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Review

Kaposi's sarcoma and HHV-8 (human herpes virus 8) in those infected with human immunodeficiency virus: has the virus disappeared and if so why?

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ABSTRACT

In the early days of the HIV epidemic, Kaposi's sarcoma (KS) was the most common HIV-presenting illness for men who have sex with men (MSM). The malignancy is caused by HHV-8 (or KSHV), a human herpes virus which can be detected in the tissues of KS lesions. After discovery of the viral etiology a number of molecular techniques were developed in attempts to identify HHV-8 and the immune response to the virus in asymptomatic individuals. It was hoped these tests would help better define the population at risk and provide tools to help to understand the pathogenesis and epidemiology of the disease. However major issues exist with the sensitivity, specificity and predictive values of these tests making comparisons between studies unreliable. Today, the diagnosis of KS is much less common. It is unclear why this malignancy is seen less frequently in MSM newly diagnosed with HIV. Although highly active antiretroviral therapy (HAART) either directly or indirectly has had a major impact, this may not be the entire explanation. Other considerations include changes in sexual practices, and the impact of HAART on cofactors that mediate HHV-8 related disease. If HAART is the major driver, it remains unclear whether it is a consequence of a decrease in HHV-8 prevalence or viral load, whether there has been an impact on transmission, or whether HAART has altered viral expression into clinical disease. In this paper we will discuss the various hypotheses, and will review the molecular tests available to test for HHV-8 and whether or not they can be used in a current cohort to better determine what has happened to HHV-8 and KS.

KEYWORDS: Kaposi's sarcoma, HHV-8, KSHV, prevalence, testing, HIV

ABBREVIATIONS

AIDS, Acquired immunodeficiency syndrome; EIA, Enzyme immunoassay; HAART, Highly active antiretroviral therapy; HIV, Human immunodeficiency virus; HHV-8, Human herpes virus 8; IFA, Immunofluorescent assay; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma herpes virus; MSM, Men who have sex with men; NHL, Non-Hodgkin's lymphoma; PCR, Polymerase chain reaction; PBMC, Peripheral blood mononuclear cells; OD, Optical density; STIs, Sexually transmitted infections

INTRODUCTION

An initial signal of the AIDS epidemic was the appearance of Kaposi's sarcoma (KS) lesions in homosexual men. In the early years, KS was both the most common AIDS defining and AIDS presenting illness in men who had sex with men (MSM) [1]. The emotional impact of having the cutaneous lesions was devastating, with issues relating to

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disclosure, stigma, discrimination, poor self-esteem and self-worth, while visceral KS led to a rapidly fatal outcome. Investigations determined that KS was caused by human herpes virus 8 (HHV-8) also known as Kaposi's sarcoma herpes virus (KSHV) [2].

Early attempts at treatment were largely unsuccessful despite use of agents such as interferons, antivirals, topical retinoic acid compounds, hormones and systemic chemotherapy [3-6]. With the advent of HAART (highly active antiviral therapy) there was a significant decline in the incidence of KS estimated at 8.8%-39% per year [7, 8]. Now, in the developed world, clinicians report only the occasional case in patients presenting with HIV disease and even fewer new cases in patients in care [9].

What has happened to KS? Is HAART entirely responsible for the decrease in KS either through direct or indirect antiviral activity, through restoration of the immune system or through the impairment of the clinical expression of HHV-8 among HIV infected populations? Could changing sexual practices have decreased HHV-8 prevalence? Has a decline in asymptomatic HHV-8 infection, particularly among MSM contributed to the fall in incidence of KS? Is it possible with the data available to determine which of these hypotheses is most likely true?

In this review, we will discuss the challenges associated with testing for HHV-8, what is known about the epidemiology of the virus in North America, the hypotheses about possible transmission and speculate about whether declining incidence of HHV-8 plays a significant role in the declining incidence of AIDS-KS.

Kaposi's sarcoma

KS is a multicentric, angioproliferative spindle cell tumor of blood vessels, specifically of endothelial cells [10] which typically presents as red to violaceous, flat or raised lesions on the skin and mucosa. Extra-cutaneous lesions can involve the lymph nodes, lung or gastrointestinal tract. KS is almost exclusively seen in MSM. As it is described only infrequently in women, injection drug users or hemophiliacs with HIV, it was initially thought to be primarily sexually transmitted. A "related" form of the disease designated classic KS presents typically in elderly persons from the Eastern Mediterranean region and Sub Saharan Africa [11].

Chang *et al.* in 1994 identified DNA fragments from KS lesions of a previously unrecognized herpes virus which was named HHV-8 [2]. The 165 Kb genome was sequenced within 2 years of virus discovery and ultimately led to the development of many molecular assays to assess for the presence of HHV-8 or an immune reaction to it.

Diagnosis of KS and HHV-8

The diagnosis of KS requires clinical and histologic evaluation. Use of a surrogate marker to detect asymptomatic infection with HHV-8 would be important to better understand the epidemiology, pathogenesis and transmission of this virus. A major challenge to the use of surrogate markers has been the lack of a standardized test, and the only "true gold standard" upon which to validate these assays is samples from subjects with documented clinical lesions. Below we discuss the difficulties in detecting asymptomatic HHV-8 infection.

Serologic assays for HHV-8

Much research on testing methods for HHV-8 infection has focused on developing and comparing different serologic tests. To date there is no official standard test or combination of tests to confirm infection with HHV-8. HHV-8 has a genome of over 85 genes which could all potentially be employed as antigens; however the host immune response is not consistent or predictable [12]. However, relative to detecting viral DNA using PCR amplification, these tests have high sensitivity in serum and are easier to perform on a large scale.

Briefly, the first generation of serologic tests developed to detect HHV-8 were immunofluorescence assays (IFA) which incorporated antigens produced from cell lines infected with HHV-8 such as BCP-1 and BCBL-1 [13, 14] which did not support Epstein Barr Virus (EBV) replication to avoid cross-reactivity. IFA tests were developed to detect antibodies using the latent nuclear antigen (LNA or LANA) sometimes referred to as orf 73 for the gene which encodes it [15]. Induction of viral replication in the BCBL-1 cell line also leads to the production of additional lytic antigens such as K8.1. The detection of antibodies to these antigens improves the IFA sensitivity but lowers specificity [16, 17].

Later research focused on developing enzyme immunoassay (EIA) tests to detect antibody, as

they are easier to conduct on large scale and, in some investigations demonstrated improved specificity or sensitivity over IFAs. Although many unique proteins have been evaluated for use in EIA diagnostic tests, the most commonly used ones are the lytic-cycle glycoprotein K8.1, the lytic-cycle capsid protein orf 65 and the latency-associated orf 73 or LANA protein, none of which are cross-reactive with EBV [18-20]. The orf 73 EIA has increasingly replaced the orf 73 IFA because it improves sensitivity while retaining high levels of specificity and is currently the only latent cycle protein with repeatedly confirmed diagnostic utility [21, 22]. EIAs have also been developed based on whole virus lysates derived from cell lines infected with HHV-8. These are reactive with multiple lytic structural HHV-8 proteins and may be complemented with a test for latent antibodies [16, 23, 24].

When comparing assay characteristics, the simplest choice of a "gold standard" positive test for determining assay sensitivity is reactivity in patients with KS lesions. Serologic tests are less sensitive to AIDS-KS than classic KS, which may relate to the impaired ability of immunocompromised hosts to mount adequate antibody responses [13, 19]. Overall, sensitivities for serologic detection of HHV-8 in blood from KS patients range from approximately 85% using the latent antibody IFA to above 95% for the lytic antibody IFA. The latent test however has very high specificity, while the specificity of the lytic IFA test is questioned as two studies employing this test found the seroprevalence of HHV-8 among North American blood donors to be 20%, well above that found by subsequent studies and with other antibody tests [14, 17, 24, 25].

Using sera from KS patients as the gold standard for a positive test is problematic because antibody profiles of HHV-8 infection and antibody titers appear to vary with the duration and stage of HHV-8 infection. As antibody titers are highest among patients with KS lesions [13], using these thresholds as the "gold standard" may lead to misclassification of asymptomatically infected patients who may have lower titers. To overcome this problem, some researchers have diluted serum from KS patients to form a "true positive". In an investigation which used this strategy, the K8.1 viral protein EIA and the latent BCBL-1 IFA (measuring orf 73 antibodies) retained better sensitivity at 4-fold and 16-fold serum dilutions than EIAs for orf 73, orf 65 and whole virus [19].

For the same reasons, selecting the optical density (OD) cut points when evaluating EIA tests poses a challenge. One example is subtracting several standard deviations from an HHV-8 positive sample, high or low risk, to determine the cut point for a positive result [21, 24, 26]. Receiver operator curves (ROC) have also been generated using "true positive and negative" group samples, to enable selection of cut points which maximize specificity, sensitivity or both [19, 24].

Longitudinal studies have illustrated that a wider variety of antibodies are detectable in patients with longer term HHV-8 infection and KS. Biggar *et al.* collected yearly samples from MSM in New York and Washington (1982-1999) and tested for HHV-8 using a latent nuclear antigen IFA to and an EIA for antibody to lytic antigen K8.1. In early samples, reactivity might only be present on one assay but over time sera were more likely reactive to both antibodies and to increase in titre, and were higher in HIV positive men. The expanding epitope recognition suggested low-grade replication of HHV-8 and not new infections. Seroreversion, to one or the other antigen was also observed in this and other longitudinal cohorts [27, 28].

Accordingly, combining assays and developing algorithms to categorize samples has been considered. For example, in one study, serum was categorized as reactive if it was seropositive for either the orf 65 or K8.1 EIA. This improved sensitivity among KS patients and those who were asymptomatic but who subsequently developed KS without diminishing specificity as measured by lack of reactivity in blood donors, the negative control [20]. Other investigations recommend coupling an assay that detects antibodies to orf 73 with a test which detects lytic antibodies [19, 22]. Classifying samples as positive if they are reactive on at least one test helps to overcome the difficulty of single antibody reactivity and changing profiles throughout HHV-8 infection. Having a testing algorithm, wherein an indeterminate test is categorized using a second test also helps to refine classifications. Western blot technology has been investigated in attempts to improve assay reliability, and to develop a confirmatory test. To date none have proved effective and they are cumbersome and costly [18].

From this discussion above, it can be seen that comparing results of studies using different assays is frought with many problems.

PCR based assays for HHV-8

As PCR has limited sensitivity in testing peripheral blood mononuclear cells (PBMC) in KS patients, it is not recommended as a reliable method for identifying patients who are infected with HHV-8 [29, 30]. Only half of those with Kaposi's sarcoma are viremic, and even in these, viremia is often intermittent when samples assessed serially. Even in saliva [29] which has been found to have a higher viral burden, shedding may be intermittent and infection often missed [18, 31]. Most investigators have found less than 10% of seropositive, asymptomatic Americans are viremic by PCR [32].

Asymptomatic HHV-8 seroprevalence

Based on the existing literature, it is challenging to determine HHV-8 prevalence in different North American populations with enough certainty to make inferences as to whether trends in the rates of HHV-8 infection could help to explain the declines in KS incidence. It is difficult to compare prevalence estimates in different studies as they could reflect differences in test methodologies and variability in population characteristics rather than true differences in infection rates.

Epidemiologic investigations of HHV-8 seroprevalence have mostly examined samples from the late 1980s to early 2000s, before the introduction of HAART. In these studies, HHV-8 prevalence differed across populations. For the general population and blood donors, reported prevalence rates vary widely from 0%-15%, and is similar in heterosexual men, hemophiliacs and women [13, 18, 26, 33]. Prevalence rates reported vary between countries studied and are as high as 35%-60% in Sub-Saharan Africa where endemic KS is common [13, 18, 34]. Studies of injection drug users report prevalences of approximately 10%, and may correlate with the frequency and duration of drug use, leading some to the conclusion that rates in these cohorts are higher than in the general population [35-37]. Prevalence rates of 8%-40% are reported among men who have had at least one lifetime sexual contact with another man [26, 38, 39]. Given these estimates, trying to assess differences in rates in a given setting over time could be markedly influenced by changes in the genetic, geographic, and demographic make-up of the group under study.

Selected epidemiologic investigations of North American populations are discussed below to give a sense of how testing methodologies contribute to the variable ranges of estimates of HHV-8 prevalence in asymptomatic populations.

Pellett *et al.* tested sera from 1000 US blood donors in six laboratories from 1994-5. Each used algorithms based on different combinations of serologic tests to categorize samples. Samples were categorized as HHV-8 positive based on two latent class analysis models and a "pseudo-gold" or consensus-derived standard (which classified samples as positive if two different labs determined the sample was positive). They determined a prevalence of HHV-8 among blood donors between 3.0 and 3.5% [25]. This contrasted with two earlier and smaller studies ($n < 100$) that used the lytic BCBL-1 IFA to estimate a 20% HHV-8 infection rate from US blood donors. These latter estimates are believed to be falsely elevated due to the low specificity of this test relative to those utilized in the larger study [14, 16, 17].

Engels *et al.* examined the prevalence of HHV-8 using samples and survey data collected as part of the National Health and Nutrition Examination Survey (NHANES) III between 1988 and 1994. This cross-sectional survey was designed to randomly sample the US general population to gather data on health and nutritional habits. Using EIA serologic responses to orf 73 and K8.1, this sub-investigation established HHV-8 seroprevalence rates between 1.8 and 7.1%, depending on the assay and OD cutoff used. Overall prevalence was similar between exclusively heterosexual men and women. Among the men in the study who reported any lifetime sexual contact with another man, testing for HHV-8 using the high specificity EIA cutoff yielded a prevalence rate of 8.2% [26]. This corroborates data from other investigations demonstrating that HHV-8 prevalence is higher among MSM [33]. The prevalence of HHV-8 in MSM in San Francisco was as high as 37.6% using a whole virus EIA that claims a 100% specificity [38].

Among heterosexuals, evidence of correlation between sexual behaviors and rates of HHV-8 infection is mixed. In the Engels study, among men who had exclusively heterosexual contact, HHV-8 seropositivity correlated with the lifetime number of sexual partners,

and co-infection with hepatitis B and herpes simplex, indicators of increased sexual activity. In contrast, there was no correlation between these factors and HHV-8 seropositivity in women [26]. Two other investigations of US women found that syphilis infection was independently associated with HHV-8 positivity, though lifetime number of sexual partners was not [37, 40].

Although HIV appears to be a risk factor for HHV-8 infection, the prevalence varies within this group by risk exposure category. HIV is most strongly correlated with HHV-8 infection among MSM with rates of 20%-60% depending on the geographic location studied and test used [13, 24, 38]. Prevalence levels in other groups infected with or at risk for HIV are not clear cut. Using a latent BCBL-1 IFA to test for HHV-8 infection, Kedes *et al.* found that among those who had acquired HIV through blood transfusion and HIV-positive hemophiliacs, the prevalence of HHV-8 was between 3 and 5%, comparable to rates in the HIV-negative blood donors they examined, while Gao *et al.* found a prevalence of 0% in this group using a latent BCP-1 IFA [13, 33]. HIV infection has been found to be an independent risk factor for HHV-8 in women, and in some studies, injection drug users [35, 37, 40].

How is HHV-8 transmitted?

Injection drug use may be correlated with higher rates of infection, suggesting that HHV-8 may be spread through blood [35, 37]. Yet among individuals who acquired HIV from infected blood, such as hemophiliacs, the prevalence of HHV-8 is not higher than the general population [13, 33]. It has been concluded that there is little risk of acquiring HHV-8 infection from blood products and screening blood products for HHV-8 infection is not recommended [25].

As KS is more common in MSM than in other HIV risk groups, researchers have theorized that HHV-8 may be sexually transmitted. In MSM with HIV, HHV-8 seropositivity is associated with the lifetime number of sexual partners and history of sexually transmitted infections [39, 41]. In one cohort the HHV-8 seroprevalence rate was 20X higher for men with > 50 partners in the 6 months prior to enrollment than for men with 0-2 partners. Investigations [41] of the types of sexual behaviors associated with HHV-8 in MSM are inconclusive as to whether certain behaviors are riskiest for HHV-8 transmission. Problems in survey based studies which attempt to link sexual practices with seropositivity include the unreliability of self-reporting of sexual behavior and the difficulty of determining what specific acts an individual was engaging in at the time they became infected with HHV-8. The collinear relationships between the practices, such as insertive and receptive penile-anal intercourse, also make it difficult to isolate any one behavior as a major risk for transmission [38, 39, 41-43]. What's more, exposure to other bodily fluids can confound these studies, such as saliva which is commonly used by MSM as a lubricant in protected and unprotected penile-anal intercourse, and traumatic sexual acts involving blood exposure such as "fisting" [44]. Although rates of HHV-8 infection are sometimes correlated with lifetime number of sexual partners and history of sexually transmitted infections (STIs) in heterosexuals, the trends are less pronounced than in MSM [26, 37, 40, 45].

To enhance the understanding of transmission, studies have attempted to determine rates and quantities of HHV-8 DNA detection in bodily fluids. A higher proportion of patients with KS lesions had HHV-8 DNA detectable in their PBMCs, plasma or sera, semen and saliva than subjects who were asymptomatic for HHV-8 infection. Saliva had detectable DNA in more samples than semen and plasma or sera from the subjects with KS lesions [18, 46]. Comparing samples where HHV-8 DNA is detectable, saliva contains statistically higher levels of copies of viral DNA than serum and semen or anal samples. Saliva contained detectable DNA in more samples than semen, plasma or sera and PBMCs among the asymptomatic HIV positive and negative subjects although detection rates of DNA in all of these samples were 10% or below [18, 31, 46, 47]. In one study, viral DNA was detected in 30% of oropharyngeal samples compared to 1% of anal and genital samples from HHV-8 seropositive MSM [31].

In a prospective study conducted by Pauk *et al.* which repeatedly sampled MSM seropositive for HHV-8 but without clinical Kaposi's sarcoma over an average of 49 days, oral shedding of HHV-8 DNA was frequent. Of 9/13 seropositive men with detectable HHV-8 DNA in any oro-pharyngeal sample, viral DNA was present in saliva on over 35% of days. Some men, both HIV positive and negative, shed

HHV-8 DNA from their oral cavities at levels > 10,000 copies per ml for extended periods.

The consistent association between seropositivity in MSM and higher number of partners could therefore be related to the cumulative risk of exposure to an individual shedding high levels of viral DNA, or exposure to an individual who sheds DNA on a high proportion of days [31].

This data, coupled with evidence of casual transmission within families in countries where HHV-8 is endemic, has contributed to the alternate hypothesis that saliva is the most common mechanism for HHV-8 transmission [18, 45]. Kissing, however, which is not always studied along with sexual behavior, is widely thought to be an inefficient means for spreading HHV-8 given the low HHV-8 seroprevalence in the general North American population particularly compared to EBV, another herpes virus transmitted primarily through saliva. Comparisons between patterns of salivary shedding of viral DNA among persons seropositive for HHV-8 infection demonstrate that DNA of EBV is detectable in significantly more subjects and shed light on why HHV-8 is not ubiquitous [48].

What has happened to KS and HHV-8?

Has asymptomatic HHV-8 virus decreased in the population, have changes in sexual practices altered its transmission, has HAART decreased the prevalence of the virus directly through antiviral activity or through immune restoration of hosts, or has HHV-8 prevalence not changed but its clinical expression been altered such that we no longer see KS?

The incidence of AIDS-related KS is considerably lower than before the advent of HAART [7, 8], and HAART reduces the risk of KS among HIV positive individuals [49, 50]. KS rarely develops in patients on HAART and when it does, it generally occurs within 6 months of initiating therapy as part of an immune reconstitution inflammatory reaction (IRIS) [51] or when there is treatment failure. However, there are increasingly reports of KS developing in HIV positive individuals with adequately suppressed viremia and high CD4 counts [52, 53]. As HAART is an element of the standard treatment for AIDS-KS, and HAART may be sufficient to induce remission of AIDS-KS [54, 55], it is not surprising that investigators have concluded that the incidence has declined since the advent of HAART in 1997. The effect could be directly antiviral as demonstrated by studies that have shown HAART may decrease levels of HHV-8 virus in the blood of HIV positive individuals [56, 57]. However, these findings are inconsistent. For example, in a study of 14 patients with Kaposi's sarcoma, Tedeschi did not find a decrease in HHV-8 viral load by PCR with the introduction of HAART, despite the observation of a pre-treatment correlation between the viral loads of HIV and HHV-8 [58]. In contrast, in another study HAART use was independently associated with lower levels of HHV-8 DNA in saliva among HIV positive MSM [59]. The effect of HAART on HHV-8 could be indirect such that HIV-infected MSM, with better controlled HIV infection and stronger immune system health could have decreased likelihood of acquiring HHV-8 from an infected partner. Alternatively better HIV control could decrease the likelihood of HHV-8 to be expressed as clinical disease.

But is HAART the only reason for the decline in KS or did changes in sexual activity contribute?

The KS incidence rates appeared to peak in the early 1990s, before the advent of HAART [60, 61]. Some authors have speculated that the pre-HAART peak in KS incidence could be explained by a changing demographic composition of new HIV infections, for example with a lower proportion of MSM and a higher proportion of IVDUs who are less likely to be HHV-8 infected. Examining data from our own clinic (Figure 1), however, demonstrates the proportion of new MSM clinic referrals with KS declined coincidentally with the introduction of HAART. There is evidence that changing prevalence of HHV-8 infection could be a factor involved in explaining declining incidence of KS throughout the HAART era as well.

A study linked AIDS diagnoses and HAART in the San Francisco area (1990-2000) with the incidence of AIDS-KS and Non-Hodgkin's Lymphoma (NHL) in the California Cancer registry [62]. The decreased risk of AIDS-KS in the later periods was significantly correlated not only with the HAART regimen, but also with more recent calendar period of HIV diagnosis. Decreased risk of NHL, however, was only correlated with HAART use. The authors speculated that less risky sexual behavior or the use of drugs that have anti-herpetic activity could explain why the later calendar period was associated with decreasing incidence

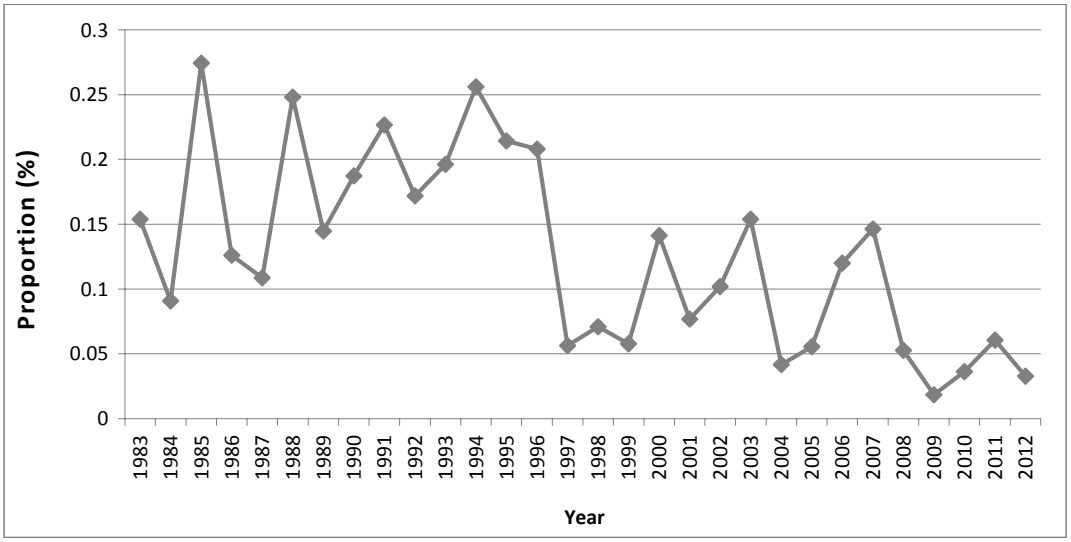

Figure 1. Proportion of Kaposi's sarcoma (KS) diagnoses of new clients of men who have sex with men (MSM) from an urban, tertiary, Immunodeficiency clinic, Toronto, Ontario.

of AIDS-KS independent of HAART regimen, although the changing demographics of the populations with HIV could also play a role.

Longitudinal studies have investigated changing rates of HHV-8 prevalence among MSM before HAART. These studies examined clearly defined subpopulations within small geographic regions using the same sets of serologic assays to minimize biases in estimates of seroprevalence between the years studied. Osmond *et al.* examined the prevalence of HHV-8 in San Francisco using blood samples from the San Francisco men and Young men's health studies (unmarried men living in census tracts where HIV prevalence was highest) for years 1984-1985 and 1995-1996, and from MSM in the San Francisco City clinic cohort (for years 1978-1980). From 1984, they also conducted interviews about recent sexual behavior. The fluctuations in the prevalence of HHV-8 between the 3 periods examined in the Men's health studies were stable at 26-30% whereas the HIV incidence decreased. In the San Francisco City Clinic Cohort, HHV-8 rates remained stable between 26.5%-33.4%, yet the HIV prevalence rose from 6.9% (1978-1979) to 24.0% (1979-1980). Prevalence rates of HHV-8 remained steady in spite of a reduction in unprotected receptive anal intercourse. This suggests sexual practices thought to be at lower risk for HIV transmission, including unprotected oral-penile intercourse which was practiced by 60%-90% of men studied after 1986, may be involved in spreading HHV-8 and behavioral changes as a consequence of the HIV epidemic, may not have played a role in reducing incidence of HHV-8 and thus KS [38]. This is also supported by a study of MSM in Italy which found near constant rates of HHV-8 prevalence among MSM in this period [63].

In contrast, a study conducted by O'Brien *et al.* tested serum yearly from a cohort of 85 MSM in New York and 160 in Washington, DC for HHV-8 (1982-1994) and for HIV (1982-1990). Overall, the prevalence of HHV-8 and HIV were 20.4% and 34.0% respectively but varied by site of enrollment and the number of sexual partners. The incidence of both HIV and HHV-8 peaked in 1983 with average incidence rates of 9.7 and 6.7 between 1982 and 1986, declining to 0.0 for HIV and 1.1 for HHV-8 between 1987-1990. The authors hypothesized that changes in sexual behavior associated with decreasing HIV incidence also decreased HHV-8 incidence [41].

More data may be needed to explain the decrease in AIDS-KS incidence (and possibly HHV-8) prior to HAART. Changes in sexual behavior with increased use of condoms associated with awareness of HIV transmission may not decrease the rates of HHV-8 infection if the virus is primarily transmitted by saliva. Although more investigation into patterns of salivary shedding of HHV-8 DNA is needed, some samples contain substantially higher levels of HHV-8 DNA than others. Any decrease in the number

of sexual partners associated with decreasing risky sexual behavior overall could potentially decrease the risk of exposure to such individuals.

Finally, clinicians have observed declines in KS as an HIV presenting diagnosis in MSM leading some to speculate that HHV-8 prevalence in MSM is lower than prior to the HAART era. Again it is unclear whether these observations are explained by changes in sexual practices that lead to less exposure or transmission of HHV-8 relative to HIV or whether HAART decreased asymptomatic HHV-8 infection in this population. Although there is an increased tendency to treat HIV infection earlier with HAART which could alter HHV-8 expression, the proportion of "late presenters" with HIV has not changed in many cohorts over the recent years [64] and yet presentation with KS is uncommon.

CONCLUSIONS AND FUTURE RESEARCH

KS has declined in MSM with HIV but the reasons for the decline remain incompletely understood, and could include any or all of HAART, either directly through antiviral activity or viral expression into clinical disease or indirectly through impact on the immune system and viral control, changing sexual patterns, decreased HHV-8 prevalence and shedding or decreased viral transmission. In spite of the challenges associated with testing that we have reviewed above and the imprecise estimates of HHV-8 prevalence from the 1990s, determining HHV-8 seroprevalence in populations of MSM, especially those with untreated HIV infection, could prove valuable to better our understanding of the reasons for the decrease in clinical KS in this population. We challenge investigators who have stored plasma banks from MSM early in the epidemic to compare rates of asymptomatic HHV-8 infection to that in contemporary cohorts. Investigators in regions where KS is still common may be better poised to study this prospectively as HAART is more widely introduced in their setting.

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CONFLICT OF INTEREST

No author has any conflict related to the contents of this manuscript. Dr. Sharon Walmsley has served on advisory boards, and has spoken at CME events for Abbvie, Merck, Janssen, ViiV, Bristol Meyers Squibb, and Gilead.

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