

RISK FACTORS FOR KAPOSI'S-SARCOMA-ASSOCIATED HERPESVIRUS (KSHV/HHV-8) SEROPOSITIVITY IN A COHORT OF HOMOSEXUAL MEN, 1981–1996

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A newly identified herpesvirus has been associated with Kaposi's sarcoma. We determined risk factors for Kaposi's-sarcoma-associated herpesvirus/human herpesvirus 8 (KSHV/HHV-8) seropositivity and incidence of infection over time in a cohort of Danish homosexual men followed from 1981 to 1996. Antibodies to a latent nuclear (LANA) and a structural (orf65) antigen of KSHV/HHV-8 were measured by immunofluorescence and ELISA/WB respectively. Through linkage with the national AIDS registry, all cohort members diagnosed with AIDS as of September 1996 were identified and their hospital records were scrutinized to record all diagnoses of KS. Overall, 21.1% (52/246) of the men were KSHV/HHV-8-seropositive in 1981. Among the initially seronegative, the rate of KSHV/HHV-8 seroconversion was highest between 1981 and 1982 and declined steadily thereafter. In a multivariate analysis of the status at enrollment in 1981, KSHV/HHV-8 seropositivity was not associated with age but was independently associated both with number of receptive anal intercourse (OR = 2.83; $p = 0.03$) and with sex with US men (OR = 2.27; $p < 0.05$). In a multivariate analysis of follow-up data, risk of KSHV/HHV-8 seroconversion was independently associated with having visited homosexual communities in the United States, and current HIV-positive status. More than 5 years' homosexual experience was associated with an insignificantly increased risk (RR = 2.68). KS occurred only in HIV-positive men who were KSHV/HHV-8-positive at or prior to their KS diagnosis. In conclusion, KSHV/HHV-8 appears to be sexually transmitted, probably by receptive anal intercourse, and may have been introduced to Danish homosexual men via sex with US men. The epidemic of KSHV/HHV-8 is now declining. These findings are concordant with the view that KSHV/HHV-8 may have been actively spread simultaneously with and by the same activities that lead to the spread of HIV. *Int. J. Cancer* 77:543–548, 1998.
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A new human herpesvirus (KSHV/HHV-8) has been described and may be the hitherto elusive infectious agent long postulated to play a role in the etiology of Kaposi's sarcoma (KS) (Beral *et al.*, 1990; Peterman *et al.*, 1993; Chang *et al.*, 1994). KSHV/HHV-8 is consistently found in all forms of AIDS and non-AIDS-associated KS (Chang *et al.*, 1994, 1996; Boshoff *et al.*, 1995; Buonaguro *et al.*, 1996; Moore and Chang, 1995; Schalling *et al.*, 1995) as well as in primary effusion lymphomas (Cesarman *et al.*, 1995). Detection of KSHV/HHV-8 in peripheral-blood mononuclear cells correlates with and, in asymptomatic HIV-infected patients, predicts the development of KS (Whitby *et al.*, 1995; Moore *et al.*, 1996).

The exact prevalence of KSHV/HHV-8 infection in the general population, as measured by currently available assays, is still controversial and shows considerable geographical variation. Infection with KSHV/HHV-8 appears to be widespread in several African countries (50–70%) and more common in some Mediterranean countries than in the UK or the US (Gao *et al.*, 1996a,b; Kedes *et al.*, 1996; Simpson *et al.*, 1996; Miller *et al.*, 1996; Lennette *et al.*, 1996; Rickinson, 1996). However, while several studies have supported the concept that sexually active groups have a higher prevalence of KSHV/HHV-8 antibodies (Gao *et al.*,

1996b; Simpson *et al.*, 1996; Lennette *et al.*, 1996), studies looking at risk factors for KSHV/HHV-8 seropositivity are lacking. Similarly, no studies have addressed time-related trends in rates of infection, although studies of homosexual men with AIDS have suggested a decrease in KS incidence since the beginning of the AIDS epidemic.

In the present study, we analyzed a cohort of Danish homosexual men followed from 1981 to 1996, with the purpose of identifying risk factors for KSHV/HHV-8 seropositivity and to determine the incidence of infection over time.

MATERIAL AND METHODS

Subjects

A cohort of 259 homosexual men was recruited in 1981 from a national homosexual organization in Denmark as described in detail elsewhere (Melbye *et al.*, 1984, 1998). In short, a letter was sent in November 1981 to all (approximately 800) members of this organization with a request to participate in a study of lifestyle habits and sexual behavior. The letter explicitly stated that one of the purposes was to study factors that might relate to the outbreak of a rare cancer among homosexual men, called Kaposi's sarcoma. Because the outbreak appeared restricted primarily to the homosexual milieu, the questionnaire design was focused on factors that might be particular to this milieu (detailed sexual-lifestyle factors, travel activity and interaction with similar milieus elsewhere, drug abuse, in particular amyl nitrites). Condom use was not assessed in the early years of this cohort study. At enrollment, the mean age of participants was 32.9 years (range 17–73), with 70% of participants being between 25 and 40 years old. The mean number of sexual partners during the past year was 22.2 (median 10 partners, range 0–150 partners), and 8.9% of the participants were HIV-seropositive. Follow-up visits occurred in 1982, 1983, 1984, 1987, 1989, and 1992, at which time the participants filled in a questionnaire, had blood drawn and underwent a physical examination. In addition, a blood specimen was obtained at the time of AIDS diagnosis. Seven men requested that their identifying information be destroyed before the end of follow-up in 1996 (3 who were KSHV/HHV-8-seropositive by 1981, 1 by 1982, and 3 KSHV/HHV-8-seronegative men). Information about vital status (alive, date of death or emigration) or whether the participant had developed AIDS was obtained through yearly linkage to the Danish Civil Registration System (CRS) and to the national AIDS registry by a personal identity number (CRS number) assigned to every citizen in Denmark.

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Serologic assays (ELISA, immunofluorescence, Western blot)

All serum specimens were given an arbitrary number and forwarded to the Department of Medical Microbiology and Genitourinary Medicine in Liverpool where testing for KSHV/HHV-8 antibody was performed. The code was broken only after the serologic results had been returned to the Department of Epidemiology Research in Denmark.

Immunofluorescence (IFA) for latent antigen(s) was carried out on infected BCP-1 cells (Gao *et al.*, 1996a) at a serum dilution of 1:150 as described by Simpson *et al.* (1996). The ELISA with a recombinant capsid-related protein (orf 65) was performed at a serum dilution of 1:100 and Western blots (WB) with the same protein at a dilution of 1:300 (Simpson *et al.*, 1996). All sera were tested by orf 65 ELISA. ELISA-reactive samples taken at entry into the cohort (1981) were re-tested by WB. All 1981 samples were also tested by IFA, unless shown to be WB-reactive. Follow-up sera were re-tested with IFA and/or WB, such that most individuals, for whom more than one serum was available, were tested by IFA on at least 2 occasions.

Both ELISA-reactive/IFA reactive-samples and ELISA-reactive/WB-reactive samples were considered positive, whereas samples reactive only by ELISA were not. As reported by Simpson *et al.* (1996), some sera may react either with orf 65 or with latent IFA antigen, but not with both. ELISA-reactive/IFA-unreactive sera were analyzed by WB on recombinant orf 65 and considered positive if the WB showed the correct pattern (Simpson *et al.*, 1996), but negative if the WB was unreactive or indeterminate. ELISA-unreactive/IFA-reactive sera were considered positive if the IFA (on the same sample) was repeatedly reactive. In the case of seroconverting individuals in whom reactivity in one assay preceded that in another, the time of seroconversion was defined using the same criteria. Of 77 individuals for whom this comparison was possible, the first positive serum was positive in both assays in 38 cases (49%), orf 65 reactivity preceded IFA reactivity in 22 cases (29%), and IFA reactivity preceded orf 65 reactivity in 17 cases (22%). The overall concordance rate between IFA and orf 65 reactivity (ELISA confirmed by WB) in this study was 81%, *i.e.*, similar to our earlier report (Simpson *et al.*, 1996); 13% of sera tested with both antigens reacted only with orf 65 protein and 6% only with IFA.

Statistical analysis

KSHV/HHV-8 seropositive and seronegative subjects in 1981 were compared with respect to behavioral variables by χ^2 test, odds ratios and trend analyses. Among men who were KSHV/HHV-8-seronegative at enrollment in 1981, we estimated the KSHV/HHV-8-seroconversion incidence using a method which addressed the interval censoring problem under the assumption of independent censoring (Becker and Melbye, 1991). The estimation is based on a log-linear model of the probability of being seronegative at any given visit, *i.e.*, the probability of remaining seronegative after 1981. In this, the probability of remaining seronegative is modeled as the product of the probabilities of remaining seronegative in each of the periods between successive planned visits. Using this approach, all men who were tested one or more times in addition to the first test in 1981 were included. Specifically, for men who, after one visit, missed one or more of the following planned visits and then turned up for a later visit, *i.e.*, in the case of interval censoring, the probability of remaining seronegative in the period between the 2 observed time points is included in the maximum-likelihood estimation as the product of the probabilities of remaining seronegative in each of the periods between successive planned visits at which the men potentially could have been tested.

This analysis did not include the results from samples taken at the time of AIDS diagnosis. Two individuals were KSHV/HHV-8-seropositive only at AIDS diagnosis, and their seroconversion date was estimated as the midpoint between this date and the date of their last negative test. Time to estimated date of KSHV/HHV-8 seroconversion was analyzed by Cox's regression model. HIV

status was analyzed as a time-dependent co-variate. The adequacy of the proportional-hazard assumption for the included covariates was checked by log(-logS) plots using stratified multivariate analyses.

RESULTS

Prevalence and incidence of KSHV/HHV-8 infection in the study cohort

In 1981, 52 (21.1%) of the 246 homosexual men from whom blood samples were available had KSHV/HHV-8 antibodies detected. Of the 194 men who were KSHV/HHV-8-seronegative in 1981, 66 did not return for any of the subsequent follow-up visits. Of the remaining 128 men who were seronegative in 1981, serostatus was available at the time of 3 or more follow-up visits for 86%, at the time of 4 or more follow-up visits for 75%, at the time of 5 or more follow-up visits for 68%, and for 52% of the men serostatus was known at the time of all 6 follow-up visits. During the follow-up visits, an additional 43 KSHV/HHV-8 seroconversions were identified. Time of seroconversion could be determined as having taken place in between 2 subsequent visits on 55% of these men, and between 3 subsequent visits on 85%. Among the individuals who were seronegative for KSHV/HHV-8 at enrollment in 1981, the hazard of seroconverting to KSHV/HHV-8 fell from above 10 (per 1,000 susceptibles per month) in 1981–82 to 5 in 1983–84 and 1.4 in the most recent period of 1989–92 (Fig. 1).

Risk factors for having antibodies to KSHV/HHV-8 at enrollment in 1981 (prevalent cases, cross-sectional analysis)

Among study participants at enrollment in 1981, KSHV/HHV-8 prevalence did not differ significantly by age group, although seroprevalence was lowest (9.5%) in those aged 45 years or more (Table I). As shown in Tables I and II, KSHV/HHV-8 seropositivity was significantly associated in univariate analyses with reported

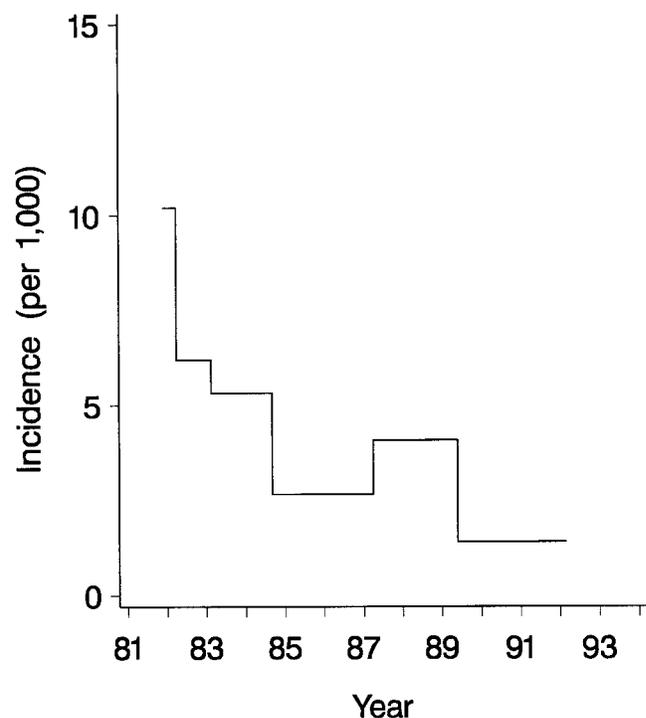


FIGURE 1 – The incidence (hazard) of HHV8 seroconversion measured as number of seroconversions per 1,000 susceptibles per month in a cohort of Danish homosexual men. Only men who were KSHV/HHV-8-seronegative at enrollment in 1981 were included in this analysis.

TABLE I – UNIVARIATE ASSOCIATIONS BETWEEN HIV SEROSTATUS, US EXPOSURE, NITRITE-INHALANT USE AND HHV-8 ANTIBODY POSITIVITY IN DANISH HOMOSEXUAL MEN AT ENROLLMENT IN 1981. A CROSS-SECTIONAL ANALYSIS BASED ON 1981 DATA¹

	HHV-8 antibody		OR	(95% CI)	
	Negative	Positive (%)			
Age (years)					
<25	46	10 (17.9)	1		
25–29	52	16 (23.5)	1.42	(0.58–3.43)	
30–34	35	10 (22.2)	1.31	(0.49–3.50)	
35–39	30	8 (21.1)	1.23	(0.43–3.46)	
40–44	12	6 (33.3)	2.30	(0.70–7.60)	
45+	19	2 (9.5)	0.48	(0.10–2.42)	<i>p</i> trend = 0.90
Educational status (years)					
<12	36	10 (21.7)	1		
13–15	40	9 (18.4)	0.79	(0.39–1.59)	
16–18	43	13 (23.2)	1.03	(0.38–2.49)	
>18	40	9 (18.4)	0.81	(0.29–2.22)	<i>p</i> trend = 0.86
HIV antibody status					
Negative	180	46 (20.4)	1		
Positive	14	6 (30.0)	1.68	(0.62–4.57)	<i>p</i> = 0.31
Visit to US homosexual community, 1980–1981					
No	163	38 (18.9)	1		
Yes	28	13 (31.7)	1.99	(0.95–4.17)	<i>p</i> = 0.07
Sex with US men, 1980–1981					
No	108	20 (15.6)	1		
Yes	70	27 (27.8)	2.08	(1.09–3.97)	<i>p</i> = 0.03
Use of nitrite inhalants (days of exposure)					
None	138	35 (20.2)	1		
1–19	33	11 (25.0)	1.31	(0.60–2.86)	
≥20	15	4 (21.1)	1.05	(0.33–3.76)	<i>p</i> trend = 0.68
Sexually-transmitted-disease history					
No	126	30 (19.2)	1		
Yes	67	21 (23.9)	1.32	(0.70–2.48)	<i>p</i> = 0.39

¹Not all subjects answered all questions, hence the numbers do not add up to the same number for all variables.

number of sexual partners in the previous year. Men who reported more than 50 partners in the previous year had a KSHV/HHV-8 prevalence of 34.5%, compared with 14.6% in men with fewer than 5 partners in the preceding year (*p*_{trend} = 0.04). Furthermore, KSHV/HHV-8 positivity was significantly associated with increasing number of years of homosexual experience, and with increasing number of reported receptive anal intercourses during the past month. Men with a history of sex with US men during 1980–1981 had significantly higher KSHV/HHV-8 seroprevalence (27.8%) than those without such contact (15.6%) (OR = 2.1; 95% CI 1.1–4.0).

Infection with KSHV/HHV-8 was weakly linked to HIV infection among participants at enrolment in 1981. Overall, 30.0% of the men who were HIV-seropositive were also KSHV/HHV-8-seropositive, compared with 20.4% of HIV-seronegative men (OR = 1.7; 95% CI 0.6–4.6). Oral receptive intercourse, rimming and number of deep kisses in the preceding month did not correlate with KSHV/HHV-8 seropositivity, nor did a history of sexually transmitted disease within the past year, educational status or use of nitrite inhalants (Tables I, II).

In a logistic-regression analysis that included all co-variables that reached significance at a *p* value of 0.05 in the univariate analysis (years of homosexual experience, number of receptive anal intercourses in the previous month, number of sexual partners, sex with US men), the number of receptive anal intercourses in the previous month (OR = 2.8; *p* = 0.03) and sex with US men (OR = 2.3; *p* < 0.05) were both independent risk factors for being KSHV/HHV-8-seropositive. Because of the close correlation between the risk factors included in the model and other variables such as receptive oral intercourse and rimming, we performed 2 additional multivariate analyses which were similar to the one mentioned above except that either receptive oral intercourse or rimming was added as a fifth co-variate. However, the significant variables remained the same.

Risk factors for seroconverting to KSHV/HHV-8 (incident cases, cohort analysis)

We also assessed risk factors for seroconverting to KSHV/HHV-8 during subsequent follow-up visits between 1981 and 1992. As shown in Table III, HIV seropositivity at any given time during this period was associated with a 2.4-fold (95% CI 1.3–4.7) increased risk of subsequent KSHV/HHV-8 seroconversion. Based on information obtained in 1981, visit to US homosexual communities in 1980–81 (RR = 2.4; 95% CI 1.3–4.6) and years of homosexual experience (RR = 3.1; 95% CI 0.97–10.1) were also associated with an increased risk of seroconverting to KSHV/HHV-8. Other sexual behavior, *e.g.*, number of sexual partners and receptive anal intercourse, did not reach statistical significance. The number of CD4⁺ cells (<100 per mm³, 100–<200, 200–<500, 500–<800, ≥800) did not significantly influence the risk of KSHV/HHV-8 seroconversion.

In a multivariate analysis (Cox's regression) which included all important variables of statistical significance in univariate analyses, visit to US homosexual communities was the strongest independent variable associated with KSHV/HHV-8 seroconversion (RR = 2.1; *p* = 0.03). However, association of HIV seropositivity with KSHV/HHV-8 seroconversion was borderline significant (RR = 2.0; *p* < 0.06), whereas for years of homosexual experience, the RR was 2.7 (*p* = 0.10). Including number of sexual partners in past year and number of receptive anal intercourses in past month in the model did not change the results.

Development of Kaposi's sarcoma

By September 1996, 41 members of the cohort had been diagnosed with AIDS, 10 of whom presented with KS at the time of AIDS diagnosis or later. All 10 men with KS were KSHV/HHV-8-seropositive prior to (8 men) or at the time of (2 men) their KS diagnosis; 3 men seroconverted to KSHV/HHV-8 prior to HIV, 5 seroconverted to HIV prior to KSHV/HHV-8, and in 2 cases

TABLE II – UNIVARIATE ASSOCIATIONS BETWEEN SEXUAL BEHAVIOR AND HHV-8-ANTIBODY POSITIVITY IN DANISH HOMOSEXUAL MEN AT ENROLLMENT IN 1981. A CROSS-SECTIONAL ANALYSIS BASED ON 1981 DATA¹

	HHV-8 antibody		OR	(95% CI)	
	Negative	Positive (%)			
Years of homosexual experience					
0-4	30	3 (9.1)	1	—	
5-9	52	11 (17.5)	2.12	(0.56-8.06)	
10-19	73	24 (24.7)	3.29	(0.97-11.17)	
20+	34	11 (24.4)	3.24	(0.86-12.19)	<i>p</i> trend = 0.05
Number of sexual partners in past year					
1-4	41	7 (14.6)	1	—	
5-19	64	15 (19.0)	1.37	(0.52-3.66)	
20-49	42	12 (22.2)	1.67	(0.60-4.67)	
50+	19	10 (34.5)	3.08	(1.04-9.16)	<i>p</i> trend = 0.04
Number of deep kisses in past month					
0-1	28	5 (15.2)	1	—	
2-9	57	12 (17.4)	1.14	(0.36-3.55)	
10-29	31	9 (22.5)	1.57	(0.46-5.20)	
30+	24	5 (17.2)	1.13	(0.29-4.37)	<i>p</i> trend = 0.65
Number of receptive anal intercourses in past month					
0	72	9 (11.1)	1	—	
1	31	11 (26.2)	2.84	(1.09-7.38)	
2-3	30	7 (18.9)	1.87	(0.64-5.44)	
4+	25	10 (28.6)	3.20	(1.20-8.54)	<i>p</i> trend = 0.04
Number of receptive oral intercourses in past month					
0	22	2 (8.3)	1	—	
1-4	43	12 (21.8)	3.07	(0.66-14.21)	
5-9	55	18 (24.7)	3.60	(0.83-15.68)	
10+	36	7 (16.3)	2.14	(0.41-11.05)	<i>p</i> trend = 0.57
Number of contacts between "your" mouth and "his" rectum					
0	95	20 (17.4)	1	—	
1-4	29	8 (21.6)	1.31	(0.52-3.29)	
5-9	15	4 (21.1)	1.27	(0.40-4.23)	
10+	19	5 (20.8)	1.25	(0.42-3.75)	<i>p</i> trend = 0.59
Number of contacts between "his" mouth and "your" rectum					
0	81	17 (17.4)	1	—	
1-4	29	9 (23.7)	1.48	(0.59-3.68)	
5-9	21	6 (22.2)	1.36	(0.48-3.88)	
10+	24	7 (22.6)	1.39	(0.52-3.74)	<i>p</i> trend = 0.45

¹Not all subjects answered all questions, hence the numbers do not add up to the same number for all variables.

TABLE III – THE RISK (RELATIVE HAZARDS) OF SEROCONVERTING TO HHV-8 DURING 1981-1992 ACCORDING TO HIV STATUS (CURRENT) AND SELECTED SEXUAL BEHAVIOR (1981). A SURVIVAL ANALYSIS OF HHV-8-SERONEGATIVE HOMOSEXUAL MEN AT ENROLLMENT FOLLOWED TO HHV-8 SEROCONVERSION

	Relative hazard (95% CI)	<i>P</i> value
HIV serostatus¹		
Negative	1	
Positive	2.44 (1.26-4.71)	<0.01
Visit to US homosexual community, 1980-1981²		
No	1	
Yes	2.44 (1.29-4.61)	<0.01
Sex with US men, 1980-1981²		
No	1	
Yes	1.66 (0.94-2.95)	0.08
Years of homosexual experience²		
<5 years	1	
≥5 years	3.12 (0.97-10.06)	0.06

¹Men who seroconverted to HIV during the follow-up contributed at risk time in the HIV-negative group until time of HIV seroconversion and thereafter in the HIV-positive group. ²Sexual behavior 1981.

seroconversion to KSHV/HHV-8 and human immunodeficiency virus was detected at the same time. None of the HIV-seronegative men in our cohort were registered in the Danish Cancer Registry or in the Pathology registry with a diagnosis of Kaposi's sarcoma.

DISCUSSION

Overall, 21.1% of Danish homosexual men in our cohort were seropositive for KSHV/HHV-8 in 1981. In comparison, only 2.5% (1/40) of a group of Danish railway workers were seropositive by the same method (data not shown). Seroprevalence rates of 3 to 5% have been reported in population samples from the US and the United Kingdom using similar technology (Kedes *et al.*, 1996; Simpson *et al.*, 1996).

Follow-up of men who were KSHV/HHV-8-seronegative at entry in 1981 documented high seroconversion rates in the beginning of the 1980s, which fell steadily over time. This decline in incidence is concomitant with a decline in sexual promiscuity and in the incidence of infection with HIV (Becker and Melbye, 1991; van Griensven *et al.*, 1993). We earlier noted a decrease in KS rates among AIDS patients during the same period (Casabona *et al.*, 1991). The uniform age distribution of KSHV/HHV-8 seropositivity in 1981 suggests that KSHV/HHV-8 only became highly prevalent in Danish homosexual men shortly before that time. If KSHV/HHV-8 had been prevalent in this community for decades, we would have expected increasing prevalence with age.

Men who were seropositive for HIV had a somewhat higher probability of being KSHV/HHV-8-seropositive at enrolment or of later seroconverting to KSHV/HHV-8. In the follow-up analysis, this association also approached significance in a multivariate analysis which included several sexual behavioral variables. How-

ever, infection with HIV is strongly correlated with sexual behavior, and one explanation for the observed association may be that we were unable to adequately adjust for all relevant sexual behavior in our study.

The strongest risk factors for KSHV/HHV-8 seropositivity were receptive anal intercourse and exposure to the US homosexual community. This corresponds very well with other studies of risk factors for AIDS-related KS among homosexual men. Already in the beginning of the 1980s, Marmor and colleagues reported AIDS-related KS to be significantly associated with the practice of receptive anal intercourse (Marmor *et al.*, 1982), while others subsequently reported increased risk of KS among HIV-positive men whose sexual practices involved fecal contact (Darrow *et al.*, 1992; Lifson *et al.*, 1990; Beral *et al.*, 1992). We were not able to establish a particular risk for KSHV/HHV-8 seropositivity associated with rimming, but other studies on risk factors for AIDS-related KS have been somewhat contradictory on this point (Lifson *et al.*, 1990; Beral *et al.*, 1992).

Sex with US men and visit to US homosexual community are strongly correlated variables, and, whereas one turned out to be the most important risk factor for KSHV/HHV-8 seropositivity in the cross-sectional analysis, the other was the more important in the cohort analysis. The independent risk associated with these exposures is in line with earlier reports on risk factors for AIDS-related KS. Thus, Archibald *et al.* (1990) found KS diagnosed in 56% of Canadian homosexual men with AIDS who reported more than 20 sex partners from large cities in the US, as compared with 21% of those who did not. Similarly, British homosexual men with AIDS were more likely to be diagnosed with KS also if they had had sex with an American man (31%) than if they had not (19%) (Beral *et al.*, 1991). From currently available data, it is not possible to judge whether the prevalence of KSHV/HHV-8 in US gay milieus was higher than in the Danish gay milieu in the early 1980s. A potential

interaction that might be associated with increased risk from sexual contact with a homosexual man from the US is the rate of co-infection of US men with KSHV/HHV-8 and HIV. This interaction might, through HIV-induced immunosuppression, lead to higher viral load of KSHV/HHV-8 and a greater risk of transmission, even when the rates of KSHV/HHV-8 in the 2 populations were the same. We note that the observed association between KSHV/HHV-8 seropositivity and exposure to the US homosexual community is similar to the experience with HIV at the beginning of the HIV epidemic (Melbye *et al.*, 1984).

In our study, KSHV/HHV-8 preceded KS in 8 out of 10 cases, and was detected simultaneously with KS diagnosis in the remaining 2 cases. While all 10 cases were also HIV-seropositive, KSHV/HHV-8 seroconversion preceded HIV seroconversion in 3 of the patients. Overall, this finding is consistent with the hypothesis that KSHV/HHV-8 infection may be causally associated with KS (Whitby *et al.*, 1995; Moore *et al.*, 1996; Gao *et al.*, 1996a,b).

In conclusion, the incidence of KSHV/HHV-8 was higher in the beginning of the 1980s than in more recent years among Danish homosexual men. The most likely route of transmission appears to have been sexual, probably by anal receptive intercourse. We note with interest that some of the factors independently associated with higher KSHV/HHV-8 seropositivity in this study, *e.g.*, contact with the US homosexual community in the early 1980s, strongly argue against the view that detection of antibodies to KSHV/HHV-8 reflects reactivation of a ubiquitous infection.

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