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ORIGINAL RESEARCH

Clinical relevance of cytomegalovirus viraemia*,†

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Background

Using new sensitive quantitative polymerase chain reaction (PCR) assays, cytomegalovirus (CMV) DNA is often detectable in the plasma of immunosuppressed patients. We investigated the prognostic value of a positive CMV DNA test for the development of CMV end-organ disease, other AIDS-defining events and mortality.

Methods

A survival analysis was performed, using the Kaplan–Meier method and Cox proportional hazards models, for patients prospectively followed in the Swiss HIV Cohort Study, from January 1996 to December 2007, who were CMV-seropositive, had a CD4 count of ≤ 100 cells/μL, and had a plasma sample available for the measurement of baseline CMV DNA with an ultrasensitive PCR. The outcome analysed was an AIDS-defining event, including CMV end-organ disease, or death. Variables analysed at the time of CMV measurement were demographic variables, CD4 cell counts, HIV-1 RNA loads, and use and type of highly active antiretroviral therapy (HAART).

Results

Of 1128 patients, 208 (18%) presented an AIDS-defining event and 246 (22%) died. A total of 368 patients (34% of samples) had detectable CMV DNA at baseline, with DNA concentrations of up to 38 800 copies/mL. In the multivariate analysis, CMV DNA predicted evolution not only towards CMV end-organ disease [hazard ratio (HR) 12.6; 95% confidence interval (CI) 4.27–37.41], but also towards other AIDS-defining events (HR 2.6; 95% CI 1.60–4.33) and death (HR 1.9; 95% CI 1.10–3.34).

Conclusion

Quantitative CMV DNA detected in the plasma of HIV-infected patients with CD4 counts ≤ 100 cells/μL is a predictor for HIV disease progression, CMV disease and death. A single low value of 80 copies/mL identifies patients at low but significantly increased risk during the following months, after the measurement.

Keywords: CMV DNA, CMV end-organ disease, HIV, mortality, opportunistic diseases

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†See editorial by Jacobson *et al.* [1] on pp. 387–388 in this same issue.

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Introduction

Infection with cytomegalovirus (CMV) is a major cause of morbidity in HIV-positive patients [2]. Although the introduction of highly active antiretroviral therapy (HAART) has led to a decline in the incidence of opportunistic diseases (ODs) in general, including CMV

infection, CMV end-organ disease continues to occur in HIV-infected patients failing HAART because of adherence problems or drug resistance, or presenting late with low CD4 cell counts [3–5].

Most studies on CMV were conducted in the pre-HAART era, using methods with high thresholds for viral detection. They showed that detection of CMV in plasma predicts CMV disease in persons with advanced AIDS [2,6]. Prophylactic use of oral ganciclovir reduced the risk of CMV disease [7,8]. Between 1996 and 2003, new studies suggested that CMV plasma viral load predicts CMV retinitis [9] and that a high CMV plasma viral load is associated with an increased risk of death [10], while successful HAART suppresses CMV viraemia [11].

Nevertheless, CMV viraemia is often detected in patients with low CD4 cell counts who do not develop CMV disease after starting HAART [9,12]. In acutely ill HIV-infected patients, detection of CMV viral load by quantitative polymerase chain reaction (PCR) was found to be a poor predictor of CMV end-organ disease: 43.5% of the patients presented with positive viraemia, but only 7.4% had end-organ CMV disease [13].

Recently, new quantitative PCR assays have been developed with increased sensitivity. The threshold of detection has decreased from 400 to 20 copies/mL in plasma. With this increased sensitivity we have often found CMV viraemia in HIV-infected patients, sometimes with elevated, albeit fluctuating CMV DNA levels, but without any evidence (in cultures or biopsies) of CMV end-organ disease.

In this study, we therefore evaluated the prognostic value of an early positive CMV viral load, using an ultrasensitive PCR (detection limit 20 copies/mL), for global mortality, CMV end-organ disease and other ODs in HIV-infected patients with CD4 counts ≤ 100 cells/μL. We also describe the incidence and prevalence of CMV end-organ disease in the Swiss HIV Cohort Study (SHCS) since 1996.

Methods

Study population, covariates and endpoints

We included patients followed in the SHCS (www.shcs.ch) after 1 January 1996 who had detectable CMV-specific immunoglobulin G (IgG), a CD4 cell count ≤ 100 cells/μL measured after or at the same time as the diagnosis of CMV seropositivity, and a frozen plasma sample available in the interval of 3 months before to 1 month after the CD4 cell count for the measurement of baseline CMV DNA. The endpoints analysed were CMV end-organ disease, global mortality and development of other ODs. An interval of at least 1 month was required between the date of baseline CMV viraemia analysis and these endpoints. The potential prognostic factors assessed were sociodemographic variables (sex, age, ethnic origin and HIV transmission category), use of any antiretroviral therapy (ART), CD4 cell counts, HIV viraemia and CMV DNA in plasma. The patients were followed from the date of the available plasma sample collection for the baseline CMV PCR to the date of the last cohort visit before 31 December 2007. The occurrence of CMV end-organ disease or another OD did not result in follow-up being terminated.

To determine the incidence and prevalence of CMV end-organ disease in the SHCS, we used data obtained for the whole population of the cohort since 1996.

Definitions

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

Detection and quantification of CMV DNA

CMV DNA was measured in plasma collected at a time when the CD4 count was ≤ 100 cells/μL. We used an automated CMV real-time PCR (Abbot Molecular, Des Plaines, IL, USA) with a threshold of detection of 20 copies/mL. This method is used routinely to monitor CMV infection in our institution and is described in recent publications [14–16].

In 216 samples, the quantity of plasma was insufficient and the plasma had to be diluted (1:4) in order to measure the CMV DNA, which was positive in 67 samples (31%). The initial threshold of detection of 20 copies/mL could not be guaranteed in these samples and we therefore considered 80 copies/mL to be our global threshold in the survival analysis.

Statistical analysis

The evolution of the annual incidence rate (assessed in person-years) of CMV end-organ disease from 1996 to 2007 was analysed using Poisson regression (with the year as predictor). The exponential of the regression parameter was interpreted as a relative decrease (or increase) of the incidence rate in a given year compared with the previous year [17]. This model allowed for different changes of the incidence rate between the periods 1996–1998 and 1999–2007, because the reduction in incidence was not linear over the whole observation period.

The performance of the CMV DNA measurement in predicting the prognosis of CMV end-organ disease, OD and

mortality was assessed using time-dependent receiver operating characteristic (ROC) curves. For each ROC curve, the area under the curve (AUC) and the confidence intervals (CIs) were assessed by bootstrap (1000 simulations). The purpose of this method [18] is to evaluate the performance of a marker in predicting the occurrence of an event, which can happen at different points in time. The closer the AUC is to a value of 1, the better the performance of the test. 0.5 represents an uninformative test.

Survival analyses were performed to assess the impact of factors on the time to occurrence of CMV end-organ disease, other ODs and mortality. Survival curves were first assessed in a univariate analysis (Kaplan–Meier method), and compared between subgroups (log-rank test). The number of CMV end-organ disease events being low, a procedure of selection of variables for the multivariate analysis was applied to avoid overfitting: the factors potentially correlated with the survival function [$P<0.20$ in the log-rank test or the univariate hazard ratio (HR)] were introduced into a multivariate Cox model. Despite this selection, four variables were retained in the model for CMV end-organ disease. We restricted the adjustment factors to age and CD4 cell count ($P<0.15$ in the univariate analysis). The CD4 count was used as a categorical variable because our inclusion criterion of CD4 count ≤ 100 cells/μL yielded a small range of values and the cut-off value of 50 cells/μL is clinically meaningful. CMV viraemia was categorized as detectable/not detectable because of a high frequency of undetectable values and the clinical importance of this information. Treatment (HAART *vs.* non-HAART) was considered a time-dependant variable. The HRs are given with the 95% CIs and Wald's tests were used to measure significance levels. The assumptions of proportional hazard were checked. The survival analyses focused on the events occurring in the first year of follow-up because the ROC curve analyses indicated that the prognostic performances were not useful beyond this time horizon (AUC <0.6).

In all cases, $P\leq 0.05$ (two-sided) was considered to indicate statistical significance. Statistical analyses were performed using SPSS 11.0 (SPSS, Chicago, IL, USA), STATA 10.0 software (STATA Corp., College Station, TX, USA) and S-PLUS 8.0 (Insightful Corp., Seattle, WA, USA).

Results

Annual incidence rate of CMV end-organ disease in the whole SHCS since 1996

The prevalence of CMV end-organ disease in the SHCS ranged from 2.6% in 1996 to 1.6% in 2007. The highest incidence rate was 3.9 per 1000 person-years in 1996 and decreased to 0.1 per 1000 person-years in 2007. The most marked drop in the incidence rate occurred between 1996 and 1998, with an estimated reduction of 63% (CI 70–55%) with each successive calendar year ($P<0.001$). The annual reduction was less pronounced after 1998 (17%), but still remained significant ($P<0.001$). The observed and predicted annual rates are shown in Figure 1.

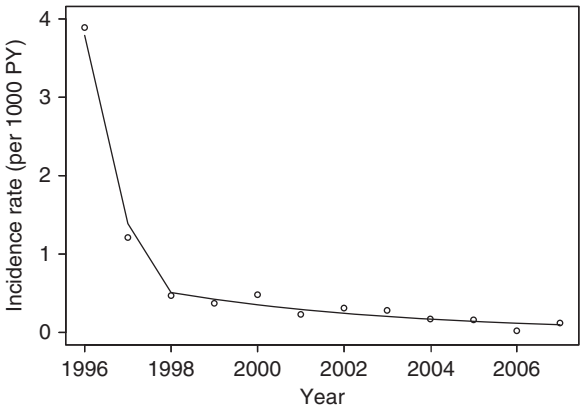

Fig. 1 Annual incidence rates of cytomegalovirus (CMV) end-organ disease. Solid line, Poisson regression model; circles, observed rates. PY, person-years.

A total of 1170 patients from the whole SHCS since 1996 met our inclusion criteria. Thirty-nine were excluded from the analysis because they had follow-up of <1 month and three others were excluded because they presented CMV end-organ disease <1 month from the baseline CMV DNA measurement.

Patient characteristics (Table 1)

A total of 1128 patients were included in the analyses. Sixty-seven per cent of the study population were men. The median age at baseline was 38 years (range 18–85 years) and the majority of the patients were white (80%). Thirty-eight per cent of patients reported that they were infected through heterosexual intercourse, 30% were men who have sex with men (MSM) and 28% were injecting drug users (IDUs). The median CD4 count at baseline was 61 cells/μL (range 0 to 100 cells/μL), and 39% of the patients had a cell count <50 cells/μL. The median HIV viral load was 98 663 HIV-1 RNA copies/mL (range <40 copies/mL to 3.5×10^7 copies/mL). Forty-one per cent of patients either were already receiving or started an antiretroviral treatment at the time of the CMV measurement. Of these, 22% had full viral suppression (<50 copies/mL) and 71% had a viral load of >200 copies/mL at baseline.

The median duration of follow-up was 4.8 years. During the complete follow-up period, CMV end-organ disease occurred in 25 patients (2.2%; retinitis in 19 patients and

Table 1 Characteristics of the patients according to the events occurring in the first year of follow-up

			Patients with at least one of the outcomes					
			OD		CMV disease		Death	
	Whole sample ($n = 1128$)		($n = 95$)		($n = 19$)		($n = 78$)	
	n	%	*n*	%	*n*	%	*n*	%
Sex								
Female	372	33.0	39	41.1	5	26.3	22	28.2
Male	756	67.0	56	58.9	14	73.7	56	71.8
Ethnic origin								
Other	117	10.4	11	11.6	2	10.5	8	10.3
White	899	79.7	74	77.9	16	84.2	67	85.9
Black	112	9.9	10	10.5	1	5.3	3	3.8
Mode of HIV infection								
Other	49	4.3	4	4.2	0	0	8	10.3
Heterosexual	429	38.0	39	41.1	10	52.6	18	23.1
IDU	310	27.5	33	34.7	2	10.5	30	38.5
MSM	340	30.1	19	20	7	36.8	22	28.2
HIV treatment*								
None	665	59.0	67	70.5	13	68.4	43	55.1
ART	121	10.7	7	7.4	3	15.8	8	10.3
HAART	342	30.3	21	22.1	3	15.8	27	34.6
Age* (years) [median (IQR)]	38.0 (33.8–45.0)		37.0 (33.0–44.0)		42.0 (35.5–52.0)		40.5 (34.3–49.8)	
CD4* (cells/μL) [median (IQR)]	61.0 (33.0–85.0)		51.0 (25.0–76.0)		39.0 (22.0–70.0)		50.5 (16.8–77.8)	
RNA* (10 000 copies/mL) [median (IQR)]	9.9 (1.2–29.3)		16.6 (3.0–45.1)		10.5 (4.7–40.9)		8.0 (0.5–21.9)	
Missing data	38	3.4	4	4.2	0	0.0	1	1.3
CMV DNA* (copies/mL)								
≥ 80	177	17.2	27	31.4	13	72.2	19	25.7
Missing data	99	8.8	9	9.5	1	5.3	4	5.1

ART, antiretroviral therapy; CMV, cytomegalovirus; HAART, highly active antiretroviral therapy; IDU, injecting drug use; IQR, interquartile range; MSM, men having sex with men; OD, opportunistic disease.
*At the time of the CMV DNA measurement.

gastrointestinal diseases in six patients) and other ODs in 183 patients (16%). A total of 246 patients died (22%). The most frequent ODs were *Candida* oesophagitis (41 patients; 22%), atypical mycobacterial diseases (23 patients; 13%), *Pneumocystis carinii* pneumonia (19 patients; 10%), Kaposi's sarcoma (14 patients; 8%) and non-Hodgkin's lymphoma (10 patients; 6%).

During the first year of follow-up, CMV end-organ disease occurred in 19 patients (1.7%) and other ODs in 95 patients (8.4%), and 78 patients (6.9%) died. The median times between the CMV DNA measurement and the development of CMV end-organ disease, other ODs and death were 141, 139 and 160 days, respectively.

Thirty-four per cent of patients (368 patients) had detectable CMV DNA in plasma at baseline, with a median of 136 copies/mL and a maximum of 38 800 copies/mL. This percentage was stable from 1996 to 2007. Amongst the patients with a detectable value, 18 (5%) experienced evolution towards CMV end-organ disease.

During the first year of follow-up, 83% of the patients who developed CMV end-organ disease had a detectable CMV DNA value at baseline, with a median positive value of 1990 copies/mL [interquartile range (IQR) 279.5–4332.5 copies/mL]. Of those who developed an OD other than CMV end-organ disease, 42% were CMV DNA-positive (median CMV DNA 179.0 copies/mL; IQR 89.8–1220.0 copies/mL), and of those who died, 38% were CMV DNA-positive (median CMV DNA 283.5 copies/mL; IQR 81.0–4117.5 copies/mL). In the group of patients who neither died nor developed CMV end-organ disease or any other OD, 32% had a detectable value, with a median of 125.5 copies/mL (IQR 51.7–740.0 copies/mL).

Prognostic performance of CMV DNA value

Using time-dependent ROC curves, we assessed the prognostic performance of the CMV DNA value at baseline in predicting our different endpoints. The areas under the curve are shown in Figure 2 for each endpoint, according to the timeframe. The optimal prognostic performance of the CMV DNA value in predicting CMV end-organ disease was achieved at 6 months (AUC 0.8; 95% CI 0.7–0.9).

For predicting other ODs, the optimal prognostic performance was achieved at 2 months (AUC 0.8; 95% CI 0.6–0.9) and for mortality it was achieved at 6 months (AUC 0.6; 95% CI 0.5–0.7).

We therefore decided to retain a 1-year timeframe for the survival analysis.

Univariate and multivariate analysis

Kaplan–Meier survival curves showing the relationship between a positive CMV DNA value in plasma at baseline and the different endpoints are shown in Figure 3. The HRs (with 95% CIs) associated with each factor in the univariate and multivariate analyses are shown in Table 2.

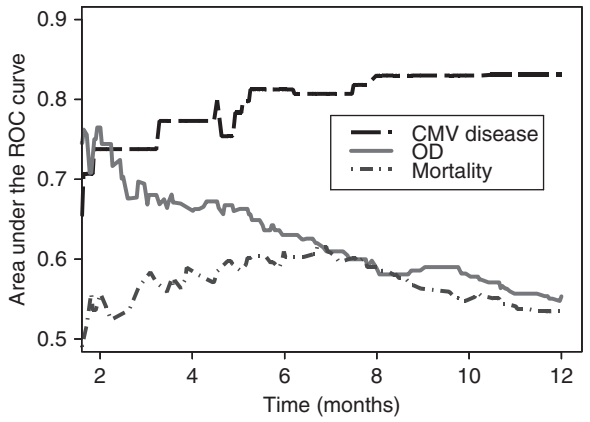

Fig. 2 Time-dependent receiver operating characteristic (ROC) curves showing the prognostic performance of cytomegalovirus (CMV) DNA. OD, opportunistic disease.

CMV end-organ disease

Age at baseline and CMV DNA were significantly associated with the development of CMV end-organ disease. Patients with a positive CMV DNA value (above 80 copies/mL) were 13 times more likely to develop the disease (HR 13.0).

Death

In the univariate analysis, IDU, age at baseline, CD4 cell count, use of HAART and CMV DNA were correlated with mortality. In the multivariate analysis, use of HAART was significantly associated with a decreased risk of death (HR 0.1), whereas, as expected, the risk of mortality increased with age (HR 1.4 per 10 years). Detectable CMV DNA at baseline was significantly associated with an increased risk of dying during the following year (HR 1.9).

ODs

Only CMV DNA was significantly associated with the development of other ODs. The risk doubled in the case of a positive value (HR 2.6). Use of HAART, in contrast, significantly decreased this risk (HR 0.4).

Not only was the detection of CMV DNA at baseline significantly associated with the three endpoints, but there was a significant relationship between the CMV DNA value

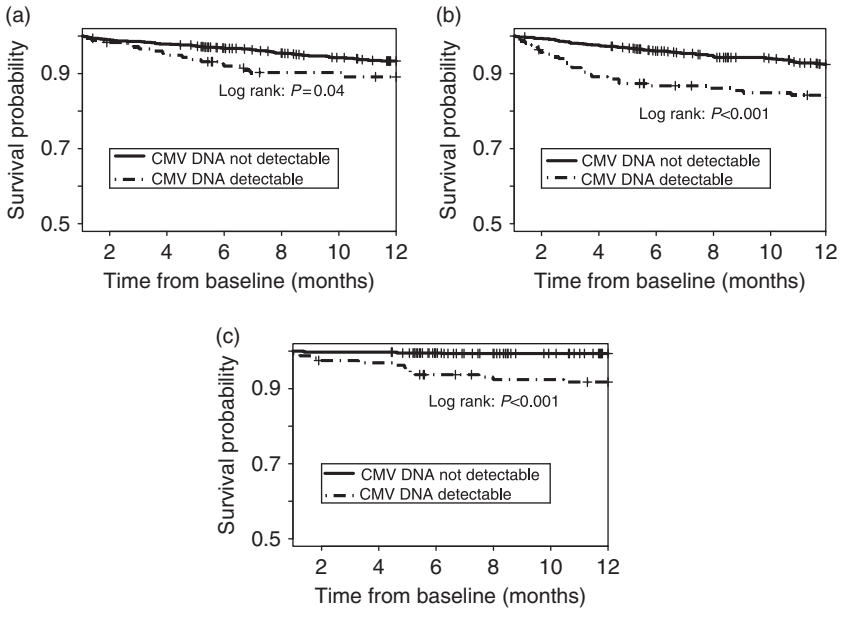

Fig. 3 Survival Kaplan–Meier curves for (a) mortality, (b) opportunistic diseases and (c) cytomegalovirus (CMV) end-organ disease, according to the CMV DNA value.

Table 2 Survival analysis (Cox proportional hazards model)

	Mortality				Opportunistic disease				CMV end-organ disease			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Sex												
Female	1				1		1		1			
Male	1.27 (0.78–2.08)	0.34			0.73 (0.48–1.13)	0.16	0.89 (0.55–1.45)	0.65	1.34 (0.48–3.75)	0.58		
Ethnic origin												
White	1		1		1				1			
Black	0.35 (0.11–1.12)	0.08	0.72 (0.21–2.45)	0.60	1.10 (0.57–2.15)	0.77			0.48 (0.06–3.61)	0.47		
Other	0.94 (0.45–1.95)	0.86	0.99 (0.47–2.11)	0.98	1.26 (0.65–2.45)	0.50			1.07 (0.25–4.69)	0.92		
		0.11†				0.79†				0.47†		
Risk												
Heterosexual	1		1		1		1		1			
IDU	2.36 (1.32–4.25)	<0.01	1.94 (0.98–3.81)	0.06	1.22 (0.75–1.98)	0.42	1.13 (0.67–1.89)	0.66	0.53 (0.21–1.34)†	0.18		
MSM	1.57 (0.84–2.93)	0.15	1.72 (0.89–3.33)	0.11	0.62 (0.34–1.11)	0.11	0.57 (0.29–1.12)	0.10				
Other	4.22 (1.84–9.70)	<0.01	4.24 (1.74–10.37)	<0.01	1.08 (0.38–3.03)	0.89	0.84 (0.26–2.73)	0.77				
		<0.01†				0.14†						
Age* (years)												
Per 10 years	1.29 (1.06–1.58)	0.01	1.42 (1.13–1.79)	<0.01	0.90 (0.71–1.12)	0.34			1.37 (0.91–2.07)	0.13	1.30 (0.87–1.96)	0.21
CD4* (cells/μL)												
CD4 < 50 cells/μL	1		1		1				1		1	
CD4 ≥ 50 cells/μL	0.66 (0.42–1.03)	0.07	0.59 (0.37–0.95)	0.03	0.79 (0.51–1.20)	0.27			0.47 (0.18–1.18)	0.11	1.00 (0.37–2.567)	0.99
HIV RNA*												
Log (RNA)	0.94 (0.82–1.08)	0.41			1.12 (0.96–1.31)	0.16	1.05 (0.89–1.24)	0.53	1.15 (0.82–1.63)	0.42		
HAART*												
No	1		1		1		1		1			
Yes	0.08 (0.05–0.14)	<0.01	0.07 (0.04–0.13)	<0.01	0.34 (0.22–0.53)	<0.01	0.35 (0.22–0.54)	<0.01	0.57 (0.22–1.48)	0.25		
CMV DNA* (copies/mL)												
<80	1		1		1		1		1		1	
>80	1.71 (1.02–2.88)	0.04	1.89 (1.09–3.28)	0.02	2.36 (1.50–3.72)	<0.01	2.64 (1.60–4.33)	<0.01	13.40 (4.80–37.6)	<0.01	13.04 (4.38–38.79)	<0.01

CMV, cytomegalovirus; HAART, highly active antiretroviral therapy; HR, hazard ratio; IDU, injecting drug use; MSM, men having sex with men.
*At the time of the CMV DNA measurement.
†Categories were grouped.

and the risk of CMV end-organ disease and death. The higher the viral load, the greater the risk of CMV end-organ disease, and the risk was especially high for values of CMV DNA above 1000 copies/mL (HR 17.1; 95% CI 6.8–49.0; $P<0.01$). In the multivariate analysis, patients with CMV DNA values above 1000 copies/mL were 15 times more likely to develop CMV end-organ disease (HR 15.3; 95% CI 5.6–42.0; $P<0.01$). The risk of dying increased significantly above 1000 copies/mL (HR 2.5; 95% CI 1.3–4.8; $P<0.01$) and was associated, in the multivariate analysis, with a fourfold increase in risk (HR 3.9; 95% CI 1.9–8.0; $P<0.01$).

We calculated the positive and negative predictive values at 6 months of a single measurement of CMV DNA. The negative predictive values for CMV end-organ disease and death, were excellent regardless of the viral load (99.5; 95% CI 99.0–99.9 and 96.8; 95% CI 95.5–98.0, respectively). The positive predictive values were low (5.9; 95% CI 2.4–9.8 and 8.5; 95% CI 4.2–12.3, respectively), but increased for viral loads above 1000 copies/mL (11.5; 95% CI 3.6–20.8 and 14.7; 95% CI 4.8–21.6, respectively).

Discussion

The objective of our study was to evaluate the clinical relevance of a detectable CMV DNA in the plasma of immunosuppressed HIV-infected patients, using an ultrasensitive PCR, in the HAART era.

Our study shows that a single positive measurement of low CMV viraemia (using DNA PCR) is significantly associated not only with the development of CMV end-organ disease but also with other ODs and death.

We found that the incidence of CMV end-organ disease in the SHCS has decreased significantly between 1996 and 2007, as have the incidences of other ODs [19,20]. This can primarily be explained by the widespread use of HAART in developed countries.

Despite this low incidence of disease, 34% of our CMV-seropositive cohort participants, with CD4 counts <100 cells/μL, had a detectable CMV viral load each year. This proportion remained stable over time. The majority (95%) of these CMV viraemic patients did not develop CMV end-organ disease. This value of 34% is twice the value reported by Deayton *et al.* [21], who used a whole-blood PCR with a sensitivity of 200 genomes/mL. It is also higher than the 20% reported by Goossens *et al.* [22], who used a detection limit of 100 copies/mL, in patients starting HAART. It clearly reflects the impact of using ultrasensitive PCRs with very low thresholds of detection, which can reveal early CMV reactivation.

In this high proportion of positive patients, the median value of CMV DNA was low (136 copies/mL). Still, these low values of viral load were significantly associated with a 12-fold increase in the risk of progression to CMV end-organ disease, and a roughly twofold increase in the risk of developing another OD or death.

The lowest value significantly associated with these different endpoints was 80 copies/mL. Unfortunately, the range of values below 80 copies/mL could not be properly explored, because of the necessity of diluting some samples. We cannot therefore exclude the possibility that the original threshold of 20 copies/mL could already be predictive of CMV, other ODs and death. No dilutions were needed for the plasma samples of the patients who developed CMV end-organ disease. In these cases, the original threshold (20 copies/mL) remained significant.

The risk of developing the different endpoints increased with the level of CMV DNA. The increase was particularly striking for CMV end-organ disease: levels of CMV DNA above 1000 copies/mL were associated with a 16-fold increase in risk. This finding is supported by a study by Tufail *et al.*, in which the six patients whose CMV DNA levels stayed persistently below 5000 genomes/mL did not develop CMV retinitis, whereas three of the four patients with levels rising above this value at some time during the follow-up did develop CMV retinitis [23].

The fact that 17% of the patients who developed CMV end-organ disease did not have detectable CMV DNA in plasma is probably explained by the limitation, in our study, entailed by the delay between the unique CMV DNA measurement and the occurrence of the disease (median 141 days).

Our results support the association between a positive viral load in plasma and evolution towards death, which was suggested by Spector *et al.* [6] and Deayton *et al.* [21]. Spector *et al.* [6] showed that a CMV DNA value >500 copies/mL at baseline was associated with a 2-fold increase in the risk of death in a univariate analysis, and Deayton *et al.* [21] reported a trend between baseline CMV DNA and risk of death. Jabs *et al.* [10] observed a comparable increased risk of mortality (relative risk 1.9) in patients with a CMV viral load >400 copies/mL.

Unlike Deayton *et al.* [21], we found a significant association between baseline CMV DNA and the progression to other ODs.

In the case of the significant association between detectable CMV DNA in plasma and ODs or death, CMV reactivation can be considered as a marker of immune suppression and impaired CD4 cell function in patients positive for CMV IgG. Panagiotakis *et al.* observed that CMV DNAemia detected in the peripheral blood lymphocytes of patients with CD4 counts <200 cells/μL was correlated with a delayed increase in CD4 count after initiating HAART [24]. CMV is also considered to function

as a cofactor as it interacts at the molecular or cellular level to promote HIV pathogenicity and the progression of AIDS [25]. Moreover, CMV encodes a large number of immunomodulatory functions which modulate both the innate and the adaptive arms of the immune response [26]. It seems that increased inflammation benefits CMV dissemination [26] and prostaglandins, such as tumour necrosis factor (TNF)-α, released during inflammation may contribute to CMV reactivation [27]. This mechanism could explain why asymptomatic CMV viraemia has been detected in critically ill immunocompetent patients and patients with septic shock [28,29].

It is therefore no surprise that the best prognostic performance of CMV DNA was achieved for CMV end-organ disease (AUC 0.81), and that the prognostic performance increased during the first 6 months. In the case of other ODs and death, the performance was acceptable (AUC 0.77 and 0.61, respectively) during the first 6 months, and then became of marginal acceptability.

Our study has several limitations inherent to retrospective analyses of prospectively collected data; in particular, the limitation of the original threshold and the impossibility of serial measurements, which may have emphasized the difference between measuring constant detectable low levels of CMV DNA and increasing levels over time. This in turn would enable determination of the best cut-off CMV DNA level in plasma to maximize its predictive value. The low frequency of CMV end-organ disease is also a limitation which may have resulted in a lack of power in the detection of factors associated with this event and a limitation in the number of adjustment factors in the Cox multivariate models. Despite this, the association between CMV viraemia and our end-points is strong and significant.

We used a cohort of patients that encompassed most of the Swiss HIV-infected population and was representative of the patients encountered in Western clinics. Compared with previous studies, our cohort of patients was larger, represented a greater number of endpoint cases, covered the period after 2003 and used a newer and more sensitive PCR.

In conclusion, despite lowering the threshold of CMV DNA detection in plasma by using ultrasensitive PCR assays, the measurement of a single low value of CMV DNA remained significantly associated with CMV end-organ disease. Moreover, it was also significantly associated with the development of other ODs and death. The positive predictive value of a single CMV viral load was low, but increased for values >1000 copies/mL. As suppressing CMV viraemia has become simpler, our results support the idea of exploring strategies of prevention of CMV end-organ disease in a subset of critically ill patients with low CD4 cell counts. Guidelines concerning the decision to start pre-emptive treatment should explore the potential of serial CMV DNA detection and the establishment of a CMV DNA cut-off value in plasma.

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