Risk Factors for Classical Kaposi Sarcoma in a Population-based Case-control Study in Sicily

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Abstract

Background: **Classical Kaposi sarcoma is a rare complication of Kaposi sarcoma-associated herpes virus (KSHV) infection. We conducted a population-based, frequency-matched case-control study in Sicily to further investigate the reported inverse relationship between smoking and classical Kaposi sarcoma and to identify other factors associated with altered risk.**

Methods: **All incident, histologically confirmed classical Kaposi sarcoma cases in Sicily were eligible. A two-stage cluster sample design was applied to select population controls. KSHV seropositivity was determined using four antibody assays (K8.1 and orf73 enzyme immunoassays and two immunofluorenscence assays). Using SAS-callable SUDAAN, we compared the characteristics of classical Kaposi sarcoma cases and KSHV-seropositive controls. Odds ratios (OR) and 95% confidence intervals (CI) are presented.**

Results: **In total, 142 classical Kaposi sarcoma cases and 123 KSHV-seropositive controls were recruited. Current cigarette smoking was associated with reduced risk of classical Kaposi sarcoma amongst males (OR, 0.20; 95% CI, 0.06-0.67). Edema was associated with classical Kaposi sarcoma, but only when it presented on the lower extremities (OR, 3.65; 95% CI, 1.62-8.23). Irrespective of presentation site, diabetes and oral corticosteroid medications were associated with increased risk (OR, 4.73; 95% CI, 2.02-11.1 and OR, 2.34; 95% CI, 1.23-4.45, respectively). Never smoking, diabetes, and oral corticosteroid medication use were all independently associated with classical Kaposi sarcoma risk.**

Discussion: **We confirmed previous reports that cigarette smoking was associated with a reduced risk of classical Kaposi sarcoma, and we found that risk was lowest among current smokers. We also found that classical Kaposi sarcoma risk was strongly and independently associated with oral corticosteroid use and diabetes. Corroboration of these observations and investigation of possible underlying mechanisms are warranted.** (Cancer Epidemiol Biomarkers Prev 2008;17(12):3435–43)

Introduction

Kaposi sarcoma-associated herpes virus (KSHV) is the primary cause of all forms of Kaposi sarcoma (1), including classical Kaposi sarcoma (2). Unlike iatrogenic or acquired immunodeficiency syndrome Kaposi sarcoma, classical Kaposi sarcoma predominately occurs in elderly men of Mediterranean or Jewish decent, with no apparent immunosuppression (3, 4). In the Mediterranean area, KSHV is thought to be mainly transmitted through early life exposure, probably to infectious saliva, with little evidence of sexual transmission except in homosexual men (5). Once infected, KSHV generally remains latent. Factors associated with dissemination of the virus *in vivo* and via shedding in saliva to susceptible individuals have not been well characterized. Patients with classical Kaposi sarcoma, as well as those with other types of Kaposi sarcoma, have much higher antibody titers and viral load in peripheral blood cells compared with KSHV-seropositive, disease-free individuals (3). Identification of other differences between classical Kaposi sarcoma patients and KSHV-seropositive controls may help to explain why only a few infected adults (approximately 1 in 3,000 men and 1 in 8,000 women) progress to classical Kaposi sarcoma annually (6).

Our group reported data from a case-control study of classical Kaposi sarcoma patients in Sicily, Naples, and Rome (1998-2001; ref. 7). Asthma, allergies in males, topical corticosteriod use, and infrequent bathing were associated with increased risk of classical Kaposi sarcoma. Interestingly, cigarette smoking was associated with a reduced risk of classical Kaposi sarcoma (7). This was most prominent in males and those who had the greatest cumulative exposure to cigarette smoking. Cigarette smoking has also been associated with a

Received 7/21/08; revised 8/27/08; accepted 9/26/08.

Grant support: Intramural Research Program of the National Cancer Institute, in part under a contract with RTI International (N02-CP-91027) and in part under contract N01-CO-12400. The Research and Development Office, Northern Ireland, funded Dr. Lesley Anderson to participate in the Cancer Prevention Fellowship Program, Office of Preventive Oncology, National Cancer Institute.

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doi:10.1158/1055-9965.EPI-08-0671

reduced risk of acquired immunodeficiency syndrome Kaposi sarcoma (8, 9) and this inverse association with smoking is supported by the reduced risk of lung cancer among patients with Kaposi sarcoma (10-12). Despite these observations, the association has not been characterized in depth, and potential mechanisms of action have not been elucidated. We conducted a case-control study in Sicily to further investigate the inverse relationship between smoking and classical Kaposi sarcoma and to identify other factors associated with the development of classical Kaposi sarcoma.

Materials and Methods

Incident, histopathologically confirmed, first primary cases of classical Kaposi sarcoma in native-born Italians, diagnosed from July 2002 through June 2006, were identified from all histopathology laboratories on the island of Sicily. Patients diagnosed with HIV were excluded from the study. A total of 177 incident cases of classical Kaposi sarcoma were identified. Of these, 35 patients did not participate for the following reasons: refusal (n = 20), deceased (n = 12), and unable to locate (n = 3). The overall participation rate of classical Kaposi sarcoma patients was 80.2%.

All residents of Italy are assigned to a primary care physician, with rosters maintained by the regional health authority. As illustrated in Fig. 1, a stratified two-stage cluster sample design was applied to select controls from the population of Sicily listed on the rosters of 4,044 primary care physicians (minimum roster size of 200 patients) with known sex and ages 31 to 92 y (n = 3,141,224). Physician practices were stratified by community size (<10,000, 10,000-100,000, and >100,000 residents), then 450 practices were randomly sampled, with the probability of selection proportional to the size of the roster. The 450 practices were randomly assigned to one of three waves, with 79 and 68 practices, on average, in eastern and western Sicily, respectively. Controls were randomly selected with different probabilities of selection from each roster in order to frequency-match them to the distribution by sex (73.6% male) and age (31-50, 51-60, 61-70, 71-80, and 81-92 y) of the anticipated classical Kaposi sarcoma cases. In wave 1, 8 patients were selected from each of the 150 practices (n = 1,200). In waves 2 and 3, we used an updated file to exclude deceased physicians (n = 13, including 4 in wave 1) and selected 12 patients per practice (n = 1,752 and 1,740 in waves 2 and 3, respectively). Of the 4,692 selected controls, 1,268 were not approached when wave 3 was terminated for lack of funds, 404 were deceased, and 14 were otherwise out-of-scope (non-Italian, insufficient blood for KSHV testing, or had classical Kaposi sarcoma). Of the 3,006 eligible patients, 1,774 were nonrespondents, including 590 who could not be approached because the physician refused, had retired, or had died. The final population of controls was n = 1,232 (41% of the eligible participants).

All subjects provided signed informed consent, and ethical approval was obtained from the U.S. National Cancer Institute, local institutions in eastern (Ragusa) and western (Palermo) Sicily, and the coordinating center (RTI International).

Exposure Assessment. Subjects were interviewed using a standardized questionnaire about demographic characteristics, socioeconomic variables, medical history, and smoking. Participants were defined as never smokers if they had smoked less than one cigarette per week in their lifetime. Current smokers were defined as those who still smoked at least one cigarette per week, and former smokers as those who had stopped smoking at least one cigarette per week. A 23 to 25 mL peripheral blood sample was obtained from all cases and controls in EDTA anticoagulated blood vacutainer tubes. Aliquots of plasma were stored at -80°C until testing. One plasma aliquot was tested for HIV-1 antibodies with a licensed immunoassay; all were negative.

Kaposi Sarcoma-Associated Herpes Virus Antibody Testing. KSHV antibody testing was done as reported previously (7). Briefly, using a 1:120 dilution of plasma and the BCBL-1 cell line with and without induction by tetradecanoyl phorbol-ester acetate, antibodies against KSHV lytic and latent nuclear antigens, respectively, were detected using immunofluorenscence assays (13). Enzyme immunoassays with recombinant proteins were used to detect KSHV antibodies against the K8.1 structural glycoprotein and open reading frame (orf) 73 at plasma dilutions of 1:20 and 1:100, respectively (7).

Subjects were considered KSHV seropositive if the latent immunofluorenscence assay was positive or the K8.1 optical density was >1.2. KSHV seronegative was defined as latent immunofluorenscence assay negative and K8.1 optical density ≤0.8 and orf73 optical density ≤0.8. Immunofluorenscence assay latent and lytic results were unavailable for four controls, all of whom were below the K8.1 and orf73 optical density cutoffs and negative in investigational KSHV antibody assays (data not presented). These four subjects were categorized as KSHV seronegative. All other subjects were categorized as having indeterminant results.

Weighting Procedures. To take into account the complex sampling used for the selection of controls, controls were weighted using the product of the reciprocal of the selection probabilities at each stage of sampling. These weights are referred to as the base weights, which were adjusted for nonresponse by first forming nonresponse categories that were a cross-classification of age (31-50 and 81-90, 51-60, 61-80 y), region (eastern, western Sicily), and gender. The base weights for enrolled controls within each nonresponse category were then multiplied by the inverse of the (base) weighted proportion of the enrolled controls to the eligible sampled controls. This involved using a two-dimension ratio-adjusted raking procedure in which the nonresponse adjusted weights first were raked to the population distribution in the cross-classified categories of zone [six zones made up of three community sizes (<10,000, 10,000-100,000, and >100,000) by two regions (eastern, western Sicily)] by age categories (31-50, 51-60, 61-70, 71-80, and 81-90 y) and then raked to the population distribution in the cross-classified categories of zone by gender. Raking is often used in survey research to reduce undercoverage bias. Raking is an iterative procedure that involves adjusting the sample weights, so that the sums of the sample weights over specified cross-classification of certain

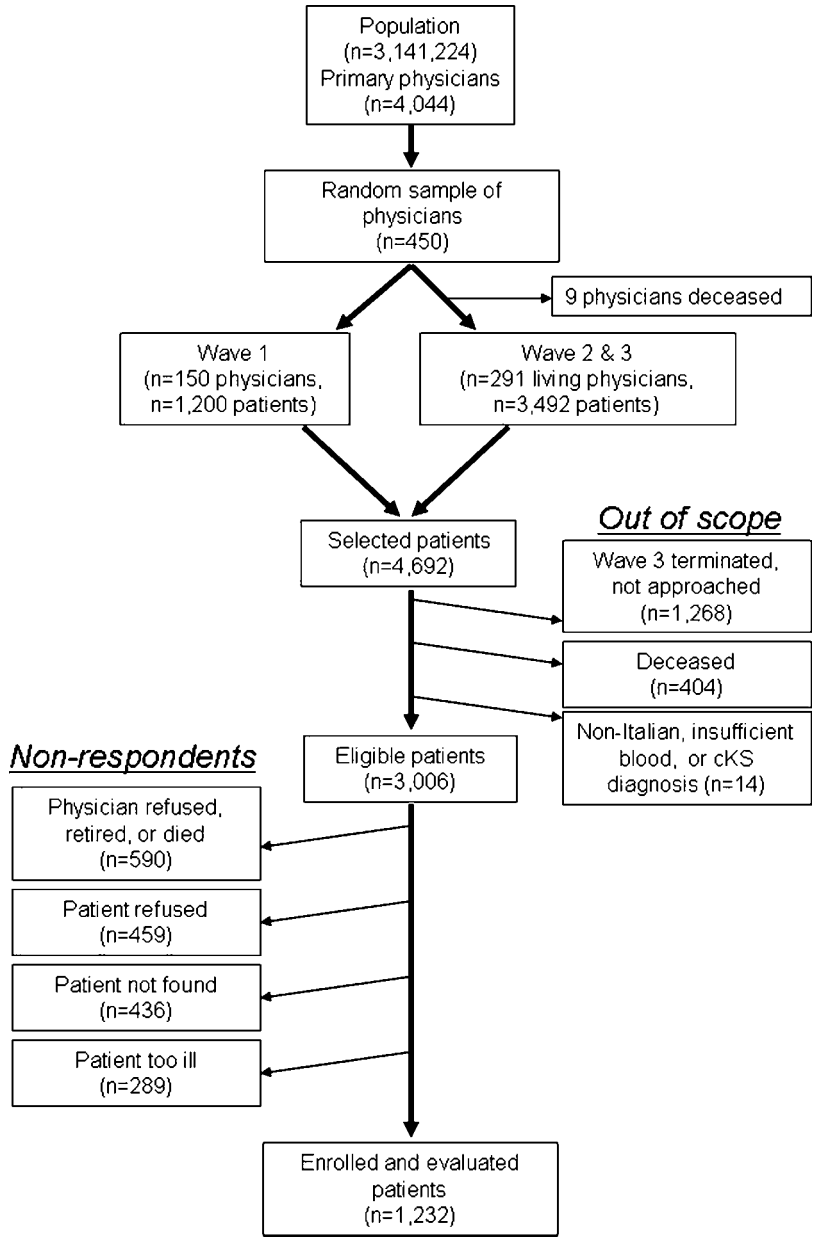

Figure 1. Two-stage sampling procedures for population-controls.

categorical variables used in the raking (in this case the cross-classification of zone by age categories and zone by gender) match the population distribution (14).

To approximate the frequency-matching of the control sample to the age, gender, and community size distribution of the cases, the nonresponse/poststratification-adjusted weights for the controls were adjusted by applying two-dimensional raking to these weights so that they matched the distribution of all the cases (including the nonresponding cases). To reflect the sample size of the enrolled controls, these ratio-adjusted weights were further scale-adjusted so that these weights summed to the sample size of the enrolled controls.

Weights were also constructed for the cases. Because cases were not sampled, the base weights were set equal to 1. These base weights were then adjusted for nonresponse ($n = 35$) in a similar manner to the controls, but the response categories were the cross-classified categories of age (≤80, >80 y) and gender. The nonresponse-adjusted case weights were further scale-adjusted so that the sum of the case weights was the number of enrolled cases ($n = 142$).

Statistical Analysis. Classical χ^2 and t-tests were used for descriptive, unweighted, and unadjusted demographic comparisons. SAS-callable SUDAAN was used to conduct logistic regression analyses that accounted for the sample weighting and the multistage stratified cluster sampling of the controls. These logistic regression analyses compared potential risk factors between patients with classical Kaposi sarcoma and the KSHV-seropositive controls. Categories for continuous measured covariates were defined *a priori*, using median or

tertile values, among KSHV-seropositive controls. All analyses were adjusted for age in quartiles (<68, 68-74, 75-80, and >80 y), education (1st-4th grade, ≥5th grade), and gender (or restricted to males). To assess the impact of serologic categorization, a statistical sensitivity analysis was conducted using only the latent immunofluorenscence assay results.

Results

Of the 142 classical Kaposi sarcoma cases recruited, 135 (95.1%) were KSHV seropositive, 4 (2.8%) were KSHV seronegative, and 3 (2.1%) had indeterminate KSHV status. Of the 1,232 controls, 123 (10.0%) were KSHV seropositive, 1,031 (83.7%) were KSHV seronegative, and 78 (6.3%) had indeterminate KSHV status. KSHV-seropositive controls were slightly older than KSHV-seronegative/indeterminate controls (mean 73.6 versus 71.1 years, respectively; $P = 0.05$), but they did not differ with respect to gender (70% versus 74% male; $P = 0.33$). KSHV-seropositive controls did not differ from classical Kaposi sarcoma cases in either age (mean 73.6 versus 74.6 years; $P = 0.45$) or gender (70% versus 63% male; $P = 0.22$). Feet or legs were the site of initial classical Kaposi sarcoma lesions in 108 (76.1%) of the patients with classical Kaposi sarcoma.

Tables 1 to 3 present odds ratios (OR) and 95% confidence intervals (CI) associated with postulated risk factors for classical Kaposi sarcoma compared with KSHV-seropositive controls that take account of the complex sample design, with adjustment for age, gender, and where appropriate, education. Classical Kaposi sarcoma patients were more likely than KSHV-seropositive controls to have been educated beyond the 4th grade (OR, 2.63; 95% CI, 1.48-4.68). Classical Kaposi sarcoma patients tended to come from childhood households with ≥6 residents, but the association did not reach statistical significance. Household crowding, as reflected by the number of people per bedroom in the childhood home, was not associated with the risk of classical Kaposi sarcoma. In addition, the number of older and younger siblings did not differ significantly between classical Kaposi sarcoma patients and KSHV-seropositive controls.

Current cigarette smoking, compared with never smoking, was strongly associated with a reduced risk of classical Kaposi sarcoma (OR, 0.22; 95% CI, 0.07-0.69), particularly in males (OR, 0.20; 95% CI, 0.06-0.67; Table 2). Because there were few current (cases, $n = 3$; controls, $n = 2$) or former (cases, $n = 2$; controls, $n = 2$) female smokers, Table 2 presents the findings for male smokers only. The reduced risk for current smokers was stronger before age 75 years (OR, 0.07; 95% CI, 0.01-0.43) than at older ages (OR, 0.66; 95% CI, 0.11-3.87).

Time since quitting smoking, compared with never smoking, was associated with a reduced risk of classical Kaposi sarcoma ($P_{trend} = 0.021$). In addition, there appeared to be a trend between increasing duration of smoking and reduced risk of classical Kaposi sarcoma ($P_{trend} = 0.058$). There were no clear trends between intensity or pack-years of smoking and risk of classical Kaposi sarcoma ($P_{trend} = 0.143$ and 0.173, respectively). Male classical Kaposi sarcoma patients were less likely than KSHV-seropositive controls to usually smoke filtered cigarettes. Cigar and pipe smoking were not associated with classical Kaposi sarcoma risk. Likewise, living or working in an enclosed environment with smokers was not associated with classical Kaposi sarcoma risk in males, females, or never-smokers (data not presented). None of the study participants had ever used nicotine patches or gum.

Table 1. Sociodemographic characteristics of classical Kaposi sarcoma cases and KSHV-seropositive controls

	No. of classical Kaposi sarcoma patients ($n = 142$)	No. of KSHV-seropositive controls ($n = 123$)	OR (95% CI)*
Community size (current)			
<10,000	21	26	1.00
≥10,000	111	97	1.09 (0.65-1.82)
Community size (most of adult life)			
<10,000	48	47	1.00
≥10,000	89	76	1.13 (0.63-2.03)
Community size (birth)			
<10,000	50	37	1.00
≥10,000	91	86	0.81 (0.45-1.45)
Education †			
1st-4th grade	44	62	1.00
≥5th grade	97	60	2.63(1.48-4.68)
No. residents in childhood home			
<6	59	65	1.00
≥6	83	58	1.65 (0.94-2.92)
No. people per bedroom			
<3	80	66	1.00
≥3	62	57	1.10 (0.62-1.96)
No. older siblings			
<2	64	66	1.00
≥2	78	57	1.36 (0.76-2.46)
No. younger siblings			
<3	82	81	1.00
≥3	59	41	1.57 (0.88-2.80)

*Adjusted for gender, age group (<68, 68-74, 75-80, >80 y) and education (1st-4th grade, ≥5th grade).
†Adjusted for gender and age group (<68, 68-74, 75-80, >80 y) only.

Table 2. Comparison of smoking habits of male patients with classical Kaposi sarcoma and KSHV-seropositive controls

	No. of male classical Kaposi sarcoma patients	No. of male KSHV-seropositive controls	OR (95% CI)*
Cigarette smoking			
Never	23	12	1.00
Former	59	55	0.50 (0.19-1.32)
Current	7	19	0.20 (0.06-0.67)
Cigarette smoking in men age <75 y			
Never	16	7	1.00
Former	23	26	0.30 (0.09-1.07)
Current	2	11	0.07 (0.01-0.43)
Cigarette smoking in men age ≥75 y			
Never	7	5	1.00
Former	36	29	0.79 (0.19-3.36)
Current	5	8	0.66 (0.11-3.87)
Years since quitting smoking			
Never smoker	23	12	1.00
≥26	23	18	0.62 (0.20-1.96)
15-25	17	19	0.42 (0.12-1.41)
<15	18	17	0.46 (0.15-1.39)
Current smoker	7	19	0.18 (0.05-0.64)
Smoking duration (y)			
Never smoked	23	12	1.00
<34	24	22	0.56 (0.19-1.64)
34-48	22	27	0.38 (0.13-1.09)
≥49	20	25	0.35 (0.11-1.07)
No. cigarettes per d			
Never smoked	23	12	1.00
<10	19	22	0.48 (0.16-1.41)
10-19	13	18	0.33 (0.10-1.10)
≥20	34	34	0.45 (0.16-1.22)
Pack-years of smoking			
Never smoked	23	12	1.00
<14.7	21	23	0.51 (0.18-1.46)
14.7-47	19	27	0.30 (0.10-0.90)
≥48	26	24	0.51 (0.18-1.46)
Cigarette type			
Never smoker	23	12	1.00
Filtered usually	29	44	0.28 (0.10-0.81)
Unfiltered usually	19	16	0.61 (0.20-1.89)
Filtered and unfiltered equally	18	14	0.63 (0.20-2.02)
Pipe			
No	79	77	1.00
Yes	10	9	0.98 (0.31-3.10)
Cigar			
No	75	75	1.00
Yes	11	14	1.18 (0.42-3.32)
Lived with ≥1 person who smoked daily †			
No	17	24	1.00
Yes	72	62	0.77 (0.38-1.55)
Worked in smoky indoor place †			
No	68	71	1.00
Yes	21	15	1.29 (0.50-3.38)

*Logistic regression analyses adjusted for age (<68, 68-74, 75-80, >80 y) and education (1st-4th grade, ≥5th grade) that account of the sample weighting and the multistage stratified cluster sampling of the controls.
†Within the past 10 y.

Classical Kaposi sarcoma cases were more likely than KSHV-seropositive controls to have been evaluated for, or diagnosed with, kidney failure/dialysis, diabetes, or edema of the legs or feet from among 16 specific medical conditions queried (Table 3). All three conditions were reported by seven classical Kaposi sarcoma patients (six of whom had taken oral corticosteroids), compared with no controls. For edema of the lower extremities, the association was limited to classical Kaposi sarcoma patients with their first Kaposi sarcoma lesion on their legs or feet (OR, 3.65; 95% CI, 1.62-8.23); Kaposi sarcoma risk was not increased with a first Kaposi sarcoma lesion at other body sites (OR, 0.99; 95% CI, 0.25-3.92). In addition, the association between edema and classical Kaposi sarcoma risk was not significant (OR, 1.63; 95% CI, 0.74-3.59) when the 5-year period before the interview date was excluded to reduce the possibility that the edema was caused by classical Kaposi sarcoma. Increased risk with kidney failure/dialysis was seen for initial Kaposi sarcoma lesions on the legs/feet (OR, 2.91; 95% CI, 0.20-41.6) and at other body sites (OR, 3.82; 95% CI, 0.19-77.1). Likewise, increased risk with diabetes was independent of the initial site of classical Kaposi sarcoma lesions (OR, 3.30; 95% CI, 0.99-10.9 for legs/feet, and OR,

Table 3. Comparison of select medical conditions and medication usage in classical Kaposi sarcoma patients and KSHV-seropositive controls

	No. of classical Kaposi sarcoma patients (n = 142)	No. of KSHV-seropositive controls (n = 123)	OR (95% CI)*
Medical conditions			
Allergies	26	24	1.01 (0.50-2.07)
Angina	9	7	0.76 (0.24-2.37)
Arthritis	4	6	0.55 (0.13-2.39)
Asthma	15	13	1.42 (0.61-3.31)
Bronchitis	41	38	0.99 (0.54-1.83)
Chilblains	38	28	1.11 (0.58-2.13)
Cirrhosis/Liver failure	15	9	2.17 (0.73-6.41)
Diabetes	45	15	4.73 (2.02-11.1)
Edema	68	31	3.11 (1.59-6.05)
>5 y prior to interview	34	18	1.63 (0.74-3.59)
Gout	14	8	1.28 (0.45-3.62)
Heart disease/stroke	43	31	0.93 (0.49-1.77)
Hypertension	87	70	0.87 (0.48-1.56)
Kidney failure/Dialysis	19	4	3.86 (1.22-12.3)
Malaria	32	35	0.84 (0.45-1.58)
Sexually transmitted disease	9	11	0.72 (0.26-1.93)
Thalassemia	3	3	1.18 (0.18-7.55)
Medications†			
Oral cortisone	53	26	2.34 (1.23-4.45)
Cortisone cream	53	36	1.45 (0.79-2.66)
Other medicated nonsteroid cream	62	57	0.81 (0.45-1.46)
Herbal treatments	26	16	1.58 (0.70-3.55)

*Logistic regression analyses adjusted for gender, age (<68, 68-74, 75-80, >80 y) and education (1st-4th grade, ≥5th grade) that account of the sample weighting and the multistage stratified cluster sampling of the controls.
†Used during the past 10 y.

2.75; 95% CI, 0.67-11.3 for other part of body). None of the other medical conditions investigated was significantly associated with classical Kaposi sarcoma. Any oral corticosteroid use during the previous 10 years was associated with a 2.34-fold increased risk of classical Kaposi sarcoma (Table 3). The use of corticosteroid cream, other topical creams, and herbal treatments was not significantly associated with classical Kaposi sarcoma risk.

Multiple variable logistic regression models, each adjusted for gender, age category, and education category, were constructed to assess the independence of variables associated with classical Kaposi sarcoma risk. Edema of the legs/feet was not included. Risk of kidney failure/dialysis remained elevated, although not significant, when adjusted for diabetes (OR, 3.03; 95% CI, 0.25-36.4). In contrast, as shown in Fig. 2, classical Kaposi sarcoma risk was independently associated with never smoking (OR, 2.66; 95% CI, 1.06-6.64), diabetes (OR, 4.02; 95% CI, 1.73-9.37,), and oral corticosteroid use (OR, 2.25; 95% CI, 1.16-4.38). The results were essentially identical in a sensitivity analysis that used only the KSHV latent immunofluorenscence assay results.

Discussion

In this population-based case-control study, we confirmed the previously reported inverse relationship between cigarette smoking and classical Kaposi sarcoma among men (7). The current study investigated this in more depth, finding that risk of classical Kaposi sarcoma was lowest among current smokers and those who smoked only filtered cigarettes. Independent of smoking, we found that higher education, diabetes, and use of oral corticosteroid medications were more common in classical Kaposi sarcoma cases compared with KSHV-seropositive controls.

Our findings are consistent with most (7-9), but not all (15, 16), studies on the relation between cigarette smoking and the risk of Kaposi sarcoma. For example, Nawar and colleagues observed a lower relative risk (0.6; 95% CI, 0.5-0.9) of acquired immunodeficiency syndrome Kaposi sarcoma among cigarette smokers in a cohort of HIV-positive, KSHV-seropositive homosexual men in the United States (8). In addition, lung cancer is uncommon in patients with Kaposi sarcoma (10-12). In unpublished data from the Surveillance Epidemiology and End Results program for the years 1973 to 2005, the risk of a second-primary lung cancer was lower than expected (0.41; 95% CI, 0.18-0.80) after a first-primary Kaposi sarcoma unrelated to acquired immunodeficiency syndrome (diagnosed before 1980 or after age 70 years; Morton, L.M., National Cancer Institute), providing population-based support for the hypothesis that most Kaposi sarcoma patients were nonsmokers.

In Italy (17), as in other developed countries (18, 19), many of our study participants had discontinued smoking. Remarkably, we found a strong and highly significant trend between smoking within the previous 15 years and reduced risk of classical Kaposi sarcoma. The lower classical Kaposi sarcoma risk was weakly related to duration of smoking, and it was not significantly related to smoking intensity or cumulative exposure, as estimated by cigarettes per day and pack-years, respectively. Perhaps the null association with cumulative exposure implies that smoking blocks an effect of aging *per se* on Kaposi sarcoma risk, which we could not evaluate because our cases and controls were matched for age. Never smokers with a high level

of education were most at risk of classical Kaposi sarcoma. As was true previously (7), classical Kaposi sarcoma risk was unrelated to cigar and pipe smoking. Passive smoking, as measured by living in a household with a smoker or working in an indoor smoky environment, was not associated with classical Kaposi sarcoma risk. However, passive smoking is difficult to measure and our null findings should not be considered definitive.

It has been postulated that the immunologic effects of cigarette smoking could reduce the risk of classical Kaposi sarcoma in smokers (9, 20). For example, nicotine can affect dendritic cell function (21-23) and alter cytokine and growth factor production involved in the propagation of Kaposi sarcoma (24). This prompted a recent randomized clinical trial to evaluate whether transdermal nicotine patches, used continuously for 15 weeks, could induce regression of classical Kaposi sarcoma lesions (20). The null results of that trial, with the null association between classical Kaposi sarcoma risk and use of topical creams and ointments in the current study, imply that exposures of the epidermis may have little impact on the pathogenesis of Kaposi sarcoma. Rather, they suggest that factors that act internally drive KSHV infection to manifest as Kaposi sarcoma. Susceptibility of cells to KSHV infection *in vitro* seems to be suppressed by cigarette smoke extract (25). Moreover, although not supported by functional data, cigarette smokers have reduced levels of serum neopterin and elevated levels of CD4 lymphocytes and other leukocytes (26, 27), which is the inverse of the pattern seen among classical Kaposi sarcoma cases (4, 28).

Despite the possibility that constituents of cigarette smoke could modulate classical Kaposi sarcoma risk, other potential explanations should be considered. Cigarette smoking is associated with significant morbidity and mortality. Because classical Kaposi sarcoma is primarily a disease of the elderly, smokers may not survive long enough to develop classical Kaposi sarcoma. If smokers who die prematurely due to smoking-related illnesses are more genetically predisposed to classical Kaposi sarcoma than smokers who survive, then the reduced risk of classical Kaposi sarcoma in smokers could be a function of differential survival bias. If true, then the risk of classical Kaposi sarcoma related to smoking would be less prominent in younger individuals who have not yet died of a smoking-related illness. However, we observed that classical Kaposi sarcoma risk was more markedly reduced in younger male smokers (OR, 0.07) than in older male smokers (OR, 0.66). In addition, there were no significant decreases in smoking-related illnesses, such as bronchitis or heart disease/stroke, in patients with classical Kaposi sarcoma, suggesting that differential survival bias does not fully explain our observations. Given the detrimental health effects of smoking, our findings should not be interpreted to recommend smoking to people infected with KSHV or anyone else.

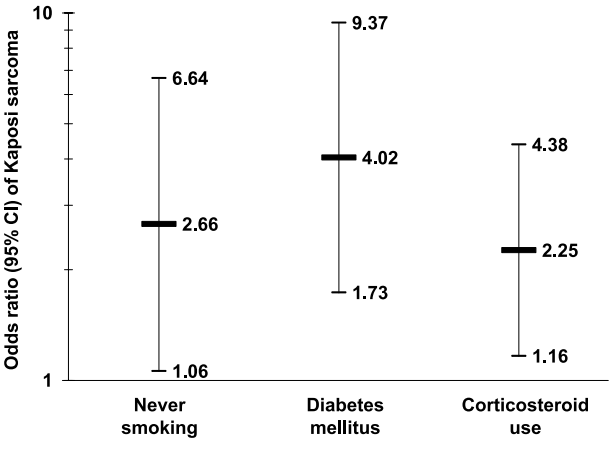

Figure 2. Multiple variable logistic regression model for risk of classical Kaposi sarcoma compared with KSHV-seropositive controls, among men and women in Sicily.

We observed strong effects of never smoking, use of oral corticosteroids, and diabetes. All three factors were independently associated with increased risk of classical Kaposi sarcoma. Our group previously reported that short-term use of oral corticosteroids was associated with an insignificantly elevated risk of classical Kaposi sarcoma (OR, 2.17; 95% CI, 0.94-5.01; ref. 7). The use of topical corticosteroid creams and ointments in the current study was not significantly related to classical Kaposi sarcoma risk, in contrast to our previous results (7). Unfortunately, the current study lacked detailed information about the duration, frequency, or indications for use of oral corticosteroids. Although not observed herein, classical Kaposi sarcoma has previously been reported to be higher in patients with asthma (7) and rheumatic diseases (29) that are commonly treated with corticosteroid medications. The immunosuppressive effects of corticosteroid medications could induce KSHV reactivation and dissemination of the virus or the malignant cells. For example, glucocorticoids have been shown to enhance Kaposi sarcoma cell growth, possibly by blocking transforming growth factor-β (30), and remission of Kaposi sarcoma has been reported with reductions in corticosteroid medication usage (31, 32). Together these studies support a role of corticosteroids in the development of classical Kaposi sarcoma.

Diabetes mellitus was also strongly associated with increased risk of classical Kaposi sarcoma in the current study and has been noted among Kaposi sarcoma patients previously (15, 33, 34). Guttman-Yassky et al. suggested a relation between diabetes mellitus and classical Kaposi sarcoma in a case-control study in Israel, although the association was not significant (OR, 1.8; 95% CI, 0.4-7.4; ref. 15). It is plausible that ascertainment bias could lead to the detection of classical Kaposi sarcoma in diabetic patients because they require careful and frequent clinical inspection of the feet, where initial classical Kaposi sarcoma lesions often arise. However, the increased risk of classical Kaposi sarcoma was not limited to patients who noticed their first Kaposi sarcoma lesion on their legs or feet. Because subjects were asked if they had ever been "diagnosed with, or investigated for" diabetes, it is possible that the true prevalence of diabetes is lower than reported. However, the prevalence of diabetes among our controls is comparable with the prevalence of type 2 diabetes in persons ages >65 years in Italy (35). How diabetes might increase the risk of classical Kaposi sarcoma is unknown, but at least two mechanisms are possible. First, impaired microvascular

circulation, as can occur in diabetics, could induce tissue hypoxia and KSHV lytic replication through hypoxia-inducible factor-1α (36). If so, this would lead to dissemination of the virus *in vivo,* increasing the total body burden of KSHV and the risk of developing classical Kaposi sarcoma. Second, hypoxia-inducible factor 1α and 2-α are not only expressed in a Kaposi sarcoma cell line, but they also are modulated by insulin-like growth factor-I, which is required for the growth of these cells (37). In addition, the transformation of dermal microvascular endothelial cells by KSHV is reported to depend on the expression of the insulin receptor (38). Further work is needed to investigate how classical Kaposi sarcoma risk is altered by the complex insulin-related pathways, taking into consideration the effects of nonsmoking and use of corticosteroids.

An elevated risk of classical Kaposi sarcoma was also observed in patients with edema. The association with classical Kaposi sarcoma was limited to patients who developed their first Kaposi sarcoma lesion on the lower extremities. Lymphedema of the lower extremities can be a consequence of Kaposi sarcoma. Even though our study was limited to patients with incident classical Kaposi sarcoma, some patients (n = 21; 15%) had Kaposi sarcoma lesions for more than two years before seeking medical attention. When we excluded the 5-year period prior to interview, the association between edema and classical Kaposi sarcoma was no longer significant, suggesting that edema did not increase the risk of classical Kaposi sarcoma. Instead, edema was likely to have been a consequence of the disease process.

This is the first population-based field study of classical Kaposi sarcoma. It has several strengths, including a broad perspective of potential risk factors, a high response rate among cases, restriction to cases who had newly diagnosed Kaposi sarcoma, inclusion of a representative sample of controls from throughout Sicily, comparison of classical Kaposi sarcoma patients with KSHV-seropositive controls to assess risk factors involved in tumor development, and weighted adjustment for nonresponse among the cases and controls.

Some limitations should also be noted. First, participation rates among the controls were poor, which may have biased some of our results. Second, because medical chart review was not undertaken, historical recollections by elderly participants may have led to misclassification of exposures. However, this is unlikely to differ greatly between cases and controls. Third, although our data suggest that diabetes may be an independent risk factor for classical Kaposi sarcoma, we cannot exclude the possibility that ketosis-prone diabetes mellitus is a serious complication of KSHV infection (39) and thus an intermediate en route to Kaposi sarcoma. Finally, imprecision of KSHV serology is an important limitation. We used four antibody assays to determine KSHV status and an algorithm combining these results that had good sensitivity (95%) in the classical Kaposi sarcoma cases and probably good specificity, given the conservative seroprevalence (10%) among the controls. Previous estimates of KSHV seroprevalence in Sicily have ranged from 12% to 38% (13, 40). Our major findings were unaltered when we categorized KSHV status using only the latent immunofluorenscence assay.

In summary, we confirmed previous reports that cigarette smoking was associated with a reduced risk of classical Kaposi sarcoma, and found that risk was lowest among current smokers. We also found that classical Kaposi sarcoma risk was strongly and independently associated with never smoking, oral corticosteroid use, and diabetes. Corroboration of these observations and study of possible underlying mechanisms are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We are grateful to G. Arena, G. Antonelli, Colleen Pelser, and Dr. Robert Biggar for helpful discussions; to Dr. Lindsay Morten for the analysis of Kaposi sarcoma risk after lung cancer; to Filippa Bonura, Anna Maria Perna, Fabio Tramuto, Anna Fidilio, Michele Massimino, Stefania Stella, and Georgina Mbisa for specimen processing and KSHV antibody testing; to Enza Viviano, Rosalia Valenti, Elisa Martorana, G. Arena, G. Antonelli, L. Leggio, G. Buscema, V. Paparazzo, M.C. DiPasquale, A. Tortorici, I. Bocchieri, and Laboratorio Brinch-Battaglia for recruitment and collection of data and specimens; to the staff at RTI International, including Mary-Anne Ardini, Dr. Barbara Kroner, Dr. Mansour Fahimi, and Liliana Preiss, for coordination, computation of weights, and analyses; and especially to Dr. Santo LoGalbo of the Assessorato Regionale della Salute for providing the population roster for Sicily.

This study would not have been possible without the Kaposi sarcoma cases reported by the following pathologists of Sicily: Professors Dr. Batolo and M. Lentini (Messina University); Professors E. Vasquez, G. Micali, S. Lanzafame, and Dr. G. Grasso (Catania University); Dr. S. Castellino (Ragusa); Dr. B. Barino (Vittoria); Dr. C. Castobello (Sircusa); Dr. Tricoli and Dr. M. Amore (Caltagirone); Dr. G. Russo (Taormina); Dr. M. Manusia, and Dr.ssa E. Salomone (Catania); Dr. Vallone (Enna), Dr.ssa Calcarco and Dr.ssa P. Napoli Nania (Messina); Professors F. Aragona and V. Rodolico (Azienda Ospedaliera Universitaria, Policlinico Paolo Giaccone, Palermo); Dr. L. Marasà (Azienda di Rilievo Nazionale e di Alta Specializzazione, Ospedale Civico e Benfratelli, G. Di Cristina e M.Ascoli, Palermo); Prof. G.Genova and Dr. C. Rimi (Azienda Ospedaliera ''Villa Sofia-C.T.O.'', Palermo); Dr. A. Rizzo and Dr. T. Mannone (Azienda Ospedaliera ''V.Cervello'', Palermo); Dr. G. Pinto and Dr. G. Becchina (Azienda USL 6, Presidio Ospedaliero G.F. Ingrassia, Palermo); Dr. D. Ientile (Ospedale ''Buccheri- La Ferla'' Fatebenefratelli, Palermo); Dr. D. Messina (Azienda. Ospedaliera ''S. Antonio Abate'', Trapani); Dr. N.A. Zarcone and Dr. Marino (Azienda Unità Sanitaria Locale n.9 Trapani Presidio Ospedaliero ''V.Emanuele'', Castelvetrano); Dr. F. Italia (Azienda Ospedale ''S. Giovanni di Dio'', Agrigento); and Dr. G. Ulrico and Dr. G. Bruno (Azienda Ospedaliera ''S.Elia'', Caltanissetta).

References

1. Moore PS, Chang Y. Kaposi's sarcoma (KS), KS-associated herpesvirus, and the criteria for causality in the age of molecular biology. Am J Epidemiol 1998;147:217–21.
2. Rady PL, Yen A, Martin RW III, Nedelcu I, Hughes TK, Tyring SK. Herpesvirus-like DNA sequences in classic Kaposi's sarcomas. J Med Virol 1995;47:179–83.
3. Brown EE, Fallin D, Ruczinski I, et al. Associations of classic Kaposi sarcoma with common variants in genes that modulate host immunity. Cancer Epidemiol Biomarkers Prev 2006;15:926–34.
4. Touloumi G, Hatzakis A, Potouridou I, et al. The role of immunosuppression and immune-activation in classic Kaposi's sarcoma. Int J Cancer 1999;82:817–21.

5. Whitby D, Luppi M, Sabin C, et al. Detection of antibodies to human herpesvirus 8 in Italian children: evidence for horizontal transmission. Br J Cancer 2000;82:702–4.
6. Vitale F, Briffa DV, Whitby D, et al. Kaposi's sarcoma herpes virus and Kaposi's sarcoma in the elderly populations of 3 Mediterranean islands. Int J Cancer 2001;91:588–91.
7. Goedert JJ, Vitale F, Lauria C, et al. Risk factors for classical Kaposi's sarcoma. J Natl Cancer Inst 2002;94:1712–8.
8. Nawar E, Mbulaiteye SM, Gallant JE, et al. Risk factors for Kaposi's sarcoma among HHV-8 seropositive homosexual men with AIDS. Int J Cancer 2005;115:296–300.
9. Hoover DR, Black C, Jacobson LP, et al. Epidemiologic analysis of Kaposi's sarcoma as an early and later AIDS outcome in homosexual men. Am J Epidemiol 1993;138:266–78.
10. Dictor M, Attewell R. Epidemiology of Kaposi's sarcoma in Sweden prior to the acquired immunodeficiency syndrome. Int J Cancer 1988; 42:346–51.
11. Iscovich J, Boffetta P, Winkelmann R, Brennan P. Classic Kaposi's sarcoma as a second primary neoplasm. Int J Cancer 1999;80:178–82.
12. Safai B, Mike V, Giraldo G, Beth E, Good RA. Association of Kaposi's sarcoma with second primary malignancies: possible etiopathogenic implications. Cancer 1980;45:1472–9.
13. Perna AM, Bonura F, Vitale F, et al. Antibodies to human herpes virus type 8 (HHV8) in general population and in individuals at risk for sexually transmitted diseases in Western Sicily. Int J Epidemiol 2000;29:175–9.
14. Little RJA, Rubin DB. Statistical analysis with missing data. New York: Wiley; 1987.
15. Guttman-Yassky E, Dubnov J, Kra-Oz Z, et al. Classic Kaposi sarcoma. Which KSHV-seropositive individuals are at risk? Cancer 2006;106:413–9.
16. Conley LJ, Bush TJ, Buchbinder SP, Penley KA, Judson FN, Holmberg SD. The association between cigarette smoking and selected HIV-related medical conditions. AIDS 1996;10:1121–6.
17. Gallus S, Pacifici R, Colombo P, et al. Prevalence of smoking and attitude towards smoking regulation in Italy, 2004. Eur J Cancer Prev 2006;15:77–81.
18. Giovino GA, Schooley MW, Zhu BP, et al. Surveillance for selected tobacco-use behaviors-United States, 1900–1994. MMWR CDC Surveill Summ 1994;43:1–43.
19. Franceschi S, Naett C. Trends in smoking in Europe. Eur J Cancer Prev 1995;4:271–84.
20. Goedert JJ, Scoppio BM, Pfeiffer R, et al. Treatment of classic Kaposi sarcoma with a nicotine dermal patch: a phase II clinical trial. J Eur Acad Dermatol Venereol 2008 [Epub ahead of print].
21. Nouri-Shirazi M, Tinajero R, Guinet E. Nicotine alters the biological activities of developing mouse bone marrow-derived dendritic cells (DCs). Immunol Lett 2007;109:155–64.
22. Nouri-Shirazi M, Guinet E. Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. Immunology 2003;109: 365–73.
23. Hogg N. Nicotine has suppressive effects on dendritic cell function. Immunology 2003;109:329–30.
24. Chang J, Renne R, Dittmer D, Ganem D. Inflammatory cytokines and the reactivation of Kaposi's sarcoma-associated herpesvirus lytic replication. Virology 2000;266:17–25.
25. Ford PW, Hamden KE, Whitman AG, et al. Cigarette smoke concentrate inhibits Kaposi's sarcoma-associated herpesvirus infection. Virus Res 2005;114:172–6.
26. Diamondstone LS, Tollerud DJ, Fuchs D, et al. Factors influencing serum neopterin and β 2-microglobulin levels in a healthy diverse population. J Clin Immunol 1994;14:368–74.
27. Tollerud DJ, Clark JW, Brown LM, et al. The effects of cigarette smoking on T cell subsets. A population-based survey of healthy caucasians. Am Rev Respir Disease 1989;139:1446–51.
28. Brown EE, Whitby D, Vitale F, et al. Virologic, hematologic, and immunologic risk factors for classic Kaposi sarcoma. Cancer 2006; 107:2282–90.
29. Louthrenoo W, Kasitanon N, Mahanuphab P, Bhoopat L, Thongprasert S. Kaposi's sarcoma in rheumatic diseases. Semin Arthritis Rheum 2003; 32:326–33.
30. Cai J, Zheng T, Lotz M, Zhang Y, Masood R, Gill P. Glucocorticoids induce Kaposi's sarcoma cell proliferation through the regulation of transforming growth factor-β. Blood 1997;89:1491–500.
31. Kroumpouzos G, Delaney T, Phillips TJ. Combined classic and iatrogenic Kaposi's sarcoma. Corticosteroid withdrawal can result in remission. Postgrad Med 2000;108:103–6.
32. Leung F, Fam AG, Osoba D. Kaposi's sarcoma complicating corticosteroid therapy for temporal arteritis. Am J Med 1981;71:320–2.
33. Weissmann A, Linn S, Weltfriend S, Friedman-Birnbaum R. Epidemiological study of classic Kaposi's sarcoma: a retrospective review of 125 cases from Northern Israel. J Eur Acad Dermatol Venereol 2000;14:91–5.
34. Lanternier F, Lebbe C, Schartz N, et al. Kaposi's sarcoma in HIV-negative men having sex with men. AIDS 2008;22:1163–8.
35. Mazzaglia G, Yurgin N, Boye KS, et al. Prevalence and antihyperglycemic prescribing trends for patients with type 2 diabetes in Italy: a 4-year retrospective study from national primary care data. Pharmacological Research 2008;57:358–63.
36. Davis DA, Rinderknecht AS, Zoeteweij JP, et al. Hypoxia induces lytic replication of Kaposi sarcoma-associated herpesvirus. Blood 2001;97:3244–50.
37. Catrina S-B, Botusan IR, Rantanen A, et al. Hypoxia-inducible factor-1α and hypoxia-inducible factor-2α are expressed in Kaposi sarcoma and modulated by insulin-like growth factor-I. Clin Cancer Res 2006; 12:4506–14.
38. Rose PP, Carroll JM, Carroll PA, et al. The insulin receptor is essential for virus-induced tumorigenesis of Kaposi's sarcoma. Oncogene 2007;26:1995–2005.
39. Sobngwi E, Choukem SP, Agbalika F, et al. Ketosis-prone type 2 diabetes mellitus and human herpesvirus 8 infection in Sub-Saharan Africans. JAMA 2008;299:2770–6.
40. Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA, Torelli G. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst 1998;90:395–7.