Inflammatory cytokines and mortality in a cohort of HIV-infected adults with alcohol problems

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Background: HIV infection leads to chronic inflammation and alterations in levels of inflammatory cytokines. The association between cytokine levels and mortality in HIV infection is not fully understood.

Methods: We analyzed data from a cohort of HIV-infected adults with alcohol problems who were recruited in 2001–2003, and were prospectively followed until 2010 for mortality using the National Death Index. The main independent variables were inflammatory biomarkers [interleukin-6 (IL-6), IL-10, tumor necrosis factor-α, C-reactive protein, serum amyloid A, monocyte chemotactic protein-1, and cystatin-C], measured at baseline in peripheral blood and categorized as high (defined as being in the highest quartile) vs. low. A secondary analysis was conducted using inflammatory burden score, defined as the number of biomarkers in the highest quartile (0, 1, 2 or ≥3). Cox models were used to assess the association between both biomarker levels and inflammatory burden with mortality adjusting for potential confounders.

Results: Four hundred HIV-infected patients were included (74.8% men, mean age 42 years, 50% hepatitis C virus-infected). As of 31 December 2009, 85 patients had died. In individual multivariable analyses for each biomarker, high levels of IL-6 and C-reactive protein were significantly associated with mortality [hazard ratio = 2.49 (1.69–5.12), $P<0.01$] and [hazard ratio = 1.87 (1.11–3.15), $P=0.02$], respectively. There was also a significant association between inflammatory burden score and mortality [hazard ratio = 2.18 (1.29–3.66) for ≥3 vs. 0, $P=0.04$]. In the fully adjusted multivariable analysis, high levels of IL-6 remained independently associated with mortality [hazard ratio = 2.57 (1.58–4.82), $P<0.01$].

Conclusion: High IL-6 levels and inflammatory burden score were associated with mortality in a cohort of HIV-infected adults with alcohol problems.

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Introduction

Individuals with HIV are living longer [1], but the survival benefit is not observed in all subgroups, particularly those who use alcohol or other drugs [2]. Besides well known mortality predictors like $CD4^+$ cell count, HIV RNA, access to and adherence to antiretroviral therapy (ART), comorbidities, and substance abuse [2–4], there is a growing interest in chronic inflammation [5,6]. Increased inflammation is a concern in an aging HIV-infected population, as HIV leads to alterations in inflammatory cytokines and coagulation marker levels despite ART [7].

Increased levels of interleukin-6 (IL-6), a pro-inflammatory cytokine, and fibrinogen, an acute-phase protein, are associated with greater HIV RNA levels [8] and advanced HIV infection [9]. IL-6 is associated with mortality in some cohorts of HIV-infected patients [5,6], but not in all [10]. Others have shown that C-reactive protein (CRP), another acute-phase reactant, and fibrinogen are predictors of mortality, even with $CD4^+$ cell counts more than 500 cells/μl [6]. Cystatin-C levels, a surrogate biomarker of kidney function, have also been associated with mortality [11]. Furthermore, studies in the early ART era found associations between IL-10 and tumor necrosis factor-α (TNF-α) and mortality [12,13]. Chronic inflammation in HIV-infected patients is believed to increase non-AIDS mortality, especially cardiovascular mortality. However, information about the impact of serum amyloid A (SAA) and monocyte chemotactic protein-1 (MCP-1), two biomarkers of atherosclerosis on mortality in HIV infection is scarce [14,15].

The majority of studies of biomarkers of inflammation and mortality in HIV infection have been performed in randomized clinical trials of ART initiation and interruption [5,16], and the association of cytokine levels and mortality in other settings is not well understood. Studies have often focused on individual biomarkers, and have rarely adjusted for biomarkers simultaneously in order to determine independent effects. Both alcohol use and chronic hepatitis C virus (HCV) infection are also associated with increased inflammation in HIV-infected patients that can be partially explained by increased intestinal permeability [17]. There is a need to study the impact of biomarkers of inflammation in HIV-infected patients with alcohol problems and high prevalence of HCV coinfection, and to adjust for these covariates in order to understand whether the effects of inflammation are independent.

In this study, we explored the association between inflammatory biomarker levels [IL-6, IL-10, TNF-α, CRP, SAA, MCP-1, and cystatin-C] and mortality in a cohort of HIV-infected patients with alcohol problems.

Participants and methods

Study design

This was a prospective analysis of patients included in an observational cohort study [HIV-Longitudinal Interrelationships of Viruses and Ethanol (HIV-LIVE)], in which assessments occurred every 6 months over a maximum of 42 months [18].

Participants

Recruitment for the HIV-LIVE cohort occurred from a previous cohort study; an intake clinic for HIV-infected patients; HIV primary care and specialty clinics at two hospitals; homeless shelters; drug treatment programs; subject referrals; and flyers. Enrollment occurred between August 2001 and July 2003, and the last study visit occurred in 2006. Eligibility criteria have been described in detail elsewhere [19]. Briefly, participants had to be HIV-infected adults with at least two affirmative responses to the CAGE alcohol-screening questionnaire [20], or physician investigator diagnosis of alcoholism; and had to speak English or Spanish. Exclusion criteria were cognitive impairment [21,22] and inability to provide informed consent [19]. The Institutional Review Boards of Boston Medical Center and Beth Israel Deaconess Medical Center approved this study.

Measures

Dependent variable

Deaths were ascertained through the National Death Index (NDI) from 2001 to the end of 2009. Methods for establishing a match can be found on the NDI website (http://www.csc.gov/nchs/ndi.htm). The primary dependent variable for the present study was overall mortality.

Independent variables

The main independent variables in this study were inflammatory biomarkers. Seven biomarkers were evaluated as potential predictors of mortality. Each biomarker was dichotomized as high vs. low, wherein high was defined as being in the highest quartile. The cytokines IL-6, IL-10, and TNF-α were chosen because they are related to immune response both in HCV and HIV infection [23,24] and are affected by increased intestinal permeability in heavy alcohol users [24–26]. CRP, cystatin-C, MCP-1, and SAA were chosen because they are been associated with cardiovascular disease, chronic kidney disease, and even mortality, both in HIV-infected patients and the general population [11,14,15,27,28].

TNF-α and IL-6 were measured using Bio-Rad Luminex Flow Cytometry (Millipore) and IL-10 was measured using Chemiluminescent ELISA (R&D Systems).

CRP, cystatin-C, and SAA were measured using a particle enhanced immunonepholometric assay (Dade Behring

Inc.). MCP-1 was measured using the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research Inc.). Laboratory testing was conducted on baseline frozen samples at the University of Vermont's Laboratory for Clinical Biochemistry Research. Further methodological details can be found elsewhere [29].

An additional independent variable of interest was inflammatory burden score, defined as the number of biomarkers (0, 1, 2, ≥3) in the highest quartile [29]. Even though IL-10 has been regarded as antiinflammatory, we included it as it is also elevated in HIV infection and high levels of IL-10 have been associated with mortality [13].

Covariates

Potential confounders controlled for in the analyses were age, gender, race (black vs. nonblack), current heavy alcohol use [30], and current cocaine/heroin use (past 6 months), smoking, $CD4^+$ cell count (categorized as <200 cells/μl and ≥200), HIV RNA load (categorized as ≤500 and >500 copies/ml), current ART, and the presence of HCV infection (defined as HCV antibody-positive result by ELISA confirmed with the presence of HCV RNA by PCR).

Statistical analysis

Descriptive statistics were used to portray the study sample overall and stratified by survival status at the end of the study period. Baseline characteristics were compared across groups using χ^2 or Fisher's exact test for categorical values and *t*-test or Wilcoxon rank-sum test for continuous variables, as appropriate.

Separate Cox proportional hazard models were fit to evaluate the association between each inflammatory cytokine and mortality. A fully adjusted model was then fit that included all cytokines that were significant in the individually adjusted models. All adjusted models controlled for age, gender, race, and HCV infection, as well as the following time-dependent covariates: heavy alcohol use, smoking, cocaine/heroin use, $CD4^+$ cell count, HIV RNA, and ART use.

Time-dependent covariates were recorded up to 2006. Adjusted hazard ratios and 95% confidence intervals (CIs) are reported for each model. Spearman correlations were used to evaluate potential colinearity: no pair of variables included in the same regression model was highly correlated ($r>0.40$). All analyses were conducted using two-sided tests and a significance level of 0.05. Because of the exploratory nature of the analyses, no adjustments were made for multiple comparisons. All statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Between 2001 and 2003, 400 participants were enrolled in the HIV-LIVE cohort and, as of 31 December 2009, 85 participants had died. Median follow-up was 6.7 years and included 2688.5 person-years; mortality rate was 3.16 deaths/100 person-years (95% CI 2.56–3.91).

Table 1 shows the baseline characteristics of the study participants. The median (interquartile range) levels of inflammatory biomarkers for the entire sample were as follows: IL-6 2.75 (1.52–4.81) pg/ml, IL-10 5.03 (3.08–8.20) pg/ml, TNF-α 7.15 (4.85–9.70) pg/ml, CRP 1.48 (0.60–3.47) μg/ml, cystatin-C 0.77 (0.67–0.91) ng/ml, MCP-1 577 (397–815) pg/ml, and SAA 3.2 (1.8–6.4) μg/ml.

Examining each biomarker separately, and adjusting for age, gender, race, heavy alcohol and cocaine/heroin use, smoking, HCV infection, ART, $CD4^+$ cell count, and HIV RNA, we found that IL-6 [hazard ratio 2.94 (1.69–5.12) $P<0.01$] and CRP [hazard ratio 1.87 (1.11–3.15), $P=0.02$] were associated with mortality (Table 2). Inflammatory burden at least 3 was also associated with mortality [hazard ratio 2.18 (1.29–3.66), $P<0.01$].

In the fully adjusted model (not shown in Table) which simultaneously included IL-6, CRP, and all other covariates, IL-6 levels were independently associated with mortality [hazard ratio (95% CI) 2.57 (1.58–4.82), $P<0.01$]. The association with CRP was attenuated and no longer significant [1.59 (0.93–2.72), $P=0.09$]. Among covariates, age, cocaine/heroin use, $CD4^+$ cell count less than 200, and HCV infection were associated with mortality [hazard ratio (95% CI) 1.04 (1.01–1.08) $P=0.01$, 1.91 (1.18–3.11) $P<0.01$, 3.13 (1.97–4.96) $P<0.01$, and 1.93 (1.12–3.32) $P=0.02$, respectively] [31]. AQ5

Discussion

In this exploratory study, high IL-6 levels were independently associated with mortality in HIV-infected adults with alcohol problems, even after adjustment for ART use, $CD4^+$ cell count, HIV RNA, alcohol, drug use, and HCV. In addition, having an inflammatory burden at least 3 was also associated with mortality.

These results are consistent with previous literature [5,32], but this study adds that IL-6 is the more prominent cytokine in independently predicting mortality. IL-6 levels are increased in those with elevated HIV RNA and in those who interrupt ART [8]. Given that IL-6 levels remain elevated after the introduction of ART [16], it seems that ART is not able to fully overcome the chronic inflammatory state present in HIV-infected patients.

Table 1. Baseline characteristics of HIV-LIVE study participants (HIV-infected adults with current or past alcohol problems).

	Overall ($n=400$)	Deceased ($n=85$)	Alive ($n=315$)	P value
Age [mean (SD)]	43 (7.4)	44 (7.9)	42 (7.2)	0.01
Female sex [*n* (%)]	101 (25.3)	23 (27.1)	78 (24.8)	0.67
Black ethnicity [*n* (%)]	166 (41.5)	36 (42.4)	130 (41.3)	0.86
$CD4^+$ cell count <200 cells/μl [*n* (%)]	75 (19.8)	31 (37.8)	44 (14.9)	<0.01
HIV RNA >500 copies/ ml [*n* (%)]	194 (53.6)	44 (57.1)	150 (52.6)	0.48
Antiretroviral therapy [*n* (%)]	248 (62)	52 (61.2)	196 (62.2)	0.86
HCV infection [*n* (%)]	200 (50.4)	60 (72.3)	140 (44.6)	<0.01
Heavy alcohol use[a] [*n* (%)]	125 (31.3)	25 (29.4)	100 (31.8)	0.67
Smoking habit [*n* (%)]	306 (76.5)	68 (81.2)	237 (75.2)	0.25
Cocaine/heroin use [*n* (%)]	199 (49.8)	43 (50.6)	156 (49.5)	0.86
IL-6 highest quartile [*n* (%)]	77 (24.9)	29 (47.6)	48 (19.4)	<0.01
IL-10 highest quartile [*n* (%)]	86 (25.1)	21 (34.8)	65 (23.7)	0.21
TNF-α highest quartile [*n* (%)]	86 (25.1)	24 (28.2)	62 (22.6)	0.05
C-reactive protein highest quartile [*n* (%)]	86 (25.1)	24 (28.2)	62 (22.6)	0.05
Serum amyloid A highest quartile [*n* (%)]	86 (26.4)	21 (32.3)	65 (24.4)	0.20
Monocyte chemotactic protein-1 highest quartile [*n* (%)]	86 (25.1)	19 (27.5)	67 (24.5)	0.80
Cystatin-C highest quartile [*n* (%)]	86 (25.1)	28 (32.9)	58 (18.4)	<0.01
Inflammatory burden[b]				<0.01
0 [*n* (%)]	139 (34.8)	25 (29.4)	114 (36.2)	
1 [*n* (%)]	93 (23.3)	12 (14.1)	81 (25.7)	
2 [*n* (%)]	71 (71.8)	15 (17.6)	56 (17.8)	
≥3 [*n* (%)]	97 (24.3)	33 (38.8)	64 (20.3)	

HCV, hepatitis C virus; IL, interleukin; TNF, tumor necrosis factor.
[a]NIAAA definition.
[b]Number of cytokines >75th percentile.

CRP was associated with mortality in the individually adjusted models, which is also consistent with prior research [6]. However, our study adds to the literature by finding that after adjustment for IL-6, CRP was not significantly associated with mortality. The finding that higher inflammatory burden (having ≥3 cytokines in the highest quartile) is associated with mortality is also consistent with prior research [32]. Previously in this cohort, investigators found that inflammatory burden was independently associated with HIV and HCV replication [29]; this study contributes further to demonstrate that inflammatory burden predicts mortality.

We also found that, in the unadjusted analysis, cystatin-C, IL-10, and TNF-α were associated with mortality, consistent with prior research [11–13]. However, in the adjusted analysis, these associations were no longer significant. Confounding may account for these results, but it is also possible that we might be underpowered to detect associations between mortality and each biomarker.

Consistent with prior studies, we observed significant associations between age, $CD4^+$ cell count, HCV, cocaine/heroin use and mortality [33–35]. Interestingly,

Table 2. Adjusted analysis of the association between cytokine levels and overall mortality in HIV-infected adults with current or past alcohol problems.

	Hazard ratio (95% confidence interval)[b]	P value
IL-6 high vs. low	2.94 (1.69–5.12)	<0.01
IL-10 high vs. low	1.30 (0.75–2.25)	0.35
TNF-α high vs. low	1.54 (0.89–2.66)	0.13
C-reactive protein high vs. low	1.87 (1.11–3.15)	0.02
Serum amyloid A high vs. low	1.17 (0.67–2.06)	0.58
Monocyte chemotactic protein-1 high vs. low	0.93 (0.511–1.68)	0.80
Cystatin C high vs. low	1.59 (0.93–2.72)	0.09
Inflammatory burden[a]		
0	1	
1	0.73 (0.37–1.45)	0.37
2	1.22 (0.64–2.32)	0.53
≥3	2.18 (1.29–3.66)	<0.01

HCV, hepatitis C virus; IL, interleukin; TNF, tumor necrosis factor.
[a]Number of cytokines >75th percentile.
[b]Adjusted by age, gender, race ethnicity, heavy alcohol use, HCV infection, smoking, cocaine/heroin use, HIV viral load, $CD4^+$ cell count, and antiretroviral therapy. Each row represents a different model.

among covariates, current heavy alcohol use and smoking were not associated with mortality, possibly because of competing risks.

Strengths of the present study are a moderate sample size, extended follow-up period, a large panel of biomarkers, and the use of inflammatory burden score. Also, participants in the HIV-LIVE cohort represent a population at risk for chronic inflammation, with frequent alcohol or other drug use and HCV coinfection, and we accounted for these cofactors in the analysis.

This study has several limitations. First, we measured biomarker levels in serum at baseline; it is possible that serum levels do not reflect the levels in other body compartments. Also, changes in biomarkers or biomarkers closer to time of death might have a greater impact on mortality [5]. Second, the modest number of outcomes might have limited our ability to detect associations between other biomarkers besides IL-6 and all-cause mortality. In addition, the relatively small numbers of outcomes did not allow us to examine relationships between biomarkers and cause-specific types of deaths (such as death from cardiovascular disease); however, the majority of deaths were related to HIV or HCV. Third, time-dependent covariates were measured up to 2006, whereas mortality data could be extracted until 2010.

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Conclusion

In summary, high levels of IL-6 are associated with mortality in a cohort of HIV-infected persons with alcohol problems and high burden of HCV coinfection. This study supports the hypothesis that chronic inflammation, and specifically elevation in levels of cytokine IL-6, may lead to an increased risk of death in patients with HIV.

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Conflicts of interest

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