HHS Public Access

Author manuscript

AIDS. Author manuscript; available in PMC 2016 April 24.

Published in final edited form as:

AIDS. 2015 April 24; 29(7): 847–851. doi:10.1097/QAD.0000000000000618.

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

MA French[1,2], **A Cozzi-Lepri**[3], **RC Arduino**[4], **M Johnson**[5], **AC Achhra**[6], **A Landay**[7], and **the INSIGHT SMART Study Group**

[1] School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Australia.

[2] Department of Clinical Immunology, Royal Perth Hospital and PathWest Laboratory Medicine, Perth, Australia.

[3] Research Department of Infection and Population Health, University College London, London, UK.

[4] Houston AIDS Research Team, Division of Infectious Diseases, The University of Texas Health Science Center at Houston, Houston, USA.

[5] HIV Clinic and Department of Respiratory Medicine, Royal Free Hospital, London, UK.

[6] Kirby Institute, University of New South Wales, Sydney, Australia.

[7] Department of Immunology and Microbiology, Rush University Medical Center, Chicago, USA.

Abstract

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 (IL)-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 SMART study who subsequently died, and 284 matched controls, were assayedfor levels of 15 cytokines and chemokines. Cytokine and chemokine levels were analysed individually and when grouped according to function (innate/pro-inflammatory response, cell trafficking and cell activation/proliferation) for their association with the risk of subsequent death.

Results—Higher plasma levels of pro-inflammatory cytokines (IL-6 and tumour necrosis factor-alpha) were associated with an increased risk of all-cause mortality but in analyses adjusted for potential confounders, only the association with IL-6 persisted. Increased plasma levels of the chemokine CXCL8 were also associated with all-cause mortality independently of HCV status but not when analyses were adjusted for all confounders. In contrast, higher plasma levels of

Contact details: Professor Martyn French Translational Immunology Unit School of Pathology and Laboratory Medicine Level 2, MRF Building Rear, 50 Murray Street erth, WA 6000 Australia martyn.french@uwa.edu.au.

cytokines mediating cell activation/proliferation were not associated with a higher mortality risk and exhibited a weak protective effect when analysed as a group.

Conclusions—While plasma levels of IL-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.

Keywords

HIV; mortality; cytokines; chemokines; IL-6; CXCL8

Introduction

Human immunodeficiency virus-1 (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 (SNAEs), 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 (IL)-6 levels, are better predictors of SNAEs [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 cytokines and chemokines changes that provide a signature of monocyte/macrophage activation, particularly increased production of IL-6 and tumour necrosis factor (TNF)-alpha [12]. It is well-established that high plasma IL-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 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, e.g., with 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 IL-6, were assayed by multiplex bead array assay (MBA) as described elsewhere [12]. Plasma IL-6 levels were assayed by ELISA because we have demonstrated a poor correlation of results for IL-6 assayed by ELISA and MBA [12]. We assayed 15 chemokines or cytokines, which were grouped by three functional categories: 1) Innate/pro-inflammatory response; 2) Cell trafficking; and 3) Cell activation/proliferation.

Statistical analysis

The association of plasma levels of each of analyte with the risk of death was assessed in separate analyses: 1) a comparison of median analyte levels in cases and controls; 2) calculation of the odds ratios of death associated with higher plasma levels of each analyte (fitted in the $\log_{10}$ scale); and 3) calculation of the odds ratios of death comparing various strata defined according to each analyte distribution quartile. For some analytes, the measured value was below the cutoff of detection of the assay (censored values). In the analysis using the markers in the $\log_{10}$ scale, we used the naïve 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 odds ratios (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 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: 1) we adopted a Bonferroni corrected p-value of 0.05/15 = 0.003 to establish significance; 2) we took advantage of the functional groupings that were identified to apply to a global test procedure proposed by O'Brien for multiple endpoints [17]. 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 while 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 SAS (Version 9.3).

Results

Analysis of demographic characteristics of study participants (Supplementary Table 1) revealed that rates of HCV co-infection, 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/pro-inflammatory, 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 IL-6, CXCL8 (also known as IL-8) and TNF-alpha 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), IL-6 and CXCL8 remained associated with the risk of death after adjustment for HCV infection status, while the association with TNF-alpha 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 IL-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), plasma levels of IL-6, CXCL8 or TNF-alpha in the 75^{th} centile remained associated with death after adjustment for HCV infection status, though 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 IL-6 was statistically significant (OR=3.4[1.56, 7.42], 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/pro-inflammatory, cell trafficking, cell activation/proliferation) and death using the method of O'Brien et al [17] (Supplementary Table 3). 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/pro-inflammatory 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, though 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 IL-6 with mortality, we undertook an analysis of the association of IL-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).

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 IL-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/pro-inflammatory group (IL-6, TNF-alpha and IL-1-beta), though this may reflect the dilution of the strong association with IL-6 by the weaker associations with TNF-alpha and IL-1-beta. In addition, when the effect of other cytokines and chemokines on the association of IL-6 with mortality was examined, CXCL8 demonstrated the greatest effect. Our findings therefore raise the possibility that increased 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.

In summary, while plasma IL-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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors are grateful for the contribution of all SMART study participants. For the complete list of SMART study investigators, please see *N Engl J Med* 2006; 355:2283-96. The SMART study was funded by NIH Grants UM1-AI068641, U01-AI046362 and U01-A1042170.

References

1. Hsu DC, Sereti I, Ananworanich J. Serious Non-AIDS events: Immunopathogenesis and interventional strategies. AIDS Res Ther. 2013; 10:29. [PubMed: 24330529]
2. Hearps AC, Martin GE, Rajasuriar R, Crowe SM. Inflammatory comorbidities in HIV+ individuals: learning lessons from healthy ageing. Curr HIV/AIDS Rep. 2014; 11:20–34. [PubMed: 24414166]
3. Bastidas S, Graw F, Smith MZ, Kuster H, Gunthard HF, Oxenius A. CD8+ T cells are activated in an antigen-independent manner in HIV-infected individuals. J Immunol. 2014; 192:1732–1744. [PubMed: 24446519]
4. Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, et al. Soluble markers of inflammation and coagulation, but not T-cell activation, are predictors of non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis. 2014; 210:1248–59. [PubMed: 24795473]
5. Wilson EM, Singh A, Hullsiek KH, Gibson D, Henry WK, Lichtenstein K, et al. Monocyte Activation Phenotypes Are Associated with Biomarkers of Inflammation and Coagulation in Chronic HIV Infection. J Infect Dis. 2014; 210:1396–406. [PubMed: 24813472]
6. Hearps AC, Maisa A, Cheng WJ, Angelovich TA, Lichtfuss GF, Palmer CS, et al. HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy. AIDS. 2012; 26:843–853. [PubMed: 22313961]
7. Burdo TH, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, et al. Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. J Infect Dis. 2011; 204:1227–1236. [PubMed: 21917896]
8. Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC. Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. AIDS. 2013; 27:1387–1395. [PubMed: 23435298]
9. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis. 2011; 203:780–790. [PubMed: 21252259]
10. Funderburg NT, Zidar DA, Shive C, Lioi A, Mudd J, Musselwhite LW, et al. Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. Blood. 2012; 120:4599–4608. [PubMed: 23065151]
11. Funderburg NT, Mayne E, Sieg SF, Asaad R, Jiang W, Kalinowska M, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. Blood. 2010; 115:161–167. [PubMed: 19828697]
12. Cozzi-Lepri A, French MA, Baxter J, Okhuysen P, Plana M, Neuhaus J, et al. Resumption of HIV replication is associated with monocyte/macrophage derived cytokine and chemokine changes: results from a large international clinical trial. AIDS. 2011; 25:1207–1217. [PubMed: 21505304]
13. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. PLoS One. 2012; 7:e44454. [PubMed: 22970224]

14. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008; 5:e203. [PubMed: 18942885]
15. Fuster D, Cheng DM, Quinn EK, Armah KA, Saitz R, Freiberg MS, et al. Inflammatory cytokines and mortality in a cohort of HIV-infected adults with alcohol problems. AIDS. 2014; 28:1059–1064. [PubMed: 24401638]
16. Strategies for Management of Antiretroviral Therapy (SMART) Study Group. El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006; 355:2283–96. [PubMed: 17135583]
17. O'Brien PC. Procedures for comparing samples with multiple endpoints. Biometrics. 1984; 40:1079–1087. [PubMed: 6534410]
18. Papadopoulou C, Corrigall V, Taylor PR, Poston RN. The role of the chemokines MCP-1, GRO-alpha, IL-8 and their receptors in the adhesion of monocytic cells to human atherosclerotic plaques. Cytokine. 2008; 43:181–186. [PubMed: 18579408]
19. Zernecke A, Weber C. Chemokines in atherosclerosis: proceedings resumed. Arterioscler Thromb Vasc Biol. 2014; 34:742–750. [PubMed: 24436368]
20. Ortego M, Bustos C, Hernandez-Presa MA, Tunon J, Diaz C, Hernandez G, et al. Atorvastatin reduces NF-kappaB activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. Atherosclerosis. 1999; 147:253–261. [PubMed: 10559511]
21. Romano M, Diomede L, Sironi M, Massimiliano L, Sottocorno M, Polentarutti N, et al. Inhibition of monocyte chemotactic protein-1 synthesis by statins. Lab Invest. 2000; 80:1095–1100. [PubMed: 10908155]
22. Breland UM, Halvorsen B, Hol J, Oie E, Paulsson-Berne G, Yndestad A, et al. A potential role of the CXC chemokine GROalpha in atherosclerosis and plaque destabilization: downregulatory effects of statins. Arterioscler Thromb Vasc Biol. 2008; 28:1005–1011. [PubMed: 18276907]
23. Pereira MM, Santos TP, Aras R, Couto RD, Atta ML, Atta AM. Serum levels of cytokines and chemokines associated with cardiovascular disease in Brazilian patients treated with statins for dyslipidemia. Int Immunopharmacol. 2014; 18:66–70. [PubMed: 24252254]

Table 1

Median values of biomarkers in cases and controls at study entry

Biomarkers, median(range)	Deaths(n=142)	Controls (n=284)	p-value*
Innate/pro-inflammatory response			
IL-6, pg/ml	3.64 (0.39, 104.8)	2.26 (0.41, 12.05)	<.001
TNF-alfa, pg/ml	12.68 (1.99, 147.8)	11.80 (1.89, 64.48)	0.009
IL-1 beta, 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			
IL-2, pg/ml	2.81 (0.07, 90.16)	3.32 (0.06, 104.1)	0.848
IL-7, pg/ml	9.86 (0.17, 144.3)	11.11 (0.17, 111.9)	0.649
IL-17, pg/ml	2.98 (0.07, 115.2)	3.03 (0.07, 200.2)	0.448
IFN-gamma, 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
IL-4, pg/ml	21.67 (0.01, 1260)	26.04 (0.00, 2354)	0.184
IL-5, pg/ml	0.55 (0.01, 9.87)	0.55 (0.01, 26.23)	0.695
IL-13, pg/ml	1.40 (0.01, 624.0)	3.92 (0.01, 1073)	0.383
IL-10, pg/ml	25.38 (0.18, 1215)	25.00 (0.18, 414.3)	0.359
IL-12p70, pg/ml	2.01 (0.04, 732.9)	2.51 (0.01, 308.1)	0.802

*from fitting a conditional logistic regression (markers in log scale). Only patients with observed values included

Table 2

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

	Adjusted[1] OR (95% CI)	p-value	Adjusted[2] OR (95% CI)	p-value*	Adjusted[3] OR (95% CI)	p-value*
Biomarkers						
Innate/pro-inflammatory response						
IL-6 (Elisa), pg/ml	2.44 (1.74, 3.44)	<.001	2.22 (1.56, 3.15)	<.001	2.15 (1.47, 3.15)	<.001
TNF-alfa, 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
IL-1 beta, 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						
IL-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
IL-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
IL-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
INF-gamma, 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
IL-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
IL-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
IL-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
IL-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
IL-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

Only patients with observed values included

*from fitting a conditional logistic regression (markers in log10 scale)

[1]adjusted for matching factors

[2]adjusted for HCV serostatus

[3]adjusted for all other potential confounders - race (black vs non-black), gender, current smoking, diabetes, use of lipid lowering drugs, previous evidence of cardiovascular disease, SMART randomisation arm, ART/viral load strata (off ART, on ART with VL ≤400 copies/mL and on ART with VL >400 copies/mL) and baseline $CD4^+$ T cell count.