphoma cells usually express activation markers such as CD30, CD38, CD71 and epithelial membrane antigen.

Figure 6. Immunoglobulin-γ end-point titres for six AIDS patients with Kaposi's sarcoma from whom three or more samples were drawn after seroconversion (immunofluorescence titre > 1:160 and a fourfold or greater rise in end-point titre)



From Gao *et al.* (1996a)

Titres remained elevated for 36–93 months after seroconversion, until onset of Kaposi's sarcoma (X), consistent with a prolonged antibody response after primary infection

These cells consistently lack the molecular defects commonly associated with neoplasia of mature B cells, including activation of the proto-oncogenes *c-myc*, *bcl-2*, *bcl-6*, *N-ras* and *K-ras* or mutations of *p53* (Cesarman *et al.*, 1995a; Carbone *et al.*, 1996a; Nador *et al.*, 1996). Cytogenetic studies have shown complex, hyperdiploid karyotypes. Alterations of the chromosomal region 1q21–q23 have been reported, which are also present in other EBV-positive AIDS-related lymphomas (Ansari *et al.*, 1996).

The levels of IL-6 and IL-10, which are involved in B-cell proliferation and differentiation, are both markedly elevated (340–16 000-fold higher than in normal human plasma) in primary effusion lymphoma. Expression of both IL-6 and IL-6 receptor transcripts in some cells suggests a paracrine mechanism for continued B-cell proliferation (Komanduri *et al.*, 1996).

In patients with AIDS, primary effusion lymphoma is a fulminant lymphoproliferation, and the median survival time is less than six months (Komanduri *et al.*, 1996; Nador *et al.*, 1996); however, a more indolent course has been documented in immunocompetent patients (Strauchen *et al.*, 1996).

Table 7. Immunophenotypic types of primary effusion lymphoma cells

CD45 (leukocyte common antigen)	+
TdT	–
Activation markers	
CD30	+
CD38	+
CD71	+
HLA-DR	+
Epithelial membrane antigen	+
T-Cell markers	
CD2	–
CD3	–
CD4	–
CD5	–
CD7	–
CD8	–
B-Cell markers	
CD19	–
CD20	–
CD22	–
CD23	–
Other markers	
CD10	–
CD14	–
CD15 (Reed-Sternberg antigen)	–

From Cesarman *et al.* (1995a,b), Ansari *et al.* (1996) and Nador *et al.* (1996)

2.2.1.2 *Descriptive epidemiology*

Very little is known about the distribution and epidemiological characteristics of primary effusion lymphoma. Because it is rare, its incidence remains to be established. Although primary effusion lymphoma was first described among AIDS patients (Knowles *et al.*, 1988), in whom it occurs mainly at an advanced stage of the disease (Komanduri *et al.*, 1996), it has also been reported in HIV-negative individuals (Nador *et al.*, 1995). In addition, like Kaposi's sarcoma, with which it is closely linked, it is seen primarily in homosexual men and seldom in other groups at risk for HIV infection (Jaffe, 1996; Nador *et al.*, 1996).

Primary effusion lymphoma is distinct from another body cavity-based lymphoma, pyothorax-related B-cell lymphoma. These large B-cell lymphomas occur in patients with long-standing pyothorax resulting from artificial pneumothorax for the treatment of pulmonary tuberculosis or tuberculous pleuritis (Iuchi *et al.*, 1987, 1989). This tumour has been identified most often in Japan, with more than 50 cases in the literature, in comparison with a single series of three cases reported from a western country, France

(Martin *et al.*, 1994). The geographical distribution of pyothorax-related B-cell lymphoma may be due to the fact that artificial pneumothorax is used more frequently as a treatment modality in Japan. In common with primary effusion lymphoma, the tumour cells in pyothorax-related B-cell lymphoma nearly always contain EBV (14 of 14 in a study by Cesarman *et al.*, 1996b); however, in pyothorax-related B-cell lymphoma, pleural mass lesions are seen, *c-myc* rearrangements are present and KSHV/HHV8 is absent (Cesarman *et al.*, 1996b).

2.2.1.3 *Case reports and case series*

Table 8 summarizes 30 case reports of primary effusion lymphoma reported in the literature. Four other cases were associated with *c-myc* gene rearrangements and thus molecularly resembled Burkitt-type lymphomas (Nador *et al.*, 1996). Since these cases also had cytomorphological features similar to those of Burkitt's or Burkitt-like lymphomas, and two of the four also involved systemic lymphoma, Nador *et al.* (1996), who originally reported these cases as primary effusion lymphomas, argued that they should be classified as Burkitt-type lymphomas, despite their body cavity involvement. [The Working Group concluded that these cases could not be considered primary effusion lymphomas.] Effusions from the 30 patients all contained KSHV/HHV8 and in 26 of these the PCR product was confirmed by Southern blot hybridization. Twenty-five of the described cases occurred in HIV-infected homosexual men and three in uninfected elderly men who did not belong to any established HIV risk group. Two cases of primary effusion lymphomas have been described in HIV-negative women (Said *et al.*, 1996b).

The median $CD4^+$ count in the HIV-infected persons was 65, indicating that they were severely immunosuppressed at the time of diagnosis of primary effusion lymphoma (Table 8). Of the patients reported to be infected with HIV, 10/25 had previously or at the same time received a diagnosis of Kaposi's sarcoma. Similarly, two of five uninfected primary effusion lymphoma patients had Kaposi's sarcoma (Nador *et al.*, 1995, 1996; Said *et al.*, 1996b; Strauchen *et al.*, 1996).

Co-infection with EBV is common in primary effusion lymphomas; EBV monoclonality has been established in most cases (Komanduri *et al.*, 1996; Cesarman *et al.*, 1995a; Nador *et al.*, 1996). It is therefore of interest that several cell lines derived from these lymphomas, with genetic and immunological markers similar to those of the original lymphomas, were latently infected with KSHV/HHV8 but not EBV (Arvanitakis *et al.*, 1996; Renne *et al.*, 1996a; Said *et al.*, 1996a; Gao *et al.*, 1996b). KSHV/HHV8 has not been consistently detected in other lymphomas (see Table 9).

2.2.2 *Castleman's disease*

2.2.2.1 *Pathology and clinical presentation*

Castleman's disease, also referred to as angiofollicular or giant lymph node hyperplasia, is a rare, usually polyclonal, non-neoplastic disorder of unknown etiology (Castleman *et al.*, 1956). Two distinct histopathological variants with different clinical characteristics have been described: the hyaline vascular type and the plasma-cell type. The more common hyaline form presents primarily as a solitary mass, most frequently in

Table 8. Demographic, clinical and virological characteristics of patients with primary effusion lymphoma

Age (years)/ sex	HIV status	HIV risk factor	KS	$CD4^+$ count (cells/μl)	Location of effusion	Other sites of disease	KSHV/ HHV8	EBV		Reference
								Type	Clonality	
46/M	+	HS	–	561	Abdominal	None	+	2	Clonal	Chadburn *et al.* (1993); Cesarman *et al.* (1995a)
31/M	+	HS	–	NR	Pleural	None	+	1	Clonal	Knowles *et al.* (1989); Cesarman *et al.* (1995a)
40/M	+	HS	+	NR	Pleural	Submandibular gland, lymph nodes	+	1	Clonal	Knowles *et al.* (1989); Cesarman *et al.* (1995a)
35/M	+	HS	–	NR	Abdominal	None	+	1	Clonal	Walts *et al.* (1990); Cesarman *et al.* (1995a)
38/M	+	HS	–	NR	Pericardial	None	+	2	Clonal	Cesarman *et al.* (1995a)
58/M	+	HS	–	NR	Pleural	None	+	1	Clonal	Cesarman *et al.* (1995a)
37/M	+	HS	+	109	Pericardial	None	+	1	ND	Nador *et al.* (1996)
42/M	+	HS	–	NR	Pleural	Oesophageal lymph node, lung	+	2	ND	Nador *et al.* (1996)
53/M	+	HS	–	84	Abdominal	None	+	2	Clonal	Ansari *et al.* (1996)
43/M	+	HS	–	34	Pleural	None	+	2	Clonal	Ansari *et al.* (1996)
44/M	+	HS	+	25	Pleural	None	+	2	Clonal	Ansari *et al.* (1996)
44/M	+	HS	+	33	Pleural	None	+	1	Clonal	Ansari *et al.* (1996)

Table 8 (contd)

Age (years)/ sex	HIV status	HIV risk factor	KS	$CD4^+$ count (cells/μl)	Location of effusion	Other sites of disease	KSHV/ HHV8	EBV		Reference
								Type	Clonality	
54/M	+	HS	+	130	Pleural	None	+	1	Clonal	Ansari *et al.* (1996)
42/M	+	HS	+	NR	Pleural	None	+	1	Clonal	Gessain *et al.* (1997)
31/M	+	HS	–	NR	Peritoneal, pleural	Small intestine	+	EBER +	ND	Gessain *et al.* (1997)
35/M	+	HS, IVDU	–	58	Abdominal, pleural	None	+[a]	–		Komanduri *et al.* (1996)
40/M	+	HS	–	65	Abdominal	None	+	1	Polyclonal	Komanduri *et al.* (1996)
32/M	+	HS	–	91	Pleural	None	+	2	Clonal	Komanduri *et al.* (1996)
42/M	+	HS	–	181	Abdominal	Left atrial mass	+	1	Polyclonal	Komanduri *et al.* (1996)
31/M	+	HS	+	34	Pericardial	None	+	–		Komanduri *et al.* (1996)
32/M	+	HS	+	65	Pleural	None	+	–		Komanduri *et al.* (1996)
47/M	+	HS, IVDU	–	20	Abdominal, pleural	None	+[a]	1	Clonal	Komanduri *et al.* (1996)
40/M	+	HS	+	190	Abdominal	ND	+	–		Komanduri *et al.* (1996)
30/M	+	HS	–	NR	Pericardial	None	+[a]	1	ND	Walts *et al.* (1990); Cesarman *et al.* (1995a)
32/M	+	HS	+	NR	Pleural	None	+[a]	1	ND	Walts *et al.* (1990); Cesarman *et al.* (1995a)

Table 8 (contd)

Age (years)/ sex	HIV status	HIV risk factor	KS	$CD4^+$ count (cells/μl)	Location of effusion	Other sites of disease	KSHV/ HHV8	EBV		Reference
								Type	Clonality	
85/M	–	–	–	288	Pleural	None	+	–		Nador *et al.* (1995, 1996)
78/M	–	–	–	NR	Abdominal	None	+	1	Clonal	Nador *et al.* (1996)
94/M	–	–	+	60	Pleural	Peritoneaum Pericardium	+	–		Strauchen *et al.* (1996)
85/F	–	–	+	NR	Pleural	None	+	–		Said *et al.* (1996b)
46/F	–	–	–	NR	Silicone breast implant	None	+	–		Said *et al.* (1996b)

M, male; F, female; NR, not reported; ND, not determined; HS, homosexual man; IVDU, intravenous drug user; EBER, Epstein-Barr virus-encoded RNA

[a] Only analysis by polymerase chain reaction

Table 9. Presence of HHV8 in lymphoid neoplasias

Histology	Positive/tested
Cases unrelated to AIDS	
Acute lymphoblastic leukaemia	0/44
Chronic lymphocytic leukaemia	0/61
Prolymphocytic leukaemia	0/10
Lymphoplasmacytoid lymphoma	0/3
Mantle-cell lymphoma	0/14
Follicular lymphoma	0/60
Monocytoid lymphoma	0/3
MALT lymphoma	0/16
Hairy cell leukaemia	0/18
Multiple myeloma and plasmacytoma[a]	0/28
Diffuse large-cell lymphoma	0/65
Small non-cleaved (including Burkitt's) lymphoma	0/57
Cutaneous T-cell lymphoma	0/9
Peripheral T-cell lymphoma	0/20
Anaplastic large-cell lymphoma	0/17
Lymphoblastic lymphoma	0/4
Adult T-cell leukaemia/lymphoma	0/13
Post-transplant lymphoproliferation	0/23
Hodgkin's disease	0/49
Primary effusion lymphoma	8/8
AIDS-related lymphomas	
Small non-cleaved lymphoma	0/42
Diffuse large-cell lymphoma	0/39
Anaplastic large-cell lymphoma	0/5
Peripheral T-cell lymphoma	0/1
Hodgkin's disease	0/14
Primary effusion lymphoma	34/35

From Chang *et al.* (1994), Pastore *et al.* (1995), Cesarman *et al.* (1995a), Karcher & Alkan (1995), Nador *et al.* (1995), Ansari *et al.* (1996), Arvanitakis *et al.* (1996), Carbone *et al.* (1996a,b), Gaidano *et al.* (1996b, 1997), Luppi *et al.* (1996b), Nador *et al.* (1996), Otsuki *et al.* (1996), Said *et al.* (1996a), Strauchen *et al.* (1996), Gessain *et al.* (1997)

MALT, mucosa-associated lymphoid tissue

[a] See also section 2.2.3 (multiple myeloma)

the mediastinum or retroperitoneum, is asymptomatic and is usually curable surgically. The rare plasma-cell type is typically characterized by generalized lymphadenopathy, immunological abnormalities and type B symptoms.

The systemic variety, also designated multicentric Castleman's disease, is primarily of the plasma-cell type, but the hyaline type has occasionally been reported in a multicentric clinical appearance (Herrada & Cabanillas, 1995; Shahidi *et al.*, 1995). Associated clinical findings are necessary to make the diagnosis of multicentric Castleman's disease, since the pathological features in lymph nodes can be nonspecific (Peterson & Frizzera, 1993; Shahidi *et al.*, 1995). EBV was reported to be present in 9 of 16 cases of localized and multicentric Castleman's disease (Barozzi *et al.*, 1996). Multicentric Castleman's disease has an aggressive clinical course with a poor prognosis, and such patients are at increased risk for Kaposi's sarcoma and lymphomas (Peterson & Frizzera, 1993). It has been suggested that some of the immunological changes observed in HIV-negative patients with multicentric Castleman's disease are similar to those in HIV-infected individuals (Lane *et al.*, 1985; Vuillier *et al.*, 1988; Birx *et al.*, 1990; Boyd & James, 1992; Ishiyama *et al.*, 1996).

2.2.2.2 *Descriptive epidemiology*

About 70% of patients with all forms of Castleman's disease are under 30 years of age, and men are affected more often than women. Patients with multicentric Castleman's disease often tend to be in their fifties or sixties and to have increased risks for non-Hodgkin's lymphoma and Kaposi's sarcoma (Peterson & Frizzera, 1993).

2.2.2.3 *Case reports and case series*

Few reports have addressed the presence of KSHV/HHV8 in Castleman's disease (Table 10). Soulier *et al.* (1995) detected KSHV/HHV8 in all of 14 HIV-positive lesions from French patients with multicentric Castleman's disease, comprising six plasma-cell type, seven mixed and one hyaline vascular type. Seven of the patients also had Kaposi's sarcoma in the same tissue sample, and an additional two at another site; 64% had Kaposi's sarcoma both in the same tissue and elsewhere. Of 17 HIV-negative multicentric Castleman's disease lesions, seven (three plasma cell, two mixed, two hyaline type) contained KSHV/HHV8. Kaposi's sarcoma was diagnosed in one of these subjects. Whereas the vast majority of cases among HIV-positive patients were found to contain the virus by Southern blot, only two of the seven cases in HIV-negative patients found to be positive by PCR were positive by Southern blot. To evaluate the significance of the positivity rate in the HIV-negative patients, reactive lymph nodes from 34 HIV-seronegative control patients were analysed; only one KSHV/HHV8-positive case was found.

Dupin *et al.* (1995b) reported the finding of KSHV/HHV8 in PMBC from two HIV-infected men with Castleman's disease, one of whom had Kaposi's sarcoma. [The authors did not specify whether these cases were multicentric.] Tirelli *et al.* (1996) found KSHV/HHV8 in lesions from an HIV-positive woman with multicentric Castleman's disease whose husband was diagnosed with Kaposi's sarcoma.

Gessain *et al.* (1996) detected KSHV/HHV8 by PCR in cryopreserved lymph node biopsy samples from one of three HIV-negative patients with multicentric Castleman's disease but in none of three with localized disease. Of the HIV-positive subjects, three of four homosexual men with multicentric Castleman's disease had KSHV/HHV8 in their

Table 10. Detection of KSHV/HHV8 in patients with Castleman's disease

Reference (country)	HIV-negative (KSHV/ HHV8 positive/total)			HIV-positive (KSHV/ HHV8 positive/total)			Method	Comments
	HV	PL	Mixed	HV	PL	Mixed		
Soulier *et al.* (1995) (France)	2/3	3/9	2/5	1/1	6/6	7/7	PCR, Southern blot	All MCD HIV-positive: 9/14 with KS HIV-negative: 1/17 with KS
Corbellino *et al.* (1996d) (Italy)	0/2	4/4					PCR, Southern blot	None with KS or lymphoma
Barozzi *et al.* (1996) (Italy)	1/11		0/5				PCR	Mixed: all MCD HV: all localized Paraffin-embedded biopsies
Gessain *et al.* (1996) (France)	0/1	1/5			3/4		PCR	HIV-positive: 3 HHV8-positive had KS in other organs HIV-negative: none had KS

HV, hyaline vascular type; PL, plasma-cell type; mixed, both HV and PL; MCD, multicentric Castleman's disease

lymph nodes. Kaposi's sarcoma was diagnosed in three HIV-positive and KSHV/HHV8-positive men but not in the KSHV/HHV8-positive but HIV-negative woman. Semi-quantitative PCR showed a high KSHV/HHV8 viral load in the lesions of patients with and without HIV infection.

In a group of Italian HIV-negative patients, Corbellino *et al.* (1996d) found high levels of KSHV/HHV8 by PCR and Southern blot hybridization in biopsy samples from all of four cases of plasma-cell type Castleman's disease but in none of two cases of the hyaline vascular type. [The authors did not specify whether these cases were multicentric.] Neither Kaposi's sarcoma nor lymphoma was diagnosed in any of the patients. None of 20 lymph node biopsy samples from 15 HIV-infected drug abusers with persistent lymphadenopathy or from five HIV-negative patients with reactive lymphadenitis contained KSHV/HHV8. Two of the four cases of plasma-cell Castleman's disease were EBV-positive by PCR.

In archival formalin-fixed, paraffin wax-embedded biopsy material from HIV-negative Italian patients with Castleman's disease, Barozzi *et al.* (1996) found KSHV/HHV8 in one of 11 patients with the localized hyaline vascular type and in none of five patients with multicentric disease. PBMC and saliva were positive for KSHV/HHV8-specific sequences in the KSHV/HHV8-positive patients, whereas serum, faeces and urine were negative.

2.2.3 *Multiple myeloma*

Rettig *et al.* (1997) demonstrated the presence of KSHV/HHV8 DNA by PCR and in-situ hybridization in the cultured bone-marrow dendritic cells of 15 patients with multiple myeloma but not in the myeloma cells (plasma cells). The authors also demonstrated by RT-PCR the expression of v-*IL-6* in three of three cultured myeloma bone-marrow dendritic cells, suggesting that KSHV/HHV8–v-IL-6 contributes to the mechanism whereby bone-marrow dendritic cells infected with KSHV/HHV8 promote myeloma growth. KSHV/HHV8 was not detected by PCR in 28 DNA samples from myeloma specimens (Cesarman *et al.*, 1995a; Pastore *et al.*, 1995; Gessain *et al.*, 1997) or bone-marrow samples (Rettig *et al.*, 1997) in previous studies; this was attributed by the authors to dilution of the sample with uninfected cells.

2.2.4 *Other lymphoproliferative disorders*

With the exception of primary effusion lymphoma, most large series of lymphoid malignancies, including a variety of immunophenotypic categories of B- and T-cell tumours, have not been shown to contain KSHV/HHV8 (Chang *et al.*, 1994; Cesarman *et al.*, 1995a; Pastore *et al.*, 1995; Luppi *et al.*, 1996b; Gessain *et al.*, 1997; see Table 9). Bigoni *et al.* (1996), however, found that with nested and semiquantitative PCR 7–9% of PBMC from non-Hodgkin's lymphoma patients and patients with Hodgkin's disease contained KSHV/HHV8.

2.3 Other tumours

In studies of angiosarcoma in HIV-negative individuals, Gyulai *et al.* (1996a,b) reported one KSHV/HHV8-positive case. McDonagh *et al.* (1996) found KSHV/HHV8 by PCR in seven cases in the United States; one case was also tested by Southern blotting. These findings were not confirmed by other investigators (Chang *et al.*, 1994; Boshoff *et al.*, 1995a,b; Dictor *et al.*, 1996; Jin *et al.*, 1996a,b).

Rady *et al.* (1995) reported the widespread presence of KSHV/HHV8 by PCR in various skin tumours from four immunosuppressed patients, but in studies in Austria, Germany, Sweden and the United Kingdom this association could not be confirmed (Adams *et al.*, 1995; Boshoff *et al.*, 1996; Dictor *et al.*, 1996; Uthman *et al.*, 1996).