Plasma levels of cytokines and chemokines and the risk of mortality in HIV-infected individuals: a case–control analysis nested in a large clinical trial

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Background: All-cause mortality and serious non-AIDS events (SNAEs) in individuals with HIV-1 infection receiving antiretroviral therapy are associated with increased production of interleukin-6 which appears to be driven by monocyte/macrophage activation. Plasma levels of other cytokines or chemokines associated with immune activation might also be biomarkers of an increased risk of mortality and/or SNAEs.

Methods: Baseline plasma samples from 142 participants enrolled into the Strategies for Management of Antiretroviral Therapy study, who subsequently died, and 284 matched controls, were assayed for levels of 15 cytokines and chemokines. Cytokine and chemokine levels were analysed individually and when grouped according to function (innate/proinflammatory response, cell trafficking and cell activation/proliferation) for their association with the risk of subsequent death.

Results: Higher plasma levels of proinflammatory cytokines (interleukin-6 and tumour necrosis factor-α) were associated with an increased risk of all-cause mortality but in analyses adjusted for potential confounders, only the association with interleukin-6 persisted. Increased plasma levels of the chemokine CXCL8 were also associated with all-cause mortality independently of hepatitis C virus status but not when analyses were adjusted for all confounders. In contrast, higher plasma levels of cytokines mediating cell activation/proliferation were not associated with a higher mortality risk and exhibited a weak protective effect when analysed as a group.

Conclusion: Whereas plasma levels of interleukin-6 are the most informative biomarker of cytokine dysregulation associated with all-cause mortality in individuals with HIV-1 infection, assessment of plasma levels of CXCL8 might provide information about causes of mortality and possibly SNAEs.

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Introduction

HIV-1 infection results in immune activation through several mechanisms. This contributes to $CD4^+$ T-cell depletion and to activation of inflammatory and coagulation pathways which contributes to the pathogenesis of serious non-AIDS events, including atherosclerotic vascular disease, osteoporosis, osteonecrosis and chronic kidney disease [1,2]. Although $CD8^+$ T-cell activation is an established marker of immune activation in HIV patients [3], plasma markers of monocyte/macrophage activation, including interleukin-6 levels, are better predictors of serious non-AIDS events [4,5]. Monocyte activation is associated with atherosclerotic vascular disease, neurocognitive decline and increased mortality [6–9], in part by activation of coagulation pathways [10,11].

Patients with HIV-1 infection receiving antiretroviral therapy (ART) who cease therapy exhibit plasma cytokine and chemokine changes that provide a signature of monocyte/macrophage activation, particularly increased production of interleukin-6 and tumour necrosis factor-alpha (TNF-α) [12]. It is well established that high plasma interleukin-6 levels are associated with morbidity and mortality in HIV patients [13–15] but unclear if increased production of other cytokines or chemokines is associated with an increased risk of death. We have, therefore, examined baseline plasma levels of cytokines and chemokines in Strategies for Management of Antiretroviral Therapy (SMART) study participants who died during study follow-up and compared them with baseline levels in matched controls.

Methods

Study participants

Baseline plasma samples were obtained from SMART study [16] participants who died during study follow-up, and from two matched controls for each case. Matching was on country, age (± 5 years), sex and approximate date of randomization (± 3 months). Age was chosen as a matching variable because of its known association with mortality; sex was chosen for consistency with other case–control studies undertaken on SMART study participants, for example, with cardiovascular disease (CVD) cases, in which sex is an established risk factor; country (site within country where possible) was chosen to control for possible differences in treatment patterns, demographics, and other factors that could vary by site or location; and date of randomization was chosen to ensure that latest levels for cases and controls were measured at approximately the same time following randomization. Controlling for other potential confounding factors was by regression adjustment. Unwitnessed deaths not attributed to violence or accidents were considered to be due to CVD.

Assay of plasma cytokines and chemokines

Plasma levels of cytokines and chemokines, with the exception of interleukin-6, were assayed by multiplex bead array assay as described elsewhere [12]. Plasma interleukin-6 levels were assayed by ELISA because we have demonstrated a poor correlation of results for interleukin-6 assayed by ELISA and multiplex bead array assay [12]. We assayed 15 chemokines or cytokines that were grouped by three functional categories: innate/proinflammatory response, cell trafficking and cell activation/proliferation.

Statistical analysis

The association of plasma levels of each analyte with the risk of death was assessed in separate analyses: a comparison of median analyte levels in cases and controls; calculation of the odds ratios (ORs) of death associated with higher plasma levels of each analyte (fitted in the $\log_{10}$ scale); and calculation of the ORs of death comparing various strata defined according to each analyte distribution quartile. For some analytes, the measured value was below the cut-off of detection of the assay (censored values). In the analysis using the markers in the $\log_{10}$ scale, we used the naive approach of replacing the observed value with the censored value. Alternatively, in a sensitivity analysis, we used the censored value divided by 2 and results were similar (data not shown). For the analysis using a categorical variable stratified according to the analyte quartiles, participants with censored values were classified in a separate group and quartiles calculated using only participants with an observed value.

Conditional logistic regression analysis for matched case–control studies was used to summarize the association of each analyte with disease progression outcomes. Unadjusted and adjusted ORs using the lowest quartile as the comparator group are cited along with 95% confidence intervals (CIs) and P values. Sequential manual adjustment for confounding was performed starting with the adjustment for matching factors alone, followed by adjustment for matching factors and hepatitis C virus (HCV) infection status, and finally for matching factors and all other confounders (see footnote of Table 2 for a list of specific factors included in the multivariable model). A separate logistic model was performed for each of the analytes.

Two approaches were taken to minimize the risk of identifying false-positive associations between analyte levels and the risk of death by highlighting subsets of individual analytes: we adopted a Bonferroni-corrected P value of $0.05/15 = 0.003$ to establish significance; we took advantage of the functional groupings that were identified to apply to a global test procedure proposed by

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O'Brien [17] for multiple endpoints. With this approach, each analyte in the raw scale within a functional grouping is ranked from lowest to highest, the ranks of the individual analytes are summed for each patient. We refer to the sum of the ranks as the 'biomarker score'. This biomarker score is then compared for patients who died versus those who did not with the conditional logistic regression models as described above (i.e. ORs for upper versus lowest quartile of the biomarker score are cited). Advantages of this procedure are simplicity and increased power if the biomarkers within a category all trend in the same direction. A disadvantage is that although the global test identifies biomarker groupings that are significant, it does not provide information on which markers are driving the statistical significance. Statistical analyses were performed using Statistical Analysis System Version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Analysis of demographic characteristics of study participants (Supplementary Table 1, http://links.lww.com/QAD/A658) revealed that rates of HCV coinfection, cigarette smoking, diabetes and previous CVD were higher in participants who subsequently died.

Plasma levels of cytokines and chemokines grouped according to functional characteristics (innate/proinflammatory, cell trafficking, cell activation/proliferation) at study entry were compared in participants who died during follow-up ($n = 142$) and in matched controls ($n = 284$) (Table 1). Plasma levels of interleukin-6, CXCL8 (also known as interleukin-8) and TNF-α were higher in participants who subsequently died compared with controls ($P < 0.001$, 0.008 and 0.009, respectively). There was also a trend towards higher plasma levels of CXCL10 [also known as interferon inducible protein-10 (IP-10)] ($P = 0.068$). None of the cytokines in the cell activation/proliferation group were associated with the risk of subsequent death.

The association between baseline plasma levels of cytokines and chemokines and death was also analysed by univariable logistic regression using a $1\log_{10}$ higher plasma level of each analyte as a continuous variable, or quartiles of the analyte levels as a categorical variable, both with adjustment for HCV infection status and other potential confounders. In the analysis of continuous variables (Table 2), interleukin-6 and CXCL8 remained associated with the risk of death after adjustment for HCV infection status, whereas the association with TNF-α was not statistically significant [OR = 2.22 (1.56, 3.15), $P < 0.001$; OR = 1.34 (1.02, 1.75), $P = 0.033$; OR = 1.41 (0.99, 2.02), $P = 0.057$, respectively]. After adjustment for all potential confounders, these associations persisted but only that for interleukin-6 was statistically significant [OR = 2.15 (1.47, 3.16), $P < 0.001$; OR = 1.21 (0.90, 1.63), $P = 0.215$; OR = 1.27 (0.85, 1.90), $P = 0.251$, respectively] at the Bonferroni-corrected level.

In the analysis of categorical variables (Supplementary Table 2, http://links.lww.com/QAD/A659), plasma levels of interleukin-6, CXCL8 or TNF-α in the 75th centile remained associated with death after adjustment for HCV infection status, although the results for CXCL8 were not statistically significant [OR = 3.9 (1.95, 7.80), $P < 0.001$; OR = 1.68 (0.93, 3.06), $P = 0.087$; OR = 1.97 (1.07, 3.62), $P = 0.030$, respectively]. After adjustment for all confounders, these associations persisted but only that for interleukin-6 was statistically significant [OR = 3.4 (1.56, 7.42),

Table 1. Median values of analytes in cases and controls at study entry.

Analyte, median (range)	Deaths ($n = 142$)	Controls ($n = 284$)	P value[a]
Innate/proinflammatory response			
Interleukin-6 (pg/ml)	3.64 (0.39 104.8)	2.26 (0.41,12.05)	<0.001
TNF-α (pg/ml)	12.68 (1.99,147.8)	11.80 (1.89,64.48)	0.009
Interleukin-1-β (pg/ml)	0.61 (0.02,15.02)	0.63 (0.02,24.40)	0.352
Cell trafficking			
CXCL8 (pg/ml)	6.84 (0.93,68.71)	5.25 (0.40,355.4)	0.008
CXCL10 (pg/ml)	348.4 (50.26,5903)	288.0 (21.63,8171)	0.068
Cell activation/proliferation			
Interleukin-2 (pg/ml)	2.81 (0.07,90.16)	3.32 (0.06,104.1)	0.848
Interleukin-7 (pg/ml)	9.86 (0.17,144.3)	11.11 (0.17,111.9)	0.649
Interleukin-17 (pg/ml)	2.98 (0.07,115.2)	3.03 (0.07,200.2)	0.448
IFN-γ (pg/ml)	4.82 (0.06,89.19)	5.39 (0.07,195.1)	0.614
GMCSF, (pg/ml)	0.86 (0.03,76.52)	0.83 (0.05,112.7)	0.150
Interleukin-4 (pg/ml)	21.67 (0.01,1260)	26.04 (0.00,2354)	0.184
Interleukin-5 (pg/ml)	0.55 (0.01,9.87)	0.55 (0.01,26.23)	0.695
Interleukin-13 (pg/ml)	1.40 (0.01,624.0)	3.92 (0.01,1073)	0.383
Interleukin-10 (pg/ml)	25.38 (0.18,1215)	25.00 (0.18,414.3)	0.359
Interleukin-12p70 (pg/ml)	2.01 (0.04,732.9)	2.51 (0.01,308.1)	0.802

IFN, interferon; GMCSF, granulocyte-macrophage colony-stimulating factor; TNF, tumour necrosis factor.
[a]From fitting a conditional logistic regression (markers in log scale). Only patients with observed values included.

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Table 2. Odds ratios of death associated with a one log_{10}pg/ml higher plasma level of each analyte.

	Adjusted[b] OR (95% CI)	*P* value[a]	Adjusted[c] OR (95% CI)	*P* value[a]	Adjusted[d] OR (95% CI)	*P* value[a]
Analyte						
Innate/proinflammatory response						
Interleukin-6 (ELISA) (pg/ml)	2.44 (1.74, 3.44)	<0.001	2.22 (1.56, 3.15)	<0.001	2.15 (1.47, 3.15)	<0.001
TNF-α (pg/ml)	1.60 (1.12, 2.26)	0.009	1.41 (0.99, 2.02)	0.057	1.24 (0.83, 1.87)	0.293
Interleukin-1-β (pg/ml)	0.98 (0.84, 1.14)	0.766	0.98 (0.84, 1.14)	0.661	0.98 (0.84, 1.14)	0.498
Cell trafficking						
CXCL8 (pg/ml)	1.43 (1.10, 1.86)	0.008	1.34 (1.02, 1.75)	0.033	1.20 (0.89, 1.62)	0.220
CXCL10 (pg/ml)	1.26 (0.98, 1.61)	0.068	1.06 (0.81, 1.39)	0.649	1.06 (0.76, 1.46)	0.739
Cell activation/proliferation						
Interleukin-2 (pg/ml)	0.99 (0.86, 1.13)	0.845	0.99 (0.86, 1.14)	0.855	0.98 (0.83, 1.15)	0.784
Interleukin-7 (pg/ml)	0.89 (0.70, 1.12)	0.320	0.94 (0.74, 1.19)	0.580	0.95 (0.73, 1.24)	0.705
Interleukin-17 (pg/ml)	0.86 (0.74, 1.01)	0.062	0.90 (0.77, 1.06)	0.201	0.89 (0.74, 1.06)	0.195
IFN-γ (pg/ml)	0.89 (0.79, 1.01)	0.065	0.90 (0.79, 1.02)	0.096	0.89 (0.77, 1.02)	0.097
GMCSF (pg/ml)	0.96 (0.86, 1.07)	0.473	0.96 (0.85, 1.07)	0.427	0.94 (0.82, 1.06)	0.313
Interleukin-4, (pg/ml)	0.95 (0.88, 1.03)	0.256	0.97 (0.89, 1.06)	0.487	0.98 (0.89, 1.07)	0.604
Interleukin-5 (pg/ml)	0.92 (0.81, 1.05)	0.235	0.92 (0.80, 1.06)	0.249	0.90 (0.77, 1.06)	0.208
Interleukin-13, pg/ml	0.97 (0.91, 1.03)	0.378	0.97 (0.91, 1.04)	0.749	0.97 (0.90, 1.04)	0.886
Interleukin-10 (pg/ml)	1.10 (0.89, 1.35)	0.346	1.04 (0.84, 1.28)	0.409	1.02 (0.80, 1.29)	0.375
Interleukin-12p70 (pg/ml)	0.97 (0.88, 1.08)	0.606	1.00 (0.89, 1.11)	0.937	0.98 (0.87, 1.10)	0.724

ART, antiretroviral therapy; CI, confidence interval; GMCSF, granulocyte-macrophage colony-stimulating factor; HCV, hepatitis C virus; IFN, interferon; OR, odds ratio; SMART, Strategies for Management of Antiretroviral Therapy.
[a]From fitting a conditional logistic regression (markers in log_{10} scale).
[b]Adjusted for matching factors.
[c]Adjusted for HCV serostatus.
[d]Adjusted for all other potential confounders – race (Black versus non-Black), sex, current smoking, diabetes, use of lipid-lowering drugs, previous evidence of cardiovascular disease, SMART randomization arm, ART/viral load strata (off ART, on ART with viral load ≤400 copies/ml and on ART with viral load >400 copies/ml) and baseline $CD4^+$ T-cell count. Only patients with observed values included.

$P = 0.002$; 1.41 (0.74, 2.70), $P = 0.294$; 1.85 (0.92, 3.70), $P = 0.082$, respectively].

We also undertook an analysis of the association between plasma levels of cytokines and chemokines grouped according to function (innate/proinflammatory, cell trafficking, cell activation/proliferation) and death using the method of O'Brien [17] (Supplementary Table 3, http://links.lww.com/QAD/A660). After adjustment for HCV infection status, the cell-trafficking group of biomarkers was associated with the risk of death [OR = 1.86 (1.08, 3.20), $P = 0.026$] and there was also a small trend for an association with the innate/proinflammatory group of biomarkers [OR = 1.66 (0.94, 2.94), $P = 0.083$]. These associations persisted after adjustment for all potential confounders but they were not statistically significant [OR = 1.56 (0.84, 2.92), $P = 0.161$ and OR = 1.41 (0.73, 2.75), $P = 0.308$, respectively]. In contrast, plasma levels of the activation/cell proliferation group of cytokines were associated with a protective effect for subsequent death after adjustment for all potential confounders, although the association was not statistically significant [OR = 0.62 (0.34, 1.14), $P = 0.126$].

Finally, to further assess the association of cytokines and chemokines other than interleukin-6 with mortality, we undertook an analysis of the association of interleukin-6 with mortality adjusted for the plasma level of each analyte individually or combined in functional groups. The adjusted OR for the risk of death decreased to the greatest extent for CXCL8 alone [from 2.44 (1.74, 3.44) to 2.32 (1.63, 3.29)] and the cell-trafficking markers [from 2.44 (1.74, 3.44) to 2.30 (1.61, 3.29)] (Supplementary Table 4, http://links.lww.com/QAD/A661).

Discussion

Our analysis of baseline plasma cytokine and chemokine levels in individuals with HIV-1 infection enrolled into the SMART study has demonstrated that none of the cytokines and chemokines examined was associated with an increased risk of all-cause mortality to the same degree as interleukin-6 [13,14]. Indeed, higher plasma levels of cytokines that induce cell activation and proliferation showed a trend towards a protective effect on all-cause mortality. However, we have provided preliminary evidence that increased production of CXCL8 might contribute to an increased mortality risk. This evidence included an analysis of analytes by functional grouping which demonstrated that the association with mortality was at least as strong for the cell-trafficking group (CXCL8 and CXCL10) as it was for innate/proinflammatory group (interleukin-6, TNF-α and interleukin-1-β), although this may reflect the dilution of the strong association with interleukin-6 by the weaker associations with TNF-α and interleukin-1-β. In addition, when the effect of other cytokines and chemokines on the association of interleukin-6 with mortality was examined, CXCL8 demonstrated the greatest effect. Our findings, therefore, raise the possibility that increased

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production of CXCL8 contributes to an increased mortality risk in individuals with HIV-1 infection.

Chemokines, including CXCL8, CCL2 [also known as monocyte chemotactic protein-1 (MCP-1)] and CXCL1 [also known as growth related oncogene-α (GRO-α)], play important roles in the migration and adhesion of monocytes to atherosclerotic plaques in vascular endothelium [18,19]. Furthermore, production of CXCL8 and other chemokines is decreased by statin therapy [20–23]. We therefore suggest that chemokines associated with the pathogenesis of atherosclerosis, such as CXCL8, are candidate biomarkers of atherosclerotic vascular disease in patients with HIV-1 infection. This should be addressed in future studies.

Conclusion

In summary, while plasma interleukin-6 levels are the most informative biomarker of cytokine dysregulation associated with an increased risk of all-cause mortality in patients with HIV-1 infection, analysis of plasma CXCL8 levels might provide information about causes of mortality. There was no evidence of an association between mortality and increased plasma levels of cytokines that mediate cell activation/proliferation.

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Conflicts of interest

There are no conflicts of interest.

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