Predicting the evolution of Kaposi sarcoma, in the highly active antiretroviral therapy era

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Background: The outcome of Kaposi sarcoma varies. While many patients do well on highly active antiretroviral therapy, others have progressive disease and need chemotherapy. In order to predict which patients are at risk of unfavorable evolution, we established a prognostic score.

Method: The survival analysis (Kaplan–Meier method; Cox proportional hazards models) of 144 patients with Kaposi sarcoma prospectively included in the Swiss HIV Cohort Study, from January 1996 to December 2004, was conducted.

Outcome analyzed: use of chemotherapy or death.

Variables analyzed: demographics, tumor staging [T0 or T1 (16)], CD4 cell counts and HIV-1 RNA concentration, human herpesvirus 8 (HHV8) DNA in plasma and serological titers to latent and lytic antigens.

Results: Of 144 patients, 54 needed chemotherapy or died. In the univariate analysis, tumor stage T1, CD4 cell count below 200 cells/μl, positive HHV8 DNA and absence of antibodies against the HHV8 lytic antigen at the time of diagnosis were significantly associated with a bad outcome.

Using multivariate analysis, the following variables were associated with an increased risk of unfavorable outcome: T1 [hazard ratio (HR) 5.22; 95% confidence interval (CI) 2.97–9.18], CD4 cell count below 200 cells/μl (HR 2.33; 95% CI 1.22–4.45) and positive HHV8 DNA (HR 2.14; 95% CI 1.79–2.85).

We created a score with these variables ranging from 0 to 4: T1 stage counted for two points, CD4 cell count below 200 cells/μl for one point, and positive HHV8 viral load for one point. Each point increase was associated with a HR of 2.26 (95% CI 1.79–2.85).

Conclusion: In the multivariate analysis, staging (T1), CD4 cell count (<200 cells/μl), positive HHV8 DNA in plasma, at the time of diagnosis, predict evolution towards death or the need of chemotherapy.

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Introduction

Kaposi sarcoma is a common opportunistic disease associated with AIDS and is responsible for severe morbidity and mortality [1,2].

Since the introduction of highly active antiretroviral therapy (HAART), the incidence of Kaposi sarcoma has decreased [3–5] and in some cases, established Kaposi sarcoma goes into remission. Incident cases of Kaposi sarcoma are prevented when treatment is started early, before advanced immune suppression. Immune reconstitution increases the level of the antibodies directed against the viral K8.1 lytic protein and restores the CD8 T-cell response against human herpesvirus 8 (HHV8) epitopes [6]. No direct effect of protease inhibitors on HHV8 has ever been demonstrated [7,8], but indinavir and saquinavir reduce the number and size of Kaposi sarcoma-like angioproliferative lesions in nude mice and inhibit bFGF or VEGF-induced formation of new vessels [7].

Nevertheless, the response of established Kaposi sarcoma to HAART is unpredictable. While many patients do well, others will have progressive disease and will need chemotherapy, which produces only short remissions. Up to 30% of patients still die following Kaposi sarcoma progression [9]. It is therefore important to define risk factors of unfavorable evolution, so as to select high-risk patients who may benefit from more aggressive treatment, while avoiding unnecessary therapy and toxicity in those who are at low risk of progression.

Few studies have tried to identify such prognostic factors and their conclusions are contradictory [9–12]. One study concluded that sex and age, CD4 cell count, level of HIV viremia and HAART-related factors such as whether patients were HAART-naive or HAART-experienced at the time of diagnosis of Kaposi sarcoma, as well as differences in the types of HAART used, did not provide prognostic information [9]. These studies [9–12] did not include information concerning HHV8, which may well be relevant as the inability to detect HHV8 in peripheral blood mononuclear cell (PBMC) predicts clinical response [13]. Specific antibodies and CD8 T-cell responses increase during HAART [14], but their prognostic value has not yet been studied. The type of HAART used may play a role, as suggested by a series of patients with flares of Kaposi sarcoma after the switch from a protease inhibitor to a nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimen [15].

We describe the incidence and prevalence of Kaposi sarcoma, in the Swiss HIV Cohort Study (SHCS) since 1996 and establish a predictive score of evolution of Kaposi sarcoma, after having assessed prognostic factors among socio-demographic variables, immunovirological factors concerning HIV and HHV8, staging and differences in the type of HAART regimens used.

Methods

Study population, covariates and endpoints

We included patients from the SHCS with a histologically confirmed or presumptive clinical diagnosis of Kaposi sarcoma made between January 1, 1996 and December 31, 2004. When available, frozen plasma samples were collected, at the time of diagnosis and at the end of the follow-up, to quantify HHV8 DNA and anti-HHV8 antibodies. Study endpoints included the initiation of chemotherapy to treat Kaposi sarcoma or death if no chemotherapy was administered before. Patients who fulfilled one or both these endpoints were considered part of the unfavorable outcome group. Potential prognostic factors were assessed among the following variables: sex, age, race, origin, sexual orientation, and intravenous drug use; tumor staging; development of multicentric Castelman's disease (MCD) or body cavity lymphoma (BCL); exposure to antiviral treatment active against herpes viruses; type of antiretroviral therapy (ART), CD4 levels, HIV viremia, quantitative HHV8 DNA, latent and lytic HHV8 antibody titers, at Kaposi sarcoma diagnosis and at the end of the follow-up.

Definitions

According to the AIDS Clinical Trials Group (ACTG) classification [16] tumor stage was classified as T0: Kaposi sarcoma confined to the skin and lymph nodes and minimal oral Kaposi sarcoma; and T1: ulcerated Kaposi sarcoma, Kaposi sarcoma-associated oedema, nodular oral Kaposi sarcoma or Kaposi sarcoma involvement of any visceral organ.

ART was defined as the use of any antiretroviral drug, either as monotherapy, dual therapy, or triple therapy (HAART); HAART as the use of three nucleoside reverse transcriptase inhibitors (NRTIs), or two NRTIs with either a protease inhibitor or a NNRTI, or four antivirals.

The exposure to antiviral treatment active against herpes, during the follow-up, included (val)ganciclovir, (val)aciclovir, adefovir, cidofovir or foscarnet.

Detection and quantification of human herpesvirus 8 DNA

HHV8 viral load in the plasma was evaluated by qualitative and quantitative PCR. DNA was extracted using an EZ1-DNA extraction robot (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's instruction. For qualitative evaluation, a nested PCR for the HHV8 ORF 26 region was amplified as previously described [17]. For quantitative PCR, HHV8 DNA was amplified by the Sequence Detection System 7500 Fast (TaqMan) (Applied Biosystems, Rotkreuz, Switzerland) amplifying a 147 bp PCR product of ORF 26 using the following primers and probe: Forward primer KS3 5′-AGC CGA AAG GAT TCC ACC AT-3′; Reverse primer KS4 5′-TCC GTG

TTG TCT ACG TCC AG-3′; KSProbe 5′-FAM-CGC TAT TCT GCA GCA GCT GTT GGT GTA CCA-TAMRA-3′. TaqMan standard conditions were applied with 40 cycles of denaturation at 95°C for 3 s and primer annealing/extension at 60°C for 30 s. All reactions were performed in duplicate. For the calculation of molecule quantity, serial dilutions of ORF 26 containing HHV8 plasmid DNA were analyzed and a standard curve established as previously described [18]. Results were expressed in copies/ml.

HHV8 serology

To determine the presence and titer of antibodies to the latent nuclear antigen (LANA) and lytic antigens at Kaposi sarcoma diagnosis and at outcome, we used CE-marked immunofluorescence assays (Panbio, Inc., Maryland, USA). Briefly, LANA-antibodies were identified in the nucleus of uninduced HHV8-infected body cavity-based lymphoma-1 (BCBL-1) cells. In induced cells, (with 12-0-tetradecanoylphorbol-13-acetate), we detected anti-HHV8 lytic antibodies, in the nucleus and cytoplasma.

Results were given as titers, ranging from 1 : 64 to 1 : 32 768. For practical reasons, an arbitrary dilution unit was defined: 1 unit is equivalent to the first titer of 1 : 64, 2 units 1 : 128, up to 10 units representing 1 : 32 768.

Statistical analysis

Patients were followed from the time of Kaposi sarcoma diagnosis to the date of the first day of chemotherapy or the date of their death. Patients who did not reach an endpoint were censored on December 31, 2004 or at their last cohort visit, whichever occurred earlier. We used the Kaplan–Meier method to estimate the survival function and the log-rank test to compare the differences between the subgroups. Differences between subgroups were also tested in a univariate analysis using the Cox proportional hazards model to compute the hazard ratio (HR) and corresponding 95% confidence interval (CI). Covariates that were found significant in the univariate analysis and those found to be biologically plausible, this is to say age and sexual orientation, were entered in a multivariate model, using a forward stepwise procedure. To establish the predictive score we used the variables, present at the time of Kaposi sarcoma diagnosis, which were found significant in the multivariate analysis. We attributed the number of points to each according to their coefficient '*B*' in the Cox model. The score ranged from 0 to 4. Poisson regression was also used to explore the changes in Kaposi sarcoma incidence rate from 1996 to 2004.

In all cases, statistical significance was claimed for $P \leq 0.05$ (two-sided). Statistical analyses were performed using SPSS 11.0 (SPSS, Chicago, Illinois, USA) and Stata 8.0 softwares (STATA Corp., College Station, Texas, USA).

Table 1. Prevalence (%) and incidence (per 1000 person-years) of Kaposi's sarcoma in the Swiss HIV Cohort Study between 1996 and 2004.

Year	Number of patients	Person-years	Number of Kaposi sarcoma reported (new cases)	Prevalence	Incidence
1996	4039	16 675.83	181 (59)	4.48%	3.5
1997	4536	19 352.33	188 (35)	4.14%	1.81
1998	4962	21 563.39	204 (26)	4.11%	1.21
1999	5329	26 166.49	212 (19)	3.98%	0.73
2000	5624	28 412.41	219 (15)	3.89%	0.53
2001	5815	31 702.13	219 (12)	3.77%	0.38
2002	5935	34 881.27	232 (19)	3.91%	0.54
2003	6057	38 103.81	228 (7)	3.76%	0.18
2004	6243	41 042.11	243 (18)	3.89%	0.44

Results

Two hundred and forty-three patients developed Kaposi sarcoma, during the study period. Two hundred and three were included in the survival analysis. Thirty-four patients were excluded because clinical and virological data on HIV were lacking and six others because of unknown outcome.

The median follow-up was 30 months (range 0–108).

Prevalence and incidence

The prevalence of Kaposi sarcoma ranged from 4.5% in 1996 to 3.8% in 2003. The incidence rate ranged from its highest value of 3.5 per 1000 person-years in 1996 to its lowest value of 0.18 per 1000 person-years in 2003 (Table 1). There was an estimated reduction of 32.18% in the incidence of Kaposi sarcoma with each successive calendar year (95% CI −37.9 to −26.4%; $P < 0.0001$). This decrease was most important between 1996 and 1999 when it was estimated to be of −53.0% (95% CI −68.8 to −37.2%; $P < 0.0001$) (Fig. 1).

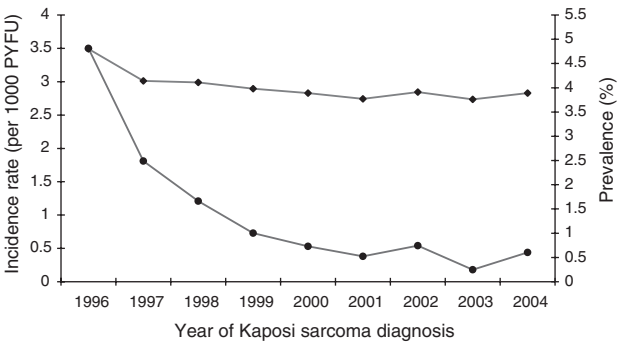

Fig. 1. Incidence rate and prevalence of Kaposi sarcoma in the Swiss HIV Cohort Study between 1996 and 2004. PYFU, person-years follow-up.

Patient characteristics

Patient characteristics are summarized in Table 2.

The median age at Kaposi sarcoma diagnosis was 41 years, ranging from 19 to 65; the majority were white men having sex with men (MSM). The overall mortality rate

Table 2. Patient characteristics at the baseline and the end of the follow-up.

	All	Unfavorable outcome	Favorable outcome
	$n = 203$	$n = 79$ (38.9%)	$n = 124$ (61.1%)
Death (%)		43 (21)	
Chemotherapy (%)		54 (27)	
Castelman's disease	7 (3)	6 (8)	1 (1)
Body cavity lymphoma	2 (1)	2 (3)	0
Age at Kaposi sarcoma diagnosis (median and range)	41 (19–65)	42 (26–65)	41 (19–63)
Men	191 (94)	74 (94)	117 (94)
Women	12 (6)	5 (6)	7 (6)
White	182 (90)	73 (92)	109 (88)
Black	17 (8)	5 (6)	12 (10)
Hispano-American	1 (0.5)	0	1 (1)
Asian	1 (0.5)	0	1 (1)
Mediterranean or African	34 (17)	12 (15)	22 (18)
Homosexual	129 (64)	46 (58)	83 (67)
Bisexual	22 (11)	10 (13)	12 (10)
Heterosexual	50 (25)	21 (27)	29 (23)
Intravenous drug use	8 (4)	5 (6)	3 (2)
T0 (%)	158 (78)	46 (58)	112 (90)
T1 (%)	45 (22)	33 (42)	12 (10)
CD4 at Kaposi sarcoma (cells/μl; median and range)	112 (0–1267)	52 (0–1267)	149 (2–782)
HIV viral load at Kaposi sarcoma (copies/ml; median and range)	76 710 (<50–2 385 000)	89 938 (<50–235 957)	65 100 (< 50–2 385 000)
ART at Kaposi sarcoma	79 (39)	31 (39)	48 (39)
Time to ART introduction (days; median and range)	1 (−3790 to 1282)		
Monotherapy	8 (4)	4 (5)	4 (3)
Dual therapy	15 (7)	7 (9)	8 (7)
Triple therapy (HAART)	56 (28)	20 (25)	36 (29)
Regimen including a protease inhibitor	55 (27)	22 (28)	33 (27)
CD4 at the end of the follow-up	284 (92–516)	54 (0–937)	364 (2–1641)
HIV viral load at end follow-up	57 (0–19 117)	9420 (0–1 000 000)	0 (0–461 784)
ART at end follow-up	149 (76)	50 (69)	99 (80)
Monotherapy	5 (3)	3 (4)	2 (2)
Dual therapy	5 (3)	4 (6)	1 (1)
Triple therapy (HAART)	139 (71)	43 (59)	96 (77)
Regimen including a protease inhibitor	86 (44)	43 (59)	43 (35)
Valganciclovir	9 (4)	7 (9)	2 (2)
Valacyclovir	51 (25)	29 (37)	22 (18)
Cidofovir	8 (4)	7 (9)	1 (1)
Foscarnet	2 (1)	2 (3)	0
Use of any antiH	57 (28)	32 (41)	25 (20)
HHV8 DNA	$n = 147$	$n = 55$ (37.4%)	$n = 92$ (62.6%)
Positive at Kaposi sarcoma (%)	38 (26)	20 (36)	18 (20)
Copies/ml (mean and SD)	803 (3468)	1888 (5501)	154 (441)
	$n = 107$	$n = 29$ (27%)	$n = 78$ (73%)
Positive at the end of the follow-up (%)	23 (22)	14 (48)	9 (12)
Copies/ml (mean and SD)	3348 (22 728)	11 822 (43 033)	198 (787)
HHV8 serology	$n = 136$	$n = 50$ (36.8%)	$n = 86$ (63.2%)
Positive latent at Kaposi sarcoma (%)	111 (82)	38 (76)	73 (84)
Median dilution and range	3 (0–10)	3 (0–10)	3 (0–9)
Positive lytic at Kaposi sarcoma (%)	126 (93)	44 (88)	82 (95)
Median dilution and range	4 (0– 8)	3.5 (0–8)	5 (0–8)
	$n = 108$	$n = 33$ (30.6%)	$n = 75$ (69.4%)
Positive latent at the end of the follow-up (%)	94 (88)	27 (84)	67 (89)
Median dilution and range	4 (0–10)	4 (0–10)	4 (0–8)
Positive lytic at the end of the follow-up (%)	104 (96)	29 (88)	75 (100)
Median dilution and range	5 (0–10)	5 (0–9)	5 (1–10)

Serologies are given in arbitrary dilution units; the first unit being equivalent to the first positive titer of 1 : 64, the second unit equivalent to 1 : 128, up to the 10th unit equivalent to 1 : 32 768. antiH, antiherpes treatment; ART, antiretroviral therapy (any type); HHV8, human herpes virus 8.

was 21%, and 27% of patients started chemotherapy. Fifty percent of these endpoints occurred during the first 3 months after diagnosis. Eighteen (33%) patients who were started on chemotherapy ultimately died.

Seven patients developed MCD and two BCL. Eight belonged to the unfavorable outcome group.

Regarding Kaposi sarcoma, 78% of the patients had a T0 disease. The median CD4 cell count at baseline was 112 cells/μl (range 0–1267). At the end of the follow-up, the median CD4 cell count increased to 284 (92–516). The median HIV viral load was 76 710 copies/ml (<50–2 385 000); by the end of the follow-up, it was 57 (<50–19 117). Thirty-nine percent of patients were using ART at the time of Kaposi sarcoma diagnosis and by the end of the follow-up, this proportion had increased to 76%. When ART was started after Kaposi sarcoma diagnosis, the median time to introduction was 42 days (1–1282). During the follow-up, 28% of patients used an antiviral drug active against herpes viruses, mainly valaciclovir.

Plasma samples for quantification of HHV8 DNA, at baseline, were available for 147 patients (72%, see Table 2). Thirty-eight (26%) of these presented a positive HHV8 viral load at baseline. The mean value was 803 copies/ml (SD 3468).

At the end of the follow-up, 107 plasma samples (53%) were available for HHV8 quantification. Twenty-three (22%) were positive, the mean value had increased to 3348 copies/ml (SD 22 728).

Baseline latent and lytic titers were analyzed in 136 patients (67%) (Table 2). One hundred and eleven (82%) and 126 (93%) of these had, respectively, a positive latent and lytic value. The median dilution unit was 3 (titer 1 : 256) for the latent and 4 (titer 1 : 512) for the lytic.

At the end of the follow-up, 108 samples (53%) were analyzed. Ninety-four (88%) and 104 (96%) had positive latent and lytic titers, respectively. The median titers increased in both (4 and 5, respectively) and the lytic values remained higher than the latent.

Dual reactivity was found in 78% of cases at Kaposi sarcoma and 86% at the end of the follow-up.

Prognostic factors

Univariate analysis

Table 3 summarizes the hazard ratios associated with each factor.

We found that evolution toward death or the necessity of chemotherapy was significantly influenced, at baseline, by tumor staging, CD4 cell count, HHV8 DNA detection and finally HHV8 lytic titers. Patients who had a T1 disease were four times more likely to present an unfavorable outcome (HR 4.23; 95% CI 2.68–6.68). Those presenting CD4 cell counts less than 200 cells/μl were at increased risk of unfavorable outcome (1.74; 1–2.95). The detection of HHV8 DNA was strongly associated with an unfavorable outcome (2.25; 1.30–3.91) and a high number of copies (>3000) even more so (7.17; 3.15–16.31). The absence of lytic antibodies was associated with a two-fold increase in risk of unfavorable outcome (2.34; 0.99–5.5) and each increase in the dilution unit reduced that risk (0.84; 0.74–0.96).

Age, sex, origin, intravenous drug use, HIV viral load, ART and latent antibody titers were not associated with a significant change in outcome.

Taking into account the evolution of certain factors during the follow-up, we noted that the risk of unfavorable outcome was significantly increased by the development of MCD or BCL (3.66; 1.59–8.47); a CD4 cell count below 200 cells/μl at the end of the follow-up (9.26; 5.47–15.7); less than a 50 CD4 cell increase from baseline (2.42; 1.37–4.27); HIV viral load values ≥5000 copies/ml at end of the follow-up (4.15; 2.62–6.57), becoming or remaining HHV8 DNA detectable (9.68; 4.01–23.36) and the absence of lytic antibodies at the end of the follow-up (19.87; 6.36–62.08). Patients without ART, at the end of the follow-up, were two times more likely to present an unfavorable outcome (2.37; 1.49–3.77). The use of a protease inhibitor was associated with an increased risk of unfavorable outcome (1.70; 1.09–2.65), as was the use of antiviral drugs active against herpes virus (2.01; 1.28–3.15).

Multivariate analysis and score

The covariates of 144 patients were entered into the final multivariate model. At baseline, the following factors predicted an unfavorable outcome: Kaposi sarcoma stage T1 (HR 5.22; 95% CI 2.97–9.18), CD4 cell count below 200 cells/μl (HR 2.33; 95% CI 1.22–4.45) and positive HHV8 DNA (HR 2.14; 95% CI 1.18–3.89) (Table 4).

To establish our predictive score of unfavorable outcome, we scaled these four factors according to their *B* coefficient, giving T1 two points; CD4 cell count below 200 cells/μl and a positive HHV8 DNA, one point each. The score ranged from 0 to 4. The receiver operating characteristic (ROC) curve has an area under the curve (AUC) of 0.77 (95% CI 0.68–0.88). Kaplan–Meier curves showing survival according to score are shown in Fig. 2. Each incremental increase in the score was associated with a HR of 2.26 (95% CI 1.79–2.85).

Discussion

The objective of our study was to establish predictive factors of death and the need of chemotherapy, among

Table 3. Univariate survival analysis of potential prognostic factors of unfavorable evolution of Kaposi sarcoma.

	Number of patients	Hazard ratio (95%CI)	*P* value
Castelman/BCL			
No	196	1 (reference)	
Yes	7	3.66 (1.59–8.47)	0.001
Staging			
T0	158	1 (reference)	
T1	45	4.23 (2.68–6.68)	<0.001
Age at Kaposi sarcoma			
<50 years old	157	1 (reference)	
≥50	46	1.13 (0.67–1.89)	0.64
Sex			
Male	191	1 (reference)	
Female	12	1.23 (0.50–3.04)	0.66
Origin			
Mediterranean or African	34	1 (reference)	
Others	169	1.07 (0.58–1.97)	0.84
Sexual orientation			
Homosexual	131	1 (reference)	
Bisexual or heterosexual	72	1.35 (0.86–2.12)	0.19
Intravenous drug use			
No	195	1 (reference)	
Yes	8	1.46 (0.59–3.62)	0.41
CD4 cell count at Kaposi sarcoma			
At least 200 cells/μl	66	1 (reference)	
Below 200 cells/μl	135	1.74 (1.0–2.95)	0.04
CD4 evolution			
Increase	164	1 (reference)	
No increase	34	1.88 (1.09–2.98)	0.02
CD4 increase			
At least 50	83	1 (reference)	
Below 50	62	2.42 (1.37–4.27)	<0.001
HIV viral load at Kaposi sarcoma			
<100 000 copies/ml	113	1 (reference)	
≥1 000 000 copies/ml	83	1.21 (0.77–1.89)	0.42
<5000 copies/ml	44	1 (reference)	
≥5000 copies/ml	152	1.22 (0.68–2.18)	0.50
ART at Kaposi sarcoma			
Yes	79	1 (reference)	
No	124	1.01 (0.65–1.60)	0.95
HAART at Kaposi sarcoma			
Yes	56	1 (reference)	
No	147	1.08 (0.65–1.80)	0.76
No protease inhibitor in regimen	148	1 (reference)	
Regimen including a protease inhibitor	55	1.04 (0.64–1.70)	0.87
CD4 at the end of the follow-up			
At least 200 cells/μl	122	1 (reference)	
Below 200 cells/μl	78	9.26 (5.47–15.70)	<0.001
HIV viral load at the end of the follow-up			
<5000 copies/ml	140	1 (reference)	
≥5000 copies/ml	59	4.15 (2.62–6.57)	<0.001
ART at the end of the follow-up			
Yes	149	1 (reference)	
No	54	2.37 (1.49–3.77)	<0.001
HAART at the end of the follow-up			
Yes	139	1 (reference)	
No	64	2.77 (1.77–4.34)	<0.001
No protease inhibitor in regimen	117	1 (reference)	
Regimen including a protease inhibitor	86	1.70 (1.09–2.65)	0.02
Use of any antiH			
No	146	1 (reference)	
Yes	57	2.01 (1.28–3.15)	0.002
PCR HHV8			
Negative at Kaposi sarcoma diagnosis	109	1 (reference)	
Positive	38	2.25 (1.30–3.91)	0.003
Negative at the end of the follow-up	84	1 (reference)	
Positive	23	6.58 (3.10–13.97)	<0.001
≤3000 copies/ml (Kaposi sarcoma)	140	1 (reference)	
>3000	7	7.17 (3.15–16.31)	<0.001
≤3000 copies/ml (end of follow-up)	100	1 (reference)	
>3000	7	10.30 (4.13–25.72)	<0.001

Continued

Table 3 (*continued*)

	Number of patients	Hazard ratio (95%CI)	*P* value
PCR evolution			
Become or stay negative	76	1 (reference)	
Become or stay positive	23	9.68 (4.01–23.36)	<0.001
Serology HHV8			
Latent at Kaposi sarcoma			
Positive	111	1 (reference)	
Negative	25	1.6 (0.82–3.01)	0.17
Per dilution increase		0.92 (0.83–1.04)	0.21
Lytic at Kaposi sarcoma			
Positive	126	1 (reference)	
Negative	10	2.34 (0.99–5.5)	0.05
Per dilution increase		0.84 (0.74–0.96)	0.01
Latent at end follow-up			
Positive	94	1 (reference)	
Negative	13	1.53 (0.59–3.98)	0.38
Per dilution increase		1.04 (0.9–1.2)	0.57
Lytic at end follow-up			
Positive	104	1 (reference)	
Negative	4	19.87 (6.36–62.08)	<0.001
Per dilution increase		0.97 (0.83–1.15)	0.76

antiH, antiherpes treatment; ART, antiretroviral therapy; BCL, body cavity lymphoma; CI, confidence interval; HHV8, human herpesvirus 8.

easily available clinical data and laboratory values, at the time of Kaposi sarcoma diagnosis, in HIV patients. We identified three factors which are T1 staging, a CD4 cell count below 200 cells/μl and a positive plasmatic HHV8 DNA value and developed a predictive score of unfavorable Kaposi sarcoma evolution in order to identify the patients who may need more aggressive treatment of Kaposi sarcoma.

Our patient characteristics are representative of the Kaposi sarcoma population encountered in occidental HIV clinics: a majority of white homosexual men, of northern European origin with no HIV treatment at the time of Kaposi sarcoma diagnosis [3].

We observed a decreasing prevalence from 4.5 to 3.8% and a reduction of 32% in the incidence of Kaposi sarcoma throughout the study period, with a faster decrease during the early period of the HAART era, from 1996 to 1999. These results are consistent with the ones described in the EuroSIDA Study [3] and studies from Australia [4] and the USA [5]. Several explanations can be put forward such as the detection of HIV seropositivity at an earlier stage before AIDS and the introduction of HAART, which prevent immunodeficiency. The question of a lower seroprevalence of HHV8 remains to be clarified. Nevertheless, the morbidity and mortality of Kaposi sarcoma remain high. In our study, more than a quarter of the patients suffered from invalidating Kaposi sarcoma, necessitating the use of chemotherapy and 21% died during the months following the diagnosis of Kaposi sarcoma, confirming the result of 25% previously published by Nasti *et al.* [9].

Our multivariate analysis revealed that T1 staging was associated with a five-fold increase in risk of unfavorable outcome, which is the highest among the different

Table 4. Significant results of a Cox proportional hazard model describing independent relation between factors present at the time of Kaposi sarcoma diagnosis and the risk of subsequent chemotherapy or death.

	Number of patients	Coefficient *B*	Hazard ratio (95%CI)	*P* value
Staging				
T0	113		1 (referent)	
T1	31	1.65	5.22 (2.97–9.18)	<0.001
CD4 level				
At least 200	50		1 (referent)	
Below 200	94	0.85	2.33 (1.22–4.45)	0.01
HHV8 DNA				
Negative	106		1 (referent)	
Positive	38	0.76	2.14 (1.79–2.85)	0.01

95% CI, 95% confidence interval; HHV8, human herpesvirus 8.

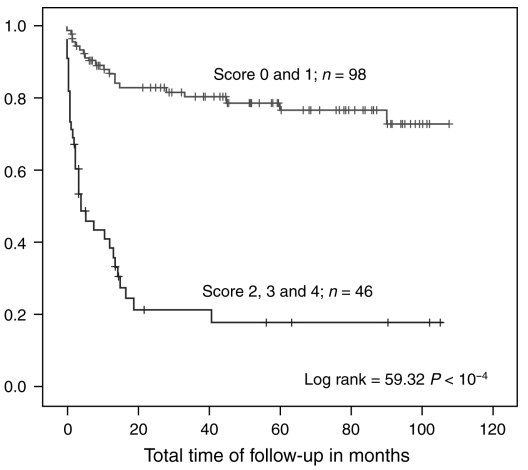

Fig. 2. Kaplan–Meier curves showing survival according to score value.

variables and that a CD4 level below 200 cells/μl and a positive plasmatic HHV8 DNA increased the risk two-fold. Similar results on the importance of tumor extension and immune status at the time of diagnosis have been reported by Krown *et al.* [12], whereas Nasti *et al.* [9] refute the predictive value of the CD4 level. We establish the importance of HHV8 DNA quantification as a predictive factor of bad evolution: not only is the detectability of the DNA a negative factor, but the higher the plasmatic load, the worse the prognosis. We chose to detect HHV8 DNA in the plasma, as it may reflect active lytic replication. Our whole plasma analysis, however, cannot distinguish viremia from free viral DNA, released from blood cells, during preparation. Plasma or serum detection of HHV8 is known to be rarer than PBMC detection [6,19]. In our study, 26% of patients were positive at the time of diagnosis, less than the previously published (41%) results, [19], but our plasma samples could have been collected earlier, during the course of Kaposi sarcoma. Few studies have tackled the importance of HHV8 DNA detection in Kaposi sarcoma, and our findings confirm these: in a study on 21 HIV patients with Kaposi sarcoma patients, Gill *et al.* [14] showed that nine of the 10 patients whose Kaposi sarcoma responded to HAART had undetectable PBMC Kaposi's sarcoma-associated herpesvirus (KSHV) load. Campbell *et al.* [20] observed an association between the extent of Kaposi sarcoma disease and the levels of HHV8 DNA in PBMC. Pellet *et al.* [13], in a study on 26 patients, suggested that an undetectable PBMC KSHV viremia was independently associated with the absence of need of chemotherapy. In non-HIV patients, the likelihood of developing classic Kaposi sarcoma was elevated with detectable PBMC KSHV DNA [21].

Although not relevant to our score, several other factors were significantly associated with an unfavorable outcome, in the univariate analysis.

Nine patients (4%) developed another HHV8-associated illness, (MCD or BCL); this was associated with a 3.5-fold increase in risk of unfavorable outcome.

We observed a 2.3-fold increase in risk associated with a negative lytic antibody titer to HHV8, at the time of Kaposi sarcoma diagnosis and each increase in dilution was associated with a significant improvement in outcome. There was only a trend toward the same effect with the latent antibody titers. This is hardly explained by the sensitivity of our test, which yields similar or even higher proportions than those observed in previously published works (96% positive for lytic and 72 or 52% positive for latent, in HIV-positive patients with Kaposi sarcoma) [22,23]. This difference between lytic and latent may suggest that the antilytic KSHV antibodies might be more effective in controlling KSHV pathogenesis than the anti-LANA antibodies, by reducing lytic viral replication [6]. Although several studies have failed to show a predictive value of KSHV seropositivity for the subsequent development of Kaposi sarcoma [22,24], the importance of the immunological response to HHV8 is suggested by the fact that patients infected with HHV8 after HIV carry a higher risk of developing Kaposi sarcoma than the ones already infected at the time of HIV seroconversion [25]. This probably reflects the incapacity of the immunosuppressed patients to mount a valuable serological response. HAART is followed by recovery of the immune response to KSHV [6]. These different findings suggest that even if neutralizing antibodies to HHV8 does not play the primary role in the protection against Kaposi sarcoma, the fact of not mounting a response at all is unfavorable.

Consistent with the previous studies [9,13,26], age, sex, sexual orientation and origin did not influence the outcome. Contrary to Stebbing *et al.* [26], age was not selected in our score, possibly because our outcome was not only survival, but also the need for chemotherapy. Although we intentionally chose to study the HAART era, only 39% of patients were using ART, at Kaposi sarcoma diagnosis. This is higher than previously published data (15% for Tam *et al.* [11]; 18% for Nasti *et al.* [9], 22% for Stebbing *et al.* [26]) and can probably be explained by the fact that the detection of HIV seropositivity is often made at the same time as the diagnosis of Kaposi sarcoma. Like Nasti *et al.* [9] and Stebbing *et al.* [26], we did not find any significant relationship between the use of ART/HAART at Kaposi sarcoma diagnosis and an unfavorable evolution.

We followed the evolution of several factors after Kaposi sarcoma diagnosis. Bearing in mind the study's timeframe limitation, the fact that some patients were not using HAART at the end of their follow-up, enabled us to confirm that starting HAART is a factor of favorable outcome. Several papers have suggested that the use of a protease inhibitors, as opposed to a NNRTI, could have an antiangioproliferative effect and therefore influence Kaposi sarcoma's evolution [7,8]. Our study does not support this assertion. It has been suggested that antiherpes treatments could have an impact on Kaposi sarcoma evolution through a direct effect on HHV8 [27,28]. In our study, however, their use was associated with an unfavorable outcome, suggesting these patients were probably sicker and that these treatments do not appear to help in the management of Kaposi sarcoma. The evolution of the HHV8 serologies and DNA quantifications during the follow-up is also important. The absence of antibodies toward the HHV8 lytic antigen, at the end of the follow-up, was associated with a bad outcome and there is a similar trend for the latent antigen. The positivity of the HHV8 DNA, at the end of follow-up, is also associated with a bad outcome, as is the fact of remaining positive or becoming positive during the study period. Higher levels of HHV8 DNA increase this risk.

Our study presents several limitations: the bias linked with the retrospective analysis of prospectively collected data; the subjectivity associated with the use of chemotherapy as one of our endpoints and all cause mortality for the other. We also chose not to study HHV8-specific T-cell responses, as, for the moment, the technique is not standardized, but it could turn out to be an interesting parameter as Guihot *et al*. [29] have demonstrated a lower specific response in patients with Kaposi sarcoma, than in asymptomatic HHV8 carriers.

Therefore, although the AUC of our ROC curve is acceptable, our score needs to be confirmed in a different cohort of HIV patients.

In conclusion, few studies have examined the various factors associated with evolution of HIV-associated Kaposi sarcoma and only one has included data on HHV8. We establish that a positive HHV8 DNA is an independent risk factor of bad evolution of Kaposi sarcoma, as is a CD4 level of below 200 cells/μl and the T1 staging. Our score is simple to use at the patient's bedside. Each point increase in the scale is associated with a two-fold increase in the risk of death or need for chemotherapy. We suggest that patients with an index of two or more have a poor risk and should be followed more closely and chemotherapy should be considered, whereas patients with a lower score could be treated with HAART alone.

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