MAJOR ARTICLE

Cytomegalovirus Coinfection Is Associated With an Increased Risk of Severe Non–AIDS-Defining Events in a Large Cohort of HIV-Infected Patients

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(See the editorial commentary by Emery on pages 169–71, and the major article by Johnson et al on pages 187–96.)

Background. Chronic cytomegalovirus (CMV) infection has been associated with immunosenescence and immunoactivation in the general population. In human immunodeficiency virus type 1 (HIV-1)–infected people, CMV coinfection, in addition to residual HIV replication and microbial translocation, has been proposed as a key factor in sustaining immune activation, even in individuals with a controlled HIV load.

Methods. Patients from the ICONA Study with at least 1 CMV immunoglobulin G (IgG) test available without active CMV disease were included in the analysis. AIDS-defining event or AIDS-related death and severe non–AIDS-defining event or non–AIDS-related death were taken as clinical progression end points. Independent predictors of CMV were identified by multivariable logistic regression. Probabilities of reaching the end points were estimated by survival analyses.

Results. A total of 6111 subjects were included, of whom 5119 (83.3%) were CMV IgG positive at baseline. Patients with CMV IgG positivity at baseline were more likely to develop a severe non–AIDS-defining event/non–AIDS-related death (adjusted hazard ratio [HR], 1.53 [95% confidence interval {CI}, 1.08–2.16]. In particular, CMV seropositivity was an independent risk factor for cardiovascular and cerebrovascular diseases (adjusted HR, 2.27 [95% CI, .97–5.32]).

Conclusions. In our study population, CMV/HIV coinfection was associated with the risk of severe non–AIDS-defining events/non–AIDS-related death, especially with cardiovascular and cerebrovascular events, independently of other prognostic factors. This finding supports a potential independent role of CMV coinfection in vascular/degenerative organ disorders in HIV-infected subjects.

Keywords. CMV infection; severe non–AIDS-defining events; cardiovascular/cerebrovascular events; HIV infection; mortality; morbidity.

Cytomegalovirus (CMV) is a beta human herpesvirus with a worldwide spread. CMV infection is often asymptomatic and acute and is followed by lifelong persistence of CMV in a latent stage in immunocompetent subjects but with potentially severe consequences in immunocompromised patients [1].

In the general population, CMV seroprevalence ranges from 50% to 90%, with the majority of individuals being infected in adulthood; the chance of acquiring CMV infection rises by 1% per year of age [2]. The infection rate is also influenced by socioeconomic status [3] and geographical location [4, 5]. In HIV-infected subjects, CMV is highly prevalent (from 75% to 90%), particularly among homosexual men [6, 7].

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CMV has been associated with the development of cardiovascular diseases (CVDs) [8, 9] and all-cause and CVD-related mortality in the general population [10].

Human immunodeficiency virus (HIV) infection seems to be an independent risk factor for atherosclerosis and end-organ disease, even in patients receiving antiretroviral therapy (ART) [11, 12], and CMV coinfection might contribute to accelerate cardiovascular complications in HIV-positive patients. Recently, several studies focused on the relationship between CMV/HIV coinfection and CVD. Hsue et al have shown that a large T-lymphocyte response to CMV is linked with an increased intima media thickness in HIV-infected patients and with atherosclerosis in both HIV-negative and HIV-positive subjects [9]. Parrinello et al have observed that CMV immunoglobulin G (IgG) antibody levels are associated with subclinical atherosclerosis in HIV aviremic women [13]. Moreover, in solid-organ-transplant recipients, the prophylactic treatment for preventing CMV reactivation reduces the risk of atherosclerosis, suggesting a potential pharmacological preventive action in this special setting [14].

Furthermore, CMV has been linked with other diseases characterized by chronic immune activation, such as dementia, cancer, and osteoporosis [15]. Finally, CMV seropositivity belongs to a cluster of immune factors constituting an immune risk profile associated with all-cause mortality in elderly individuals [16, 17].

The aim of the present study was to evaluate the seroprevalence and predictors of CMV infection in a prospectively followed Italian national cohort of HIV-infected subjects. Moreover, we studied whether CMV-seropositive status was associated with the risk of developing either AIDS-defining events or severe non–AIDS-defining events and AIDS-related death or non–AIDS-related death.

METHODS

Study Population

The study population was selected from the ICONA Foundation Study cohort. The ICONA study is an Italian multicenter prospective observational study of HIV-1–positive persons that was set up in April 1997. At the time of analysis, 10 129 subjects naive to antiretroviral drugs at baseline were enrolled after providing written informed consent via a standard form.

Demographic, clinical, and laboratory data, as well as therapies, are collected for all participants and recorded in an online database (http://www.icona.org). All data are updated at the occurrence of any clinical event and, in their absence, at least every 6 months. Details of the cohort and data collection have been previously reported [18].

Patients included in this analysis had at least 1 CMV IgG test available and, at the time of the test, were free from any reported CMV-related disease, were free from any non–AIDS-defining events listed below, and had at least 1 follow-up visit after the first CMV IgG test result was recorded. The date of the database freezing for statistical analysis was 1 October 2012. Baseline for the analysis was set as the date of the first CMV IgG test. CMV serological testing was performed in the different centers, using commercially available kits.

The ICONA study was approved by the institutional review boards or ethics committees of each clinical site and of the University of Milan.

Statistical Analysis

Patients with CMV IgG–positive and CMV IgG–negative results of serological tests were compared in terms of baseline characteristics by χ^2 analysis or the Wilcoxon test, as appropriate. Factors independently associated with CMV positivity at baseline were identified by a multivariable logistic regression model. The following variables were considered a priori as potential cofactors/confounders and were included in the multivariable logistic model, using a 1-step manual adjustment: age, sex, ethnicity (white vs other), hepatitis C virus (HCV) antibody positivity, hepatitis B virus (HBV) surface antigen (HBsAg) positivity, mode of HIV transmission (male-male sex, heterosexual sex, injection drug use, and other/unknown), time from HIV diagnosis, Centers for Disease Control (CDC) disease stage (A/B vs C), use of ART, $CD4^+$ T-cell count, and $CD4^+$/$CD8^+$ T-cell ratio. For the categorical variables, persons with missing data were included in a separate group, categorized as "unknown," to minimize the selection bias.

We then investigated time to AIDS-defining event/AIDS-related death and, in a separate model, time to severe non–AIDS-defining event/non–AIDS-related death. AIDS-defining events were defined according to the 1993 CDC classification [19]. Among severe non–AIDS-defining events we included non–AIDS-defining malignancies, cardiovascular and cerebrovascular events (myocardial infarction, coronary artery bypass graft, coronary angioplasty, carotid endarterectomy, non–myocardial infarction coronary disease, stroke, cerebral hemorrhage, peripheral vascular disease, and pulmonary hypertension), nonvascular neurological diseases (peripheral neuropathies, myelitis, epilepsy, and non–HIV-associated neurocognitive disorders), and end-stage renal disease [20]. Event data collection in the ICONA cohort follows the operative procedure protocol D:A:D MOOP, version 1.4. Non–AIDS-related deaths included all deaths due to the listed severe non–AIDS-defining events. We decided to exclude hepatic failure, liver cancer, and liver-related deaths that are clearly related to HCV/HBV coinfections and for which the impact of CMV could be at best summative more than resulting from a direct action.

Because we used composite end points, the time of the event was defined as the earliest day at which one of the event-defining conditions occurred. Only 1 event per person was counted (the first occurrence). We used a competing-risk approach: the

follow-up of patients who experienced an event other than the one under evaluation as well as any events not of interest, were censored at the end of follow-up. Competing-risk survival analyses using Kaplan–Meier estimates were used to estimate the cumulative probability of developing an AIDS-defining event/AIDS-related death and severe non–AIDS-defining event/non–AIDS-related death by a certain time after the date of the first CMV IgG test. The log–rank test was used to compare the risk of developing the end point, according to the CMV coinfection status. Multivariable Cox proportional hazards survival analysis was used to assess the association between CMV coinfection at baseline and each end point; the same baseline characteristics as for the multivariable logistic regression were considered a priori as possible confounders and were included in the multivariable model, using a 1-step manual adjustment, with the exception of the $CD4^+/CD8^+$ T-cell ratio and with the addition of baseline HIV RNA levels. In an additional model, ART use, HIV RNA levels, and $CD4^+$ T-cell counts were also handled as time-dependent covariates. To test whether smoking habits could possibly influence the risk of severe non–AIDS-defining events/non–AIDS-related deaths, a further analysis was conducted that included the subset of patients for whom this information was available; covariates included were the same as those used in the main analysis. Cox-Snell residuals were used to test the overall fit of the Cox models, and the plots of the integrated hazard based on these residuals against the hazard rates estimated from the model showed a 45-degree slope, suggesting that the models were appropriate.

RESULTS

Cross-sectional Analysis

A total of 10 129 HIV-positive subjects were enrolled in the ICONA Foundation cohort at the time of analysis: 6111 met the specified criteria and were included in the study. Patients excluded from the analyses were comparable to the whole cohort in terms of demographic characteristics (data not shown). Most of the patients underwent CMV serological testing at the time of enrollment in the cohort, whereas 10% were tested after a median time of 17 months (interquartile range [IQR], 6–45 months).

A total of 5119 subjects were positive for CMV IgG, giving a prevalence of 83.3%. The baseline characteristics of the population, according to CMV serological status, are shown in Table 1. CMV-infected patients, compared with uninfected patients, were older (median age, 36 years [IQR, 32–42 years] vs 35 years [IQR, 31–40 years]; $P < .0001$), had a lower prevalence of positivity for HCV antibody (32.7% vs 37.6%; $P = .0028$), had a lower prevalence of advanced HIV disease (AIDS, 10.8% vs 13.3% [$P = .02$]; CD4+ T-cell count, 448 cells/μL

Table 1. Demographic and Clinical Characteristics, According to Cytomegalovirus (CMV) Serostatus at Baseline, Among Human Immunodeficiency Virus (HIV)–Infected Patients

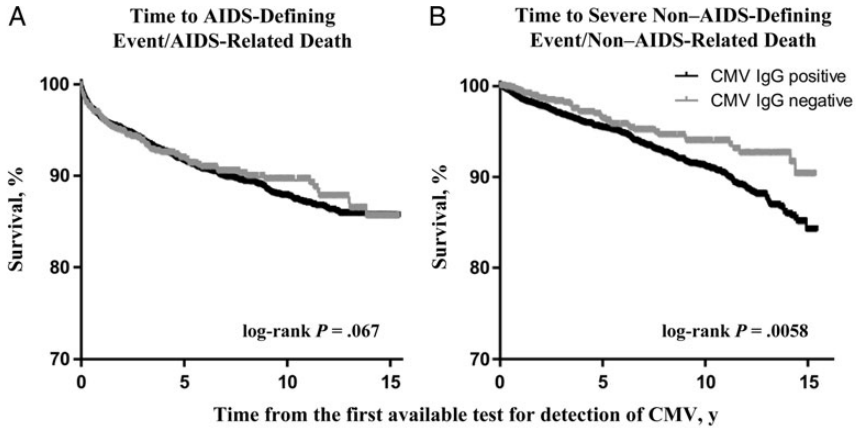
Characteristic	Total Population (n = 6111)	CMV Positive (n = 5119)	CMV Negative (n = 992)	*P* Value[a]
Age, y	36 (32–42)	36 (32–42)	35 (31–40)	<.0001[b]
Female sex	1745 (28.6)	1446 (28.3)	299 (30.1)	.23
Ethnicity				.06
White	5588 (91.4)	4658 (90.9)	930 (93.8)	
Black	285 (4.7)	252 (4.9)	33 (3.3)	
Asian	33 (0.6)	30 (0.6)	3 (0.3)	
Hispanic	145 (2.3)	125 (2.4)	20 (2.0)	
HCV positive	2047 (33.5)	1674 (32.7)	373 (37.6)	.0028
HBsAg positive	330 (5.4)	284 (5.6)	46 (4.7)	.24
Mode of HIV transmission				.0002
Injection drug use	1829 (29.9)	1495 (29.2)	334 (33.7)	
Heterosexual sex	2337 (38.2)	1942 (37.9)	395 (39.8)	
Male-male sex	1564 (25.6)	1363 (26.6)	201 (20.3)	
Time since HIV diagnosis, y	1 (0–7)	1 (0–7)	2 (0–7)	.40[b]
AIDS	683 (11.2)	551 (10.8)	132 (13.3)	.02
Receiving ART	755 (12.4)	637 (12.4)	118 (11.9)	.63
$CD4^+$ T-cell count, cells/μL	443 (270–634)	448 (279–636)	417 (225–620)	.0017[b]
$CD4^+/CD8^+$ T-cell ratio	0.46 (0.27–0.71)	0.46 (0.27–0.70)	0.49 (0.27–0.77)	.010[b]
HIV RNA load, $\log_{10}$ copies/mL	3.74 (2.62–4.60)	3.74 (2.62–4.59)	3.76 (2.65–4.66)	.47[b]

Data are median (interquartile range) or no. (%) of subjects.

Abbreviations: ART, antiretroviral therapy; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus.

[a] By the χ^2 test, unless otherwise indicated.

[b] By the Wilcoxon test for independent variables.



CMV-negative patients, no.	992	501	248	17	992	506	250	18
CMV-positive patients, no.	5119	2413	1116	76	5119	2438	1135	80

Figure 1. Kaplan–Meier survival curve for AIDS-defining event/AIDS-related death and severe non–AIDS-defining event/non–AIDS-related death, by cytomegalovirus (CMV) serostatus. *A,* CMV-infected patients did not show an increased risk of developing AIDS-defining events/AIDS-related death. *B,* CMV-infected patients had an increased risk of developing severe non–AIDS-defining events/non–AIDS-related death. Abbreviation: IgG, immunoglobulin G.

[IQR, 279–636 cells/μL] vs 417 cells/μL [IQR, 225–620 cells/μL; $P = .017$]), but had a lower $CD4^+/CD8^+$ T-cell ratio (0.46 [IQR, 0.27–0.70] vs 0.49 [IQR, 0.27–0.77]; $P = .010$).

In a multivariable logistic analysis, age (per 10-year increase; adjusted odds ratio [OR], 1.42 [95% confidence interval {CI}, 1.33–1.52]; $P < .0001$), male-male sex (vs injection drug use; adjusted OR, 1.67 [95% CI, 1.36–2.06]; $P = .0001$), and a higher $CD4^+$ T-cell count at baseline (per 100 cells/μL increase; adjusted OR, 1.04 [95% CI, 1.02–1.06]; $P = .0005$) were associated with CMV seropositivity, whereas white ethnicity (adjusted OR, 0.51 [95% CI, .39–.66]; $P = .001$) and a higher $CD4^+/CD8^+$ T-cell ratio (adjusted OR, 0.85 [95% CI, .77–.95]) were negatively associated with CMV seropositivity.

Time to AIDS-Defining Event/AIDS-Related Death

During a median follow-up period of 5.2 years (IQR, 1.79–9.66 years), 490 patients reached the end point of an AIDS-defining event/AIDS-related death: 413 experienced an AIDS-defining event, and 77 died of an AIDS-related cause. The most prevalent events were esophageal candidiasis (14.8%), *Pneumocystis jirovecii* pneumonia (10.6%), tuberculosis (13.1%; 6.8% pulmonary and 6.3% extrapulmonary), and Kaposi sarcoma (8.5%). There were not significant differences in the distribution of AIDS-defining events/AIDS-related deaths between CMV-seropositive and CMV-seronegative subjects (data not shown).

The 10-year estimated proportion experiencing an AIDS-defining event/AIDS-related death was 10.9% (95% CI, 8.3%–13.5%) for CMV-negative individuals and 12.4% (95% CI, 11.1%–13.6%) for CMV-positive individuals ($P = .67$, by the log–rank test; Figure 1*A*). CMV seropositivity was not a predictor of AIDS-defining event/AIDS-related death by multivariable Cox analysis. As expected, classical factors such as AIDS at baseline (adjusted hazard ratio [HR], 2.42 [95% CI, 1.90–3.04]; $P < .0001$) and higher HIV RNA load (per 1 $\log_{10}$ copies/mL increase; adjusted HR, 1.23 [95% CI, 1.14–1.33]; $P < .0001$) had a role in predicting AIDS-defining events/AIDS-related deaths, whereas higher $CD4^+$ T-cell counts (per 100 cells/μL increase) at baseline were a protective factor (adjusted HR, 0.80 [95% CI, .76–.84]; $P < .0001$; Figure 2).

Time to Severe Non–AIDS-Defining Event/Non–AIDS-Related Death

During a median follow-up period of 5.6 years (IQR, 2.03–10.06 years), 338 patients reached the end point: 326 experienced severe non–AIDS-defining events, and 12 died of non–AIDS-related causes. All events are listed in Table 2.

The 10-year estimated proportion reaching the end point was 6.2% (95% CI, 4.1%–8.3%) for CMV-negative subjects and 8.9% (95% CI, 7.7%–10.1%) for CMV-positive subjects ($P = .0058$, by the log–rank test; Figure 1*B*). After control for a number of potential confounders by Cox regression analysis, CMV seropositivity remained an independent risk factor for severe non–AIDS-defining events/non–AIDS-related death, with an adjusted HR of 1.53 (95% CI, 1.08–2.16; $P = .016$). Other predictive factors were older age (adjusted HR, 1.65 for each 10-year increase [95% CI, 1.47–1.85]; $P < .0001$), and AIDS at baseline (adjusted HR, 1.49 [95% CI, 1.09–2.03]; $P = .011$; Figure 2). In a different model that considered ART use,

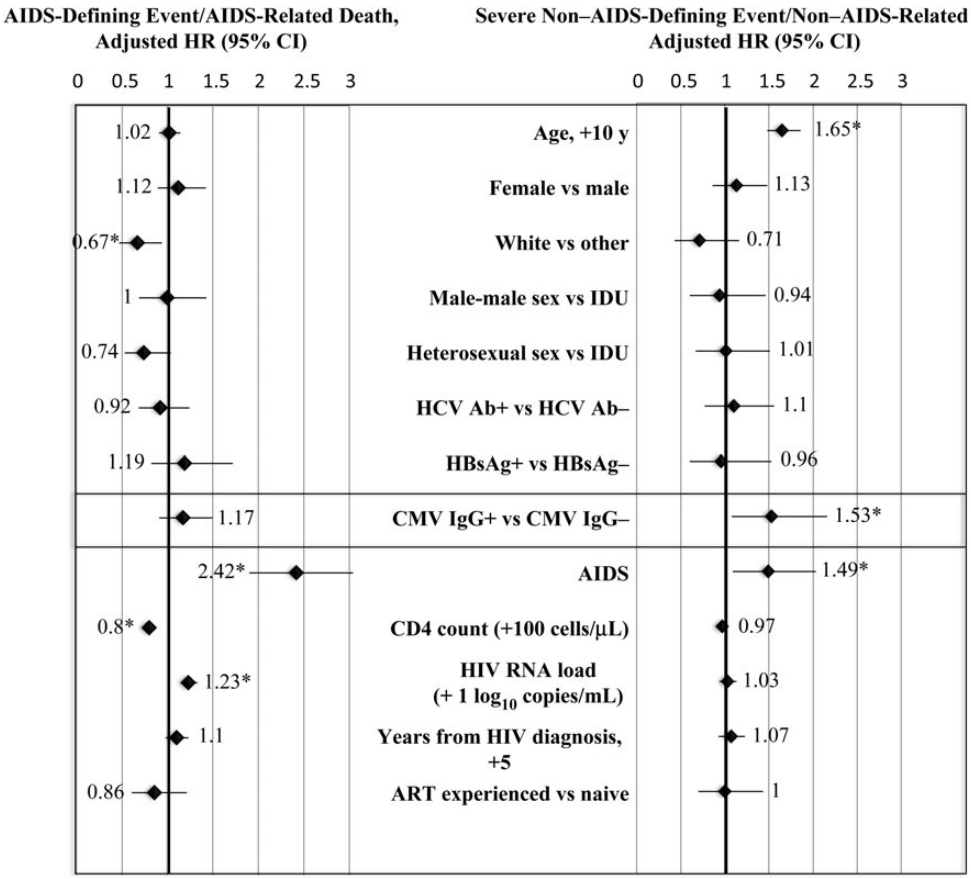


Figure 2. Factor predictive of severe non–AIDS-defining events/non–AIDS-related death and AIDS-defining events/AIDS-related death, by Cox regression analysis. Cytomegalovirus (CMV) seropositivity, AIDS at baseline, and older age were independent risk factors for severe non–AIDS-defining events/non–AIDS-related death (right). Moreover, AIDS, $CD4^+$ T-cell count, and human immunodeficiency virus (HIV) RNA load at baseline were independent risk factors for AIDS-defining events/AIDS-related death (left). Data are expressed as adjusted hazard ratios (HRs) and 95% confidence intervals (CIs). *$P < .01$. Abbreviations: Ab, antibody; ART, antiretroviral therapy; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; IDU, injection drug use; IgG, immunoglobulin G.

HIV RNA level, and $CD4^+$ T-cell count as time-dependent confounders, CMV positivity still remained an independent risk factor for non–AIDS-defining events (adjusted HR, 1.53 [95% CI, 1.08–2.16]; $P = .015$).

We did not detect significant associations between CMV seropositivity and non–AIDS-related malignancies (adjusted HR, 1.98 [95% CI, .73–5.36]; $P = .17$) or nonvascular neurological diseases (adjusted HR, 0.94 [95% CI, .54–1.62; $P = .82$) by multivariable analysis. Conversely, CMV seropositivity was an independent risk factor for cardiovascular and cerebrovascular disease, with an adjusted HR of 2.27 (95% CI, .97–5.32; $P = .058$).

To determine a possible confounding effect of tobacco smoking, we analyzed information about smoking habits at baseline, which was available for 3470 of 6111 patients (56.8%). The proportions of cigarette smokers among CMV-positive and CMV-negative individuals were comparable (51.2% vs 50.1%; $P = .64$). After adjustment for smoking status, CMV coinfection remained associated with a higher hazard of severe non–AIDS-defining events/non–AIDS-related death (adjusted HR, 1.77 [95% CI, .92–3.40]; $P = .08$). The marginally nonsignificant association was probably due to the smaller sample size, as suggested by the increase in the CI. Further, being a cigarette smoker at baseline was independently associated with severe non–AIDS-defining events/non–AIDS-related death (adjusted HR, 1.53 [95% CI, .07–2.21]; $P = .02$); the estimates of the other variables included were comparable to those from the main analysis (data not shown).

Finally, we performed an additional analysis to eliminate a possible effect of other viral comorbid conditions, using the main multivariable model but excluding all HCV antibody–positive and/or HBsAg-positive subjects. A total of 189 events in 3865 patients were observed. Results confirmed that CMV positivity was strongly associated with severe non–AIDS-defining events/non–AIDS-related death (adjusted HR, 2.43 [95% CI, 1.38–2.49]; $P = .0021$).

Table 2. Severe Non–AIDS-Defining Events and Non–AIDS-Related Death During Follow-up

Outcome	No. (%)[a]
Severe non–AIDS-defining event	326 (96.4)
Cardiovascular/cerebrovascular diseases	91 (28.2)
AMI/coronaropathy	35 (38.4)
Ischemic stroke	4 (4.4)
Hemorrhagic stroke	2 (2.2)
Peripheral vasculopathy	16 (17.6)
Dilated cardiomyopathy	3 (3.3)
Heart failure	7 (7.7)
Acute pulmonary edema	1 (1.1)
Pulmonary hypertension	10 (11.0)
Other cardiovascular diseases	13 (14.3)
Cancer	117 (36.2)
Hodgkin lymphoma	22 (18.8)
Lung	13 (11.1)
Head, neck	12 (10.3)
Bladder	10 (8.5)
Colorectal	7 (5.9)
Breast	5 (4.3)
Anal	4 (3.4)
Kidney	3 (2.6)
Melanoma	3 (2.6)
Seminoma	3 (2.6)
Metastasis	3 (2.6)
Prostate	2 (1.7)
Others	30 (25.6)
Neurologic events	115 (35.6)
Peripheral neuropathy	79 (68.7)
Epilepsy	8 (6.9)
Cerebral atrophy	4 (3.5)
Encephalopathy (not ADC)	4 (3.5)
Other neurologic symptoms	9 (7.8)
Other neurological disorders	11 (9.6)
End-stage renal disease	3 (0.9)
Non–AIDS-related death	12 (3.6)
AMI	2 (16.7)
Cardiovascular/cerebrovascular disease	6 (50.0)
Neoplasia	4 (33.3)

Abbreviations: ADC, AIDS dementia complex; AMI, acute myocardial infarction.

[a] The percentage was calculated in comparison with each parent group.

DISCUSSION

The extended access to ART has led to a remarkable improvement in terms of mortality and morbidity, thereby increasing the life expectancy of HIV-infected individuals [21]. Consequently, patterns of morbidity and mortality among HIV-infected subjects receiving ART are changing, with an increase in the proportion of deaths due to non–HIV-related disorders, including cardiovascular disease, liver disease, and non–AIDS-defining cancers [22, 23]. Lower $CD4^+$ T-cell count, anemia, and uncontrolled viral load, along with the classical potentially modifiable risk factors, such as cigarette smoking, diabetes, and hypertension, seem to play a driving role in developing severe non–AIDS-defining events, although available data are partially contrasting [24, 25]. Studies on modifiable and non-modifiable risk factors for non–AIDS-defining events in HIV-infected persons are still an important unmet research issue.

The aim of this study was to determine the seroprevalence of CMV antibodies among HIV-positive subjects enrolled in the ICONA cohort and to define the impact of CMV serostatus on AIDS and severe non–AIDS-defining events/non–AIDS-related death. The seroepidemiology of CMV was examined in 6111 HIV-positive patients (the CMV IgG test is not a mandatory test for HIV-infected people, so it is up to the treating center to decide whether to perform it at enrollment or later), and 83.3% were coinfected with CMV and HIV. The seroprevalence in HIV-infected people has been already described, but very few data are available from cohort studies. A higher rate of CMV infection has been found in HIV-infected patients, compared with the HIV-negative population, with a seroprevalence peaking at 90% in the French Seroco group [26].

In our cohort, the seroprevalence was lower than that in other cohorts, possibly because of differences in HIV transmission routes, age, or nationality [27]. Predictive factors of CMV seropositivity were older age, nonwhite ethnicity, male-male sex as transmission category, higher $CD4^+$ T-cell count at baseline, and lower $CD4^+/CD8^+$ T-cell ratio. Regarding the acquisition age of CMV infection, a gradual increase in risk during life has been demonstrated, with the highest risk of CMV seroconversion being in individuals aged 30–35 years [2].

In the general population, CMV seroprevalence tends to be highest in South America, Africa, and Asia and lowest in Western Europe and the United States. Some of the nonwhite groups had CMV seroprevalences approaching 100% [27–29]. In agreement with these findings, in our cohort of HIV-positive individuals, the seroprevalence was lower in white individuals, compared with black Africans, Asian, and Hispanics, even if the proportion of nonwhite individuals was small in this patient set.

Higher prevalence rates among homosexual men can be attributed to the already described increased risk of exposure associated with receptive anal intercourse [30]. In our cohort, a higher $CD4^+$ T-cell count and a lower $CD4^+/CD8^+$ T-cell ratio were independent predictors for CMV seropositivity, but comparison of the CMV-positive and CMV-negative groups revealed that the median $CD4^+$ T-cell count and $CD4^+/CD8^+$ T-cell ratio were very similar and that the increased risk was minimal.

We could not find an association between CMV infection and the development of AIDS-defining events/AIDS-related death. It is important to note that patients with active CMV disease were excluded. Similar results were reported in cohorts of HIV-infected hemophiliacs [31, 32]. In the pre-ART era, CMV infection was associated with disease progression, especially in

subjects with detectable CMV viremia, but this scenario changed after ART introduction. In fact, the natural history of CMV has changed in terms of viral replication and reactivation owing to the suppression of HIV replication and improved immunity among patients. However, chronic CMV infection could maintain its ability to act as a cofactor for other disorders [33, 34].

In line with this, CMV/HIV-coinfected subjects showed an approximately 50% higher risk of severe non–AIDS-defining events/non AIDS death. Indeed, after control for a number of potential confounders, CMV infection was still an independent risk factor for severe non–AIDS-defining events, suggesting a potential role of CMV infection in the morbidity and mortality of this population. The association was particularly evident for cerebrovascular and cardiovascular events, which is in agreement with several studies reporting an increased risk of all-cause and cardiovascular-related mortality in the CMV-infected HIV-negative population [8–10]. To exclude the effect of other HCV and HBV coinfections, a supplementary analysis was performed only in HCV antibody–negative and/or HBsAg-negative subjects: this confirmed the increased risk of developing severe non–AIDS-defining events/non-AIDS-related death for CMV-infected patients.

To our knowledge, this is the first HIV cohort study in which CMV/HIV-coinfected individuals were compared to HIV-monoinfected individuals in terms of morbidity and mortality, resulting in a higher risk for severe non–AIDS-defining events/non–AIDS-related death in CMV/HIV-coinfected subjects.

When we consider as end-point the different disease categories, CMV seropositivity independently increased the risk of developing cardio-cerebral vascular diseases reaching a AHR of 2.27, even if the *P* value was .058. Multiples lines of evidence suggest a direct or indirect role of CMV infection in the development of cardiovascular disorders. In the HIV-negative population, an association between CMV seropositivity or the CMV IgG antibody level and cardiovascular disease [35, 36] and cardiovascular mortality [37–39] has been reported. In a mouse model, murine CMV infection caused a significant increase in arterial blood pressure in vivo, independent of a high-cholesterol diet, and the virus has been found in atherosclerotic plaques of the mouse aorta [40]. CMV seropositivity has been associated with subsequent cardiac mortality only in patients with an interleukin 6–mediated inflammatory response [41]. In our study, we did not compare patients according to the level of CMV antibodies. Instead, we analyzed a population of patients with or without CMV infection. Whether higher IgG antibody levels represent a marker of more pronounced or more frequent subclinical CMV reactivation from latency remains a matter of debate [38]; moreover, the choice of a quantitative CMV IgG titer cutoff is often arbitrary. In our study, blood pressure, lipids, diet, and soluble markers of inflammation were not considered, but we were able to study the impact of CMV infection on clinical events in HIV-positive with or without CMV infection, according to their serostatus.

In HIV-infected individuals, a higher prevalence of atherosclerosis has been demonstrated, even after control for traditional risk factors [11, 42]. Previous studies have associated such higher rates of atherosclerosis with HIV itself, the use of ART [43], higher levels of T-cell activation [44, 45], or dysfunctional dendritic cell homing [46]. It is now widely accepted that the initiation and progression of atherosclerotic lesions involves a chronic inflammatory response, even if the source of the inflammation is still unclear. Some evidence suggests that CMV may contribute to inflammation in plaques [40]. Long-term successfully treated HIV-infected patients have been shown to present high levels of CMV-specific effector cells, similar to those observed in the elderly population [47], suggesting an important role of the CMV-specific inflammatory response in immunosenescence and non–AIDS-related morbidity and mortality. In fact, in ART recipients, a robust, age-independent anti-CMV T-cell response has been shown to alter T-cell reconstitution, owing to thymic involution or mobilization of resources, inducing a senescent phenotype [47].

Moreover, CMV infection not only induces a large fraction of antigen-specific T cells [48, 49], it is also associated with a higher frequency of subclinical atherosclerosis in HIV-infected women and men [9, 13]. Together, this suggests a potential role for CMV-specific immunity in the atherosclerosis observed in patients infected with HIV. Future studies will be needed to clarify the immunological mechanisms involved in this phenomenon and to better understand the interaction between CMV and HIV in the pathogenesis of severe non–AIDS-defining events.

Finally, to adjust our analyses for the effect of potential confounders linked to cardiovascular and cerebrovascular risk, we analyzed smoking status, which was reported only by 56.8% of patients. The association between CMV seropositivity and severe non–AIDS-defining events/non–AIDS-related death showed the same trend, although it did not reach statistical significance, presumably because of the reduced sample size.

A possible limitation of our study is that CMV testing was done only once for most patients, usually at enrollment, which therefore excludes the influence of new incident CMV infections. However, we can assume from previous observations that the seroconversion rate during follow-up is low, around 1% per 1 year of age, therefore minimizing its influence. Moreover, we assumed that chronic CMV infection would take several years to influence the analyzed clinical outcomes.

In summary, our findings from the ICONA cohort suggest that chronic CMV infection increases the risk of severe non–AIDS-defining events/non–AIDS-related death. Cardiovascular and cerebrovascular events seem to be strictly linked to CMV seropositivity, confirming the potential atherogenic role of CMV in HIV-infected subjects.

In the management of patients with HIV infection, CMV serostatus it is not routinely evaluated, probably because of the

lack of an obvious intervention for CMV-seropositive individuals. Despite this, on the basis of our findings, CMV-seropositive status should be considered a negative prognostic factor, and closer monitoring for cardiovascular and cerebrovascular diseases, as well as management of other modifiable risk factors, could be considered in CMV/HIV-coinfected individuals. The inclusion of this parameter in available prognostic scores [50] should be evaluated.

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Notes

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