Plasma HHV8 DNA predicts relapse in individuals with HIV-associated multicentric Castleman disease

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HIV-associated multicentric Castleman disease (HIV-MCD) is a rare lymphoproliferative disorder caused by infection with human herpesvirus-8. The disease follows a relapsing and remitting clinical course, with marked systemic symptoms during an active attack, which can prove fatal. Its incidence is rising, and new data indicate the utility of the anti-CD20 monoclonal antibody rituximab at inducing remissions in both first- and second-line settings, although biomarkers associated with relapse have not been previously identified. In 52 individuals with a histologic diagnosis of HIV-MCD, we performed univariate and multivariate analyses to predict factors associated with an HIV-MCD attack. Although a younger age (< 50 years) was associated with an attack, the strongest association was observed with plasma levels of human herpesvirus-8 DNA. Rising levels predicted an attack (hazard ratio = 2.9; 95% confidence interval, 1.3-6.7), and maintenance therapy with rituximab should be considered in these individuals. (*Blood*. 2011;118(2):271-275)

Introduction

Multicentric Castleman disease (MCD) is an uncommon lymphoproliferative disorder, which behaves more aggressively and occurs at a higher frequency in patients with HIV infection.[1] HIV-MCD is characterized by the presence of large abnormal plasmablasts within the mantle zones of involved lymph nodes,[2] and active HIV-MCD presents with a multitude of clinical symptoms, including fever, sweats, weight loss, peripheral neuropathy, enlargement of the spleen and liver, and multifocal lymphadenopathy.[3]

Human herpesvirus-8 (HHV8), also known as Kaposi sarcoma-associated herpesvirus, was first found to be the causative agent of HIV-MCD in 1995,[4] only a year after its association with Kaposi sarcoma was reported.[5] This γ-herpesvirus resides and replicates in the plasmablasts of MCD lymph nodes,[6] and patients with HIV-MCD are at higher risk for other virus-associated disorders, including HHV8-related non-Hodgkin lymphoma and hemophagocytic syndrome.[7-9]

HIV-MCD is diagnosed by its histopathologic features, including cells that stain positively for the viral protein Kaposi sarcoma-associated herpesvirus-associated latent nuclear antigen-1.[10,11] It is a remitting-relapsing disease, and clinical symptoms help to diagnose an active attack of MCD. Although there is no evidence-based standard by which to diagnose active MCD by clinical criteria, the CastlemaB study classified patients who presented with fever, raised serum C-reactive protein (CRP) levels, and 3 of 12 additional clinical findings as having active MCD.[12] However, the percentage of patients with these symptoms was unreported in their manuscript. In our cohort, we recently reported that, although many patients present with raised CRP (92%), fever (98%), an enlarged spleen (95%), and peripheral lymphadenopathy (100%), other symptoms were less common (range, 8%-66%), and a small proportion of our patients with histologically confirmed MCD-HIV and clinical symptoms would not fulfill this set of criteria. HHV8 levels may be a potential biomarker for a clinical diagnosis of HIV-MCD.[13,14]

Rituximab has proven itself an effective treatment in 2 open-label clinical trials: it sustained remission in 22 patients (92%) after 60 days off treatment, and a separate cohort demonstrated a relapse-free survival rate of 79% at 2 years.[12,15] In individuals receiving rituximab, in addition to clinical and radiologic response, a reduction from baseline values was observed for CRP and plasma HHV8 viral loads.[16,17] Further rituximab after relapse has been shown to lead to further clinical remission, but it remains unclear whether patients should receive maintenance therapy during remission.[18,19]

Previous studies have not examined factors that predict relapse in HIV-MCD. Because HHV8 viral loads are decreased during remitted HIV-MCD, it is plausible that an increase in HHV8 viral loads without clinical symptoms could predict presentation of active MCD. Predicting relapse and identifying patients with a higher risk of relapse are relevant for clinical decision-making, as these patients may benefit from therapy during remission. We performed a multivariate analysis, which considered CRP levels, HHV8 viral loads, and age during remission, to establish the risk of relapse in a large cohort of individuals with remitted HIV-MCD.

Methods

Study population

Fifty-two individuals (46 male) diagnosed with a histologically confirmed HIV-MCD were recruited, and plasma samples for CRP and HHV8 DNA estimations were undertaken at least every 3 months during regular clinical reviews. In addition, patients were encouraged to attend if they developed fevers or other symptoms associated with active MCD, and on these occasions blood samples were also analyzed for plasma levels of HHV8 and

Table 1. Patient characteristics

	No. (%) or median (IQR)
Patient demographics at enrollment (52 patients)	
Median age, y (range)	42 (23-69)
Sex, % male	88
Ethnicity, % white/black/other	58/35/8
Serum CRP, mg/L	75 (7-136)
Plasma HHV8, copies/mL	30 000 (60.5-1 000 000)
Active MCD, %	81
MCD-related information during follow-up (610 sample points)	
Median serum CRP, mg/L	6 (0-24)
Median plasma HHV8 viral load, copies/mL	890 (0-2400)
Median CRP during attack	38.5 (8-110.75)
Median CRP during remission	3 (0-9)
Median plasma HHV8 viral load during attack	27 000 (2700-400 000)
Median plasma HHV8 viral load during remission	0 (0-1950)
Percent follow-up with CRP > 10 mg/L during attack	73.4
Percent with CRP > 10 mg/L during remission	19.6
Percent with detectable plasma HHV8 during attack	90.5
Percent with detectable plasma HHV8 during remission	38.6

IQR indicates interquartile range; CRP, C-reactive protein; HHV8, human herpesvirus-8; and MCD, multicentric Castleman disease.

serum CRP along with other clinically relevant investigations. The median age at MCD diagnosis of the patients was 42 years (range, 23-69 years), and 19 (37%) were established on highly active antiretroviral therapy at diagnosis of HIV-MCD, of whom 10 (53%) had undetectable HIV plasma viral loads. The median CD4 cell count at diagnosis was 216 mm^3 (range, 37-834 mm^3). Forty-two patients received rituximab-based treatment with (n = 14) or without etoposide (n = 28). Three patients with concomitantly diagnosed non-Hodgkin lymphoma received anthracycline-based combination chemotherapy, 3 patients were treated with splenectomy, 1 each IFN and anti-IL 6 receptor antibody, and 2 received best supportive care only. Of the 52 patients, 7 died early of progressive disease and 45 achieved a clinical remission of HIV-MCD. Of these 45 remitters, 3 have died in remission (1 anal cancer, 1 lymphoma, 1 opportunistic infection), 31 remain in remission, and 11 have developed biopsy-confirmed relapses of HIV-MCD. The median follow-up is 49 months (range, 5-205 months). For the 45 patients who entered remission, the 2- and 5-year remission-free survivals are 89% (95% confidence interval, 79%-99%) and 66% (95% confidence interval, 55%-88%), respectively.

Plasma HHV8 measurement

Plasma HHV8 DNA viral load was measured using LightCycler quantitative polymerase chain reaction (Roche Diagnostics) on DNA extracted from whole blood using primers specific to the HHV8 ORF-7 gene, as previously described.[20] Institutional review board approval was obtained from the Riverside Ethics Committee in accordance with the Declaration of Helsinki.

Statistical analysis

Data were prepared in a counting process format, which incorporated the results of all measurements of HHV8 and CRP in time-dependent covariates. Data were prepared with a Perl script and analyzed with the R computer language. Curves for overall duration of survival were plotted by the Kaplan-Meier approach.

With respect to age, the patients were divided into 2 groups: younger than 50 years of age or 50 years of age and older at the date of diagnosis. Representation of continuous variables by a categorical variable can lead to loss of information and, if chosen by reference to the data, can lead to overfitting. Accordingly, we took steps to ensure the validity of the categorical age variable. First, the cut-off point of 50 years of age was chosen according to previous clinical considerations without specific reference to the data. Second, cut-off point analysis was used to determine the optimal cut-off point for the age covariate. As the determination of a cut-off point might be unstable with respect to

perturbations of the data, this analysis was confirmed using nonparametric bootstrapping; we took 2000 samples from the original distribution with replacement and repeated the cut-off point analysis. This procedure produced an optimal cut-off point of 49.6 years, which is consistent with our clinical approach.

Cox multivariate modeling was used to identify independent variables predictive of survival. As well as the inclusion of time-dependent covariates, an individual frailty term was included in the analysis to take account of the possibility that different individuals may have a different underlying susceptibility. The multivariate model also used a multistate model to distinguish the 2 possible transitions: from remission to relapse and from relapse to remission.

Results

A total of 610 samples were available from the 52 patients, including 190 samples obtained from patients while they had clinically active HIV-MCD and 420 samples during clinical remission. Three samples for serum CRP were missing, and 14 samples for plasma HHV8 were assayed nonquantitatively and were described as detectable. At enrollment, 42 patients had active MCD, whereas 10 patients were in remission and the median plasma HHV8 DNA load was 30 000 copies/mL (interquartile range [IQR], 60.5-1 000 000) and median CRP was 75 mg/L (IQR,7-136 mg/L). During follow-up, patients in remission had a median HHV8 viral load below the level of detection (< 50 copies/mL; IQR, 0-1950 copies/mL) and a median CRP below the level of detection (< 5 mg/L; IQR, 0-9 mg/L). Patients experiencing an active attack of MCD had a median HHV8 DNA viral load of 27 000 copies/mL (IQR, 2700-400 000 copies/mL) and a median CRP count 38.5 (IQR, 8-110.75; Table 1). CRP levels greater than 10 mg/L were found in 73.4% of patients with active MCD and 19.4% of patients in remission. Plasma HHV8 DNA was detected in 90.5% of patients with active MCD and 38.6% of patients in remission.

In our multivariate analysis, the probability of relapse over time is demonstrated in Figure 1. Three factors (age, HHV8 viral load, and CRP) were included in a multivariate Cox regression model, and 2 factors were statistically significant (Table 2). In patients with detectable HHV8 viral loads (> 50 copies/mL) during remission, the risk of relapse was significantly higher (hazard ratio = 2.9;
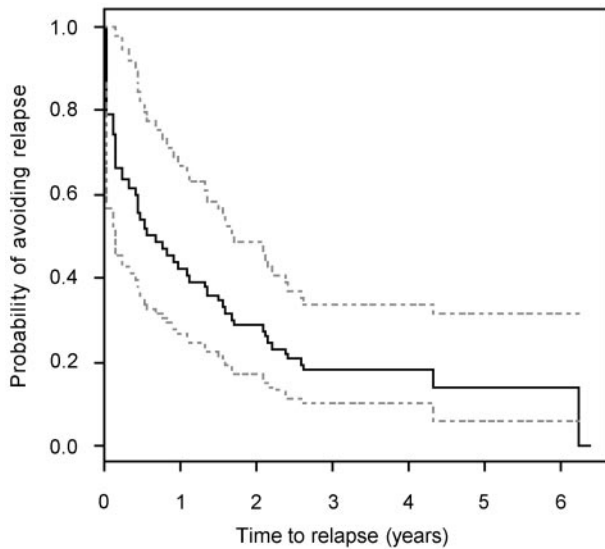
Figure 1. Kaplan-Meier curve showing the overall time to relapse. Hyphenated lines represent the 95% confidence interval.

95% confidence interval, 1.3-6.7) than those with undetectable HHV8. The confidence interval for the bootstrapped coefficients was similar to those in the original analysis, although wider, as expected.

The initial analysis suggested that when age was considered as a discrete covariable (cut off at 50 years), younger patients with inactive HIV-MCD had a significantly increased risk of relapse (Figure 2). However, age proved to be unstable after bootstrap analysis in 2 separate modes, and we cannot safely conclude that it is a significant predictor of relapse. Lastly, there was no evidence that serum CRP predicted relapse, and it did not show up in the final model.

Discussion

MCD is an HHV8-associated lymphoproliferative disorder that, despite the improved management of HIV in the post-highly active antiretroviral therapy era, has increased in frequency in patients with HIV infection. Diagnosis of HIV-MCD is currently based on histopathologic criteria; but, as it is a relapsing-remitting disease, clinical symptoms are necessary to confirm an active attack.[21] Although a French group set down a list of criteria by which to diagnose active HIV-MCD, their report did not include the prevalence of these symptoms in their patients.[12] Previous studies have demonstrated that HHV8 DNA is almost always detectable in the blood of patients with active MCD and that levels correlate with symptomatic disease.[13,14,22-26] We have previously observed that more than 80% of patients with HIV-MCD had detectable plasma

HHV8 viremia compared with 36% of HIV-seropositive patients with Kaposi sarcoma, 3% with lymphoma, and none of 53 HIV-positive control patients. Furthermore, the plasma levels of HHV-8 DNA were higher in individuals at MCD diagnosis (median, 41 000 copies/mL) than at Kaposi sarcoma diagnosis (median, 3500 copies/mL).[27]

Our data support findings in other studies that HHV8 DNA is significantly higher in MCD than in other HHV8-associated tumors (including Kaposi sarcoma, primary effusion lymphoma, and plasmablastic lymphoma) probably because of the fact that HHV8 persists in B-lymphoid cells in its lytic form (while remaining latent in Kaposi sarcoma- and primary effusion lymphoma-infected cells).[11,27,28] We suggest that that HHV8 positivity in combination with raised CRP levels, fever, multifocal lymphodenopathy, and enlargement of the spleen may be used as the main criteria for a diagnosis of HIV-MCD.

In addition to its use as a biomarker at diagnosis, HHV8 viral loads can differentiate between active and remitted HIV-MCD. Early studies established that HHV8 viral loads are associated with an active attack of HIV-MCD.[13,14] Here, we find that HHV8 viral loads decrease during remission (median, < 50 copies/mL; IQR, 0-1950 copies/mL), compared with viral loads during an attack (median, 27 000 copies/mL; IQR, 2700-400 000 copies/mL). In this study, we postulated that HHV8 viral load could be used as a biomarker to monitor disease. At present, there is no well-established treatment to prevent attacks and continuous or intermittent therapy is not the standard at this time, as continuous treatments of rituximab have been linked with adverse pulmonary events (eg, interstitial pneumonitis[29] and acute respiratory failure[12]) and progressive multifocal leukoencephalopathy,[30] and low-dose chemotherapy degrades quality of life, promotes the formation of secondary tumors (eg, acute myeloid leukemia),[31] and may be followed itself by rapid relapse.[32]

In our cohort of patients with remission of histologically confirmed MCD, a multivariate Cox regression model found that a detectable HHV8 viral load during periods of inactive MCD significantly increased the risk of relapse. Our model agrees with previous qualitative observation, where in the CastlemaB trial HHV8 was detected in 4 of 6 patients who relapsed, at the time or just before the recurrent episode.[12] We postulate that this reflects a rise of infected B-lymphoid cells that are actively replicating the virus before an attack that presents with clinical symptoms. In support of our argument, retreatment with rituximab induces remission in patients with relapsed MCD, suggesting that relapse may not be the result of progression of resistant MCD but rather a failure to completely eradicate HHV8 harbored in B cells and a subsequent lytic HHV8 infection of plasmablasts.[6,18]

No previous biomarkers associated with the probability of relapse have been identified. A prospective trial where patients presenting with detectable HHV8 during remission are selected to

Table 2. Validation of coefficients in Cox analysis of MCD data

	Coefficient	Exp(coefficient)	SE	Z	P
Multivariate analysis					
HHV8 load (> 50 copies/mL)	1.083	2.954 (1.288-6.774)	0.424	2.557	.011
Age (< 50 y)	13.971	13.973 (2.222-87.851)	0.938	2.811	.005
Bootstrap validation					
HHV8 load (> 50 copies/mL)	1.111	3.037 (1.322-6.291)	0.396	2.805	.005
Age (< 50 y)	8.965	7821 (4.323e−07-42)	8.4	1.067	.286

MCD indicates multicentric Castleman disease; and HHV8, human herpesvirus-8.
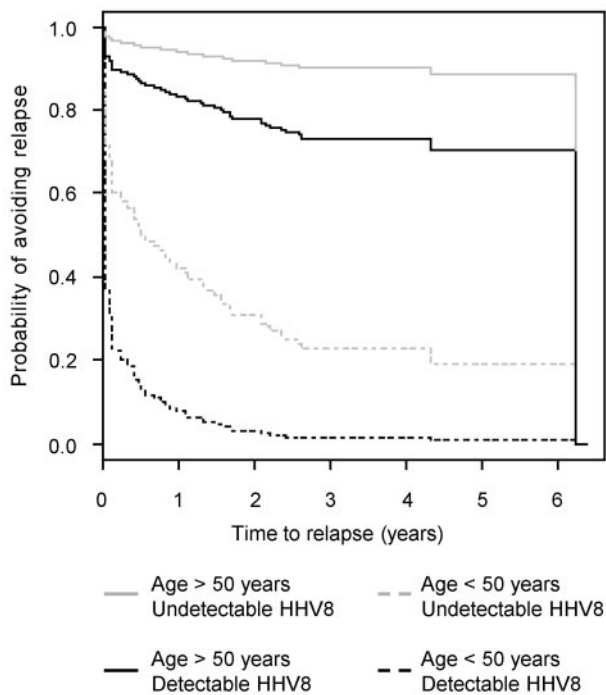
Figure 2. Kaplan-Meier curves showing the time to relapse dependent on patient age and HHV8 viral load. Gray solid line represents patients older than 50 years and undetectable plasma HHV8 viremia; gray hyphenated line, patients younger than 50 years and undetectable plasma HHV8 viremia; black solid line, patients older than 50 years and detectable plasma HHV8 viremia; and black hyphenated line, patients who were both younger than 50 years and had detectable plasma HHV8 viremia.

receive maintenance therapy would determine the utility of HHV8 as a biomarker and the efficacy of maintenance rituximab in preventing the onset of active MCD. For example, rituximab could be administered to a patient with a sudden spike in HHV8 viral loads, or for those patients who continuously present with HHV8 plasma positivity; in addition, the duration of rituximab beyond HHV8 normalization would also require investigation.

Although our data suggested that younger patients may be more at risk for a recurrent attack, we could not conclude this with certainty in a second bootstrapped analysis. We have previously found that plasma HHV8 viral load at diagnosis did not influence overall survival (log-rank, all $P > .1$)[27]; however, we establish here that HHV8 viral loads are an important biomarker in the pathogenesis and monitoring of HIV-MCD. Not only do HHV8 viral loads aid in the confirmation of active HIV-MCD, but they also predict relapse in those with disease in remission. A clinical trial is necessary to determine the potential benefit of maintenance rituximab or valganciclovir in remitted HIV-MCD patients, with the aim to prevent relapse, prolong survival, and improve quality of life.

Authorship

Contribution: M.B., J.S., C.A., and A.S. performed the statistical work; and all authors designed the study, performed research, analyzed the data, and approved and contributed to the final paper.

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