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Viremic and virologically suppressed HIV infection increases age-related changes to monocyte activation equivalent to 12 and 4 years of ageing respectively.

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ABSTRACT

Background: Chronic inflammation and immune activation occur in both HIV infection and normal ageing and are associated with inflammatory disease. However, the degree to which HIV influences age-related innate immune changes, and the biomarkers which best reflect them, remains unclear.

Methods and Results: We measured established innate immune ageing biomarkers in 309 individuals including 88 virologically-suppressed (VS) and 52 viremic (viral load ≤ and >50 copies/ml respectively) HIV+ individuals. Levels of soluble (ie. CXCL10, soluble CD163, neopterin) and cellular (ie. proportions of inflammatory CD16+ monocytes) biomarkers of monocyte activation were increased in HIV+ individuals and were only partially ameliorated by viral suppression. Viremic and VS HIV+ individuals show levels of age-related monocyte activation biomarkers that are similar to uninfected controls aged 12 and 4 years older respectively. Viremic HIV infection was associated with an accelerated rate of change of some monocyte activation markers (eg. neopterin) with age, whilst in VS individuals, subsequent age-related changes occurred at a similar rate as in controls, albeit at a higher absolute level. We further identified CXCL10 as a robust soluble biomarker of monocyte activation, highlighting the potential utility of this chemokine as a prognostic marker.

Implications: These findings may partially explain the increased prevalence of inflammatory, age-related diseases in HIV+ individuals and potentially indicate the pathological mechanisms underlying these diseases which persist despite viral suppression.

Key words: HIV, innate immune activation, monocyte, ageing

INTRODUCTION

The advent of effective combination antiretroviral therapy (cART) has made HIV infection a manageable, chronic condition for HIV+ individuals who maintain long term viral suppression. However, as this population ages it is becoming increasingly apparent that even virologically suppressed (VS) HIV+ individuals suffer ongoing immune activation and dysfunction which is not reflected by traditional biomarkers of HIV disease such as viral load and CD4+ T cell count. HIV infection induces immunological changes which are characteristic of the normal ageing process including increased inflammation (as indicated by inflammatory markers such as TNF and IL-6, reviewed in [1]), an expansion of senescent T cells with shortened telomeres [2,3], and an increase in markers of adaptive and innate immune activation [4]. Beyond being simply biomarkers of ageing or HIV infection, many of these age-related parameters are of clinical significance and are increasingly being shown to be predictive of inflammatory, age-related morbidities which HIV+ individuals are at heightened risk of developing [1,5]. Markers of inflammation such as IL-6 [6] and high-sensitivity C-reactive protein [7] are associated with increased risk of cardiovascular disease (CVD) in HIV+ individuals and the general population (reviewed in [8]). Monocytes/macrophages play critical roles in the pathogenesis of inflammatory disease such as CVD [9] and we have previously shown that monocyte activation markers increase during ageing [10] and in both VS and viremic HIV infection [5,11]. Elevated levels of monocyte activation biomarkers including the proportion of inflammatory CD16+ monocytes and levels of soluble (s)CD163 and sCD14 (shed from activated monocytes) are associated with indices of CVD including non-calcified plaques [12], arterial inflammation [13], coronary artery calcification [14] and progression of carotid intima-media thickness (cIMT, a surrogate clinical measure of atherosclerosis) [15].

Whilst adaptive immune parameters such as CD4+ T cell counts may be useful prognostic markers in untreated HIV infection, it is becoming increasingly clear that innate markers of inflammation and monocyte activation may more accurately predict morbidity and mortality than T cell parameters in cART-treated individuals [16-18]_ENREF_16. However, the relative contributions of both ageing and HIV infection on these immunological changes are unclear.

Studies investigating the link between monocyte activation and clinical outcomes in HIV infection use various cellular and soluble markers of monocyte activation including the proportions of monocyte subsets (particularly the 'inflammatory' $CD16^+$ subsets), expression of activation markers such as CD11b or tissue factor and plasma levels of molecules either shed from or secreted by activated monocytes/macrophages (eg sCD14, sCD163, CXCL10, MCP-1). Importantly, it remains unclear which of these parameters best indicates monocyte activation and to what extent these biomarkers may overlap and indicate common underlying immunological mechanisms.

Here, we evaluated a combined dataset of commonly tested biomarkers associated either directly or indirectly with monocyte activation in order to a) identify the most robust and indicative markers of monocyte activation and b) quantitate the individual effects of ageing and viremic and VS HIV infection on changes to monocyte activation which are thought to underpin development of inflammatory diseases in these populations.

METHODS

Participant recruitment

HIV+ individuals were recruited from the Alfred Hospital Infectious Diseases Unit with informed consent and IRB approval. Blood collected into EDTA-coated tubes was used for

fresh whole blood immunophenotyping of monocytes, whilst plasma was prepared and stored for future analysis as previously described [5]. Healthy control individuals were recruited from the community. This study utilised data from newly recruited individuals (n=31) plus biomarker data from previously published cross-sectional cohort studies [5,11,19,20]

Experimental procedures and statistical analysis

Monocyte subsets and soluble plasma markers of innate immune activation/inflammation including CXCL10, neopterin, sCD163, sCD14 and LPS were determined as previously described [5,11,19]. To evaluate the continuous effect of ageing on the biomarkers tested, data from cross-sectional studies were combined with additional patient recruitment for analysis. Linear regression analysis was used to identify monocyte biomarkers which were significantly altered with age and determine the influence of sex, current smoking status and either viremic or VS HIV infection on this relationship. Spearman's correlation analysis was used to determine the association between individual monocyte activation biomarkers. To quantitatively estimate the impact of viremic and VS HIV infection on monocyte activation in the context of ageing, linear regression modelling was used to estimate the age difference between VS and viremic HIV+ individuals with HIV- controls for a given level of each biomarker. Differences in coefficients due to HIV status were analysed using a Student's t test ($p<0.05$ considered significant) whilst differences in slope between sample groups were determined by comparing models fitted with or without an interaction term (likelihood ratio test). Where the slope differed between groups, the linear equations were solved for a given parameter level taking into account the interaction term. Linear regression analysis was performed using Stata v.11 (STATA Corp) and correlations assessed using GraphPad Prism (version 6.02).

RESULTS

Data from a total of 309 participants were analysed including 169 HIV seronegative controls (median age [range] 48 [20-84], 67.5% male), 88 VS HIV+ individuals with undetectable viral load (<50 RNA copies/ml, 97.7% receiving cART, median age 48 [20-70], 81.8% male) and 52 viremic HIV+ individuals with detectable viral load (VL >50 copies/ml [range 80 to >100,000 copies/ml], 19.2% receiving cART, median age 40 [19-63], 82.7% male). Clinical and demographic information is detailed in Table 1.

To identify markers of monocyte activation which altered significantly with age, we performed linear regression modelling on all biomarker data and adjusted for HIV infection, sex (which we have previously shown influences a number of these biomarkers [10]) and current smoking status. Plasma levels of CXCL10, sCD163, neopterin and the proportions of monocyte subsets (defined as classical; $CD14^{++}CD16^{-}$, intermediate; $CD14^{++}CD16^{+}$, and non-classical; $CD14^{+}CD16^{++}$) were all significantly altered with age independent of HIV infection ($p<0.05$ for all, see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A642), confirming and extending our previous findings using age as a categorical variable that these parameters represent biomarkers of age-related monocyte activation [10]. Although routinely used as biomarkers of monocyte activation in HIV studies, neither sCD14 nor LPS levels were significantly associated with age in this study. Sex affected the relationship between a number of the biomarkers analysed (including monocyte subsets, CXCL10, sCD163 and LPS, see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A642) and age, confirming the need to adjust for sex in these analyses, although adjustment for smoking did not alter the relationship between any of the biomarkers and age in the control cohort (data not shown).

We have previously shown in a small cross-sectional study that younger HIV+ individuals (aged <45 years) exhibit higher levels of monocyte activation than age matched controls, and this effect is only partially ameliorated by viral suppression[5]. We thus used linear regression to extend these findings in an expanded cohort of HIV+ and HIV seronegative individuals aged 19-84 using statistical analysis which allowed appropriate adjustment for the effect of sex and smoking status (due to the increased prevalence of current smokers in many HIV+ cohorts including ours; Table 1). We also sought to determine whether the impact of HIV on monocyte activation is amplified at older ages. Biomarkers which were significantly altered with age in control individuals showed a similar trend in HIV+ individuals (data not shown). Table 2 shows the impact of HIV infection in addition to age-related changes on each parameter, and indicates that proportions of intermediate monocytes were significantly increased, and the proportions of classical monocytes consequently decreased, in viremic HIV infection ($p=0.002$ and 0.015 respectively, column "P value vs HIV-") but not in VS individuals. In contrast, levels of soluble age-related monocyte activation markers CXCL10, neopterin and sCD163 were significantly elevated in both viremic ($p\leq0.001$ for all, column "P value vs HIV-") and VS individuals ($p<0.001$ for all). Although it was not significantly altered by age, sCD14 was increased in both viremic and VS HIV+ individuals as compared to controls ($p\leq0.001$ for both). We also determined whether the slope of the regression curve, which indicates the rate at which these parameters change with age, differed between HIV+ and control individuals (column "P value slope", Table 2). The rate of change for non-classical monocytes was altered in HIV+ individuals, however the overall effect of HIV infection on this parameter was not significant. There was a significant difference in the slope of the regression curve between viremic and control individuals for neopterin ($p=0.012$, Table 2 and Figure 1), indicating age-related changes to neopterin occur at a greater rate in those with uncontrolled HIV replication. However, no such differences in slope were detected for

soluble biomarkers in VS HIV+ individuals ($p>0.05$, Table 2 and Figure 1; Note similar slope of the regression curves for VS HIV+ and uninfected individuals), indicating that although HIV increases the overall level of these markers, subsequent age-related changes occur at the same rate in VS HIV+ individuals as in uninfected controls. These data confirm that HIV significantly alters age-related changes to monocytes independent of sex and smoking, and that viral suppression associated with cART does not normalise levels of soluble monocyte activation markers.

To assess the level of redundancy of each marker and determine whether one or more markers could be used to accurately predict other markers of monocyte activation, we next analysed associations between each of the monocyte activation markers. CXCL10 levels significantly correlated with all other soluble monocyte activation markers measured except sCD14, and also with proportions of classical and intermediate monocyte subsets (Table 3). Neopterin levels also correlated significantly with a number of soluble markers (CXCL10, sCD163 and sCD14) and intermediate but not classical monocyte subsets. In this cohort, sCD14 showed a significant association with only neopterin, and not with monocyte subsets. Taken together, these data indicate that CXCL10, and to a lesser extent neopterin, levels are indicative of other soluble monocyte activation markers and monocyte subset proportions.

To determine whether CXCL10 also correlated with other commonly measured cellular markers of monocyte activation, in a subset of participants we measured the association between CXCL10 levels and expression of functional/activation markers including CD38 [21], which is upregulated on monocytes from HIV+ individuals irrespective of cART (unpublished data). We found CXCL10 levels correlated significantly with expression of the activation markers CD11b and CD38 and the pro-coagulant protein tissue factor on classical and intermediate monocyte subsets (Table 3). sCD14 levels did not correlate with any of these markers, whilst neopterin and sCD163 correlated with CD11b and tissue factor levels

only. Taken together, these data suggest that CXCL10, but not sCD14, is a robust and easy to measure plasma marker of monocyte activation that can be used to indicate other soluble, and importantly the more difficult to analyse cellular markers, of monocyte activation under both healthy and inflammatory states.

To quantify the effect of HIV infection on monocyte activation and estimate the age at which HIV+ individuals reach a given level of monocyte activation, we used a similar regression model as above but where age was the outcome of the regression equation. We selected CXCL10 to use in this analysis, having established it above as a robust biomarker of age and HIV-related monocyte activation and an accurate indicator of other monocyte activation markers. In this analysis, the coefficient for the viremic or VS HIV adjustment term indicated the difference in age between HIV+ individuals and uninfected controls for any given level of CXCL10. In VS HIV+ individuals, the value of the coefficient was -4.0 (95% confidence interval (CI) -9.1 to 1.1, see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A642), indicating that VS HIV+ individuals exhibit the same level of CXCL10 4 as seronegative controls aged approximately 4 years older. The coefficient for viremic HIV+ individuals indicated a more dramatic effect, in that these individuals reached this level of activation 12.0 (95% CI -18.9 to -4.9) years earlier than uninfected controls. We validated this statistical approach using other biomarkers shown to be elevated in HIV infection, and found strikingly similar coefficient values (ranging from -9.7 to -12.9 and -1.8 to -3.7 years for viremic and VS HIV infection respectively, see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/A642). In summary, these data indicate that viremic HIV+ individuals exhibit levels of monocyte activation markers similar to those seen in seronegative controls aged up to 12 years older, and whilst viral suppression significantly

reduces this effect, VS HIV+ individual still experience levels of monocyte activation similar to those observed in controls aged up to 4 years older.

DISCUSSION

Whilst HIV infection is known to be associated with innate immune dysfunction and the premature appearance of age-related changes, the extent to which age and HIV contribute to these changes in HIV+ individuals, and whether these effects are completely reversed by cART, has remained unclear. We report that viremic HIV+ individuals display a similar level of monocyte activation to HIV- controls aged 12 years older, and whilst viral suppression significantly reduced the level of biomarkers such as CXCL10, VS HIV+ individuals still show an equivalent level of monocyte activation 4 years earlier. Strikingly similar estimated effects of HIV in year equivalents were observed for a range of soluble and cellular markers of monocyte/innate immune activation, supporting the validity of this analysis. These findings suggest that in addition to the effects of ageing, viremic and VS HIV infection have an additional burden of innate immune activation equivalent to up to 12 and 4 years of normal ageing respectively.

In addition to increasing the absolute level of monocyte activation biomarkers, viremic HIV infection was associated with an accelerated rate of change of the biomarker neopterin with age, indicating a potentiation of age and HIV viremia on factors driving the production of this biomarker. For all other biomarkers, our analysis revealed that HIV infection heightened monocyte activation, but did not accelerate the rate of subsequent age-related changes (indicated by parallel regression curves in Figure 1). This suggests that HIV infection is associated with a higher absolute level of monocyte activation irrespective of age, but that age-related changes continue to occur at a rate similar to uninfected controls.

Whilst cross-sectional studies such as these are a useful, predictive way of estimating likely age-associated effects, the observations made here require confirmation in a longitudinal study of individuals infected at a similar age. However, due to the decades-long follow-up period required to conduct such analyses, a retrospective study design will likely be required.

Initiating cART early after infection (within 6 months) has been shown to reduce the extent of T cell activation as compared to those who initiate cART later [22], although whether early cART initiation would similarly reduce subsequent innate immune activation has not been determined. Duration of HIV infection and duration of viral suppression are both likely to impact the degree of innate immune activation, although the former cannot usually be determined with accuracy in cross-sectional studies. Nadir CD4 T cell count is often used in VS individuals as a surrogate marker for the extent of immune damage incurred prior to cART initiation. In our cohort, there was no significant difference in CXCL10 or neopterin levels in VS HIV+ individuals with nadir CD4 T cell counts above versus below 200 cells/uL ($p>0.05$ for both, data not shown). The estimated median duration of cART for VS patients in this study was 4.8 years, however this only included treatment received at the attending clinic, thus we did not adjust for duration of therapy in our study. We consider the effect of both early cART initiation and duration of therapy on immune activation is best investigated using a contemporary cohort of well-characterised patients receiving optimal cART regimens.

In this analysis, we adjusted for sex, which we have shown affects monocyte activation biomarkers levels [10], and also determined the effect of current smoking status, due to its established effects on inflammation [23]. Whilst smoking status did not alter the relationship

between the biomarkers and age in control individuals, it did influence levels in HIV+ individuals; plasma LPS levels were significantly elevated in viremic and VS HIV+ individuals as compared to controls before but not after adjustment for smoking (data not shown), demonstrating the necessity to control for smoking status when comparing LPS levels in HIV studies. Whilst BMI was not significantly different between HIV+ and seronegative individuals, concurrent illness including hepatitis C co-infection (more prevalent in HIV+ individuals), socio-demographic differences and other lifestyle factors may also affect our results.

Cytomegalovirus (CMV) infection significantly impacts immunosenescence associated with ageing [24] and CMV seropositivity is ubiquitous in most HIV+ populations, making it difficult to delineate the discrete effects of CMV and HIV. CMV reactivation can occur during HIV infection and healthy ageing, but it is not known how the timing and/or frequency of reactivation impacts on immune activation. For these reasons, CMV status was not adjusted for here, and whilst these results clearly demonstrate that immune dysfunction occurs in HIV+ individuals, it is entirely possible that this may result from the combined effects of HIV and CMV.

Studies investigating monocyte activation in HIV infection typically measure plasma levels of sCD14, LPS or the proportion of monocyte subsets (or a combination of) as biomarkers of monocyte activation. Accurate determination of plasma LPS levels can be influenced by inhibitory binding proteins in plasma [25] and a lack of robustness in the analytical assays used, and is therefore a less reliable biomarker. sCD14 is produced following monocyte stimulation and is thus considered indicative of monocyte activation, however it can act systemically to inhibit LPS-responses [26], complicating the interpretation of elevated plasma sCD14 levels.

We have shown both here and previously [10] that whilst monocyte activation biomarkers such as CXCL10 and neopterin are increased during ageing, sCD14 levels are not, suggesting these soluble biomarkers may indicate different types of monocyte activation. Indeed, in our analysis sCD14 levels correlated only with neopterin levels but no other soluble biomarkers or proportions of monocyte subsets. $CD16^+$ monocyte subsets are often considered 'inflammatory' [27] and thus a marker of inflammation-induced monocyte activation as they produce large amounts of pro-inflammatory cytokines following stimulation [28] and are present at elevated levels in various inflammatory settings [29], including viremic HIV infection. The lack of correlation between sCD14 levels and proportions of inflammatory monocyte subsets shown here suggest that sCD14 levels may only partially predict the extent of monocyte activation and should therefore be interpreted with caution. In contrast, CXCL10 levels were associated with all other soluble biomarkers measured (except sCD14 as discussed above), with proportions of intermediate and classical (although not non-classical) monocyte subsets, and also with other cellular markers of monocyte activation/coagulation (ie. CD11b, CD38 and tissue factor). These data suggest CXCL10 can be used to indicate a range of monocyte activation outcomes and is thus a useful biomarker warranting inclusion in studies of monocyte activation. CXCL10 can be produced from many different cell types including neutrophils and endothelial cells following IFNγ stimulation, and is therefore not an exclusive marker of monocyte/macrophage activation. However, our data suggests CXCL10 levels may be a more sensitive marker than monocyte-specific factors such as sCD14 in this setting. Furthermore, monocytes are the primary source of CXCL10 from PBMC in HIV+ individuals and plasma CXCL10 levels correlate significantly with CXCL10 mRNA levels in monocytes[30]. In the general population, plasma CXCL10 levels are increased in individuals with a range of conditions including colorectal cancer [31], frailty [31,32], hypertension and coronary heart disease and correlate significantly with traditional CVD

biomarkers including CRP, IL-6 and fibrinogen [33,34]. Plasma CXCL10 levels are increased in HIV infection (including in HIV controllers[35]) and are associated positively with T cell activation and negatively with CD4 T cell counts[35,36]. CXCL10 is a neurotoxic factor which is elevated in the cerebrospinal fluid of HIV+ individuals and is further increased in those with AIDS-related dementia [37,38]_ENREF_28; further work is required to assess the predictive value of plasma CXCL10 levels in morbidities such as frailty and CVD in HIV infection.

Whilst it has been speculated that HIV may accelerate biological ageing, this is likely an oversimplification of the diverse and incompletely understood mechanisms which contribute to immunological ageing. The data presented here indicate that HIV infection heightens age-related changes such that levels of monocyte activation reflect those observed in HIV- individuals who are significantly older, and that this effect is only partially ameliorated by cART. Given the established associations between these activation biomarkers and inflammatory disease, this likely confers increased disease risk, although this requires investigation. Determining the pathological consequences of these changes, and the mechanism underlying them, is required to prevent inflammatory diseases in the increasingly ageing VS HIV+ population.

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REFERENCES

1. Deeks SG, Tracy R, Douek DC. Systemic Effects of Inflammation on Health during Chronic HIV Infection. *Immunity*. Oct 17 2013;39(4):633-645.

2. Effros RB, Allsopp R, Chiu CP, et al. Shortened telomeres in the expanded CD28-CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *AIDS (London, England).* Jul 1996;10(8):F17-22.

3. Desai S, Landay A. Early immune senescence in HIV disease. *Curr HIV/AIDS Rep.* Feb 2010;7(1):4-10.

4. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *The Journal of pathology*. Jan 2008;214(2):231-241.

5. Hearps AC, Maisa A, Cheng WJ, et al. HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy. *AIDS (London, England).* Apr 24 2012;26(7):843-853.

6. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med*. Oct 21 2008;5(10):e203.

7. Tien PC, Choi AI, Zolopa AR, et al. Inflammation and mortality in HIV-infected adults: analysis of the FRAM study cohort. *Journal of acquired immune deficiency syndromes (1999).* Nov 1 2010;55(3):316-322.

8. Hearps AC, Martin GE, Rajasuriar R, Crowe SM. Inflammatory Co-morbidities in HIV+ Individuals: Learning Lessons from Healthy Ageing. *Curr HIV/AIDS Rep.* Jan 11 2014.

9. Crowe SM, Westhorpe CL, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M. The macrophage: the intersection between HIV infection and atherosclerosis. *Journal of leukocyte biology*. Dec 1 2009;87(4):589-598.

10. Hearps AC, Martin GE, Angelovich TA, et al. Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. *Aging Cell.* Oct 2012;11(5):867-875.

11. Martin GE, Gouillou M, Hearps AC, et al. Age-associated changes in monocyte and innate immune activation markers occur more rapidly in HIV infected women. *PloS one.* 2013;8(1):e55279.

12. Burdo TH, Lo J, Abbara S, et al. Soluble CD163, a Novel Marker of Activated Macrophages, Is Elevated and Associated With Noncalcified Coronary Plaque in HIV-Infected Patients. *The Journal of infectious diseases.* Oct 2011;204(8):1227-1236.

13. Subramanian S, Tawakol A, Burdo TH, et al. Arterial inflammation in patients with HIV. *JAMA.* Jul 25 2012;308(4):379-386.

14. Althoff KN, Jacobson LP, Cranston RD, et al. Age, comorbidities, and AIDS predict a frailty phenotype in men who have sex with men. *J Gerontol A Biol Sci Med Sci.* Feb 2014;69(2):189-198.

15. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. *Journal of Infectious Diseases.* 2012;206(10):1558-1567.

16. Mutlu-Turkoglu U, Ilhan E, Oztezcan S, Kuru A, Aykac-Toker G, Uysal M. Age-related increases in plasma malondialdehyde and protein carbonyl levels and

lymphocyte DNA damage in elderly subjects. *Clinical biochemistry*. Jul 2003;36(5):397-400.

17. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble Markers of Inflammation and Coagulation but Not T-Cell Activation Predict Non-AIDS-Defining Morbid Events During Suppressive Antiretroviral Treatment. *The Journal of infectious diseases.* Oct 15 2014;210(8):1248-1259.

18. Sandler NG, Wand H, Roque A, et al. Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection. *The Journal of infectious diseases.* Jan 20 2011;203(3):780-790.

19. Lichtfuss GF, Cheng WJ, Farsakoglu Y, et al. Virologically suppressed HIV patients show activation of NK cells and persistent innate immune activation. *J Immunol.* Aug 1 2012;189(3):1491-1499.

20. Westhorpe CL, Maisa A, Spelman T, et al. Associations between blood monocyte markers and carotid atherosclerosis in HIV-positive patients. *Immunology and cell biology*. 2013;In press.

21. Lande R, Urbani F, Di Carlo B, et al. CD38 ligation plays a direct role in the induction of IL-1beta, IL-6, and IL-10 secretion in resting human monocytes. *Cellular immunology*. Nov 2002;220(1):30-38.

22. Greene M, Valcour V, Miao Y, et al. Geriatric Syndromes Are Common Among Older HIV-Infected Adults. Paper presented at: Conference On Retroviruses And Opportunistic Infections2014.

23. Bakhru A, Erlinger TP. Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. *PLoS Med.* Jun 2005;2(6):e160.

24. Pawelec G, Derhovanessian E, Larbi A, Strindhall J, Wikby A. Cytomegalovirus and human immunosenescence. *Reviews in medical virology.* Jan 2009;19(1):47-56.

25. Funderburg NT, Jiang Y, Debanne SM, et al. Rosuvastatin treatment reduces markers of monocyte activation in HIV-infected subjects on antiretroviral therapy. *Clin Infect Dis.* Feb 2014;58(4):588-595.

26. Kitchens RL, Thompson PA. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. *Journal of endotoxin research.* 2005;11(4):225-229.

27. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *Journal of leukocyte biology.* Mar 2007;81(3):584-592.

28. Belge KU, Dayyani F, Horelt A, et al. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. *J Immunol.* Apr 1 2002;168(7):3536-3542.

29. Fingerle G, Pforte A, Passlick B, Blumenstein M, Strobel M, Ziegler-Heitbrock HW. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. *Blood.* Nov 15 1993;82(10):3170-3176.

30. Simmons RP, Scully EP, Groden EE, et al. HIV-1 infection induces strong production of IP-10 through TLR7/9-dependent pathways. *AIDS (London, England).* Oct 23 2013;27(16):2505-2517.

31. Dimberg J, Skarstedt M, Lofgren S, Zar N, Matussek A. Protein expression and gene polymorphism of CXCL10 in patients with colorectal cancer. *Biomedical reports.* May 2014;2(3):340-343.

32. Qu T, Yang H, Walston JD, Fedarko NS, Leng SX. Upregulated monocytic expression of CXC chemokine ligand 10 (CXCL-10) and its relationship with serum interleukin-6 levels in the syndrome of frailty. *Cytokine.* Jun 2009;46(3):319-324.

33. Yang T, Li ZN, Chen G, et al. Increased levels of plasma CXC-Chemokine Ligand 10, 12 and 16 are associated with right ventricular function in patients with idiopathic

pulmonary arterial hypertension. *Heart & lung : the journal of critical care.* Jul-Aug 2014;43(4):322-327.

34. Rothenbacher D, Muller-Scholze S, Herder C, Koenig W, Kolb H. Differential expression of chemokines, risk of stable coronary heart disease, and correlation with established cardiovascular risk markers. *Arteriosclerosis, thrombosis, and vascular biology.* Jan 2006;26(1):194-199.

35. Noel N, Boufassa F, Lecuroux C, et al. Elevated IP10 levels are associated with immune activation and low CD4(+) T-cell counts in HIV controller patients. *AIDS (London, England).* Feb 20 2014;28(4):467-476.

36. Liovat AS, Rey-Cuille MA, Lecuroux C, et al. Acute plasma biomarkers of T cell activation set-point levels and of disease progression in HIV-1 infection. *PloS one.* 2012;7(10):e46143.

37. Yuan L, Qiao L, Wei F, et al. Cytokines in CSF correlate with HIV-associated neurocognitive disorders in the post-HAART era in China. *J Neurovirol.* Apr 2013;19(2):144-149.

38. Cinque P, Bestetti A, Marenzi R, et al. Cerebrospinal fluid interferon-gamma-inducible protein 10 (IP-10, CXCL10) in HIV-1 infection. *J Neuroimmunol.* Nov 2005;168(1-2):154-163.

FIGURE LEGENDS

Figure 1: Regression analysis of plasma levels of CXCL10 (A) and Neopterin (B) in Viremic (black circles and line) and virologically suppressed (grey triangles and line) HIV+ individuals and uninfected controls (open squares, dashed line) and age. Curves were fitted to unadjusted data. Adjusted regression equations for CXCL10 including adjustment for viremic or virologically suppressed HIV infection were CXCL10 = 1.29[age] + 204[viremic HIV] + 62.2[sex] - 28.1[smoking] - 2.40 and CXCL10 = 1.29[age] + 66.5[VS HIV] + 62.2[sex] – 28.1[smoking] – 2.40 respectively, where HIV status, smoking and sex were categorical variables.

Table 1: Demographic details of study participants.

	HIV-	Viremic HIV+		VS HIV+	
			P value		P value
Participants (n)	169	52		88	
Age (years)					
Median (IQR)	48 (36-66)	40 (35-47)	<0.001[2]	48 (39-54)	NS[2]
Range	20-84	19-63		20-70	
Sex (% male)	67.5%	82.7%	0.034[3]	81.8%	0.015[3]
Body Mass Index[1]					
Median (IQR)	24.5 (22.0-27.5)	24.1 (22.5-27.2)	NS[2]	25.7 (22.7-29.5)	NS[2]
Range	16.1-42.0	16.0-37.1		16.0-43.9	
Missing data (n)	18	21		19	
Smoking status					
Current	6.5%	46.2%	<0.001[3]	37.5%	<0.001[3]
Previous	26.0%	23.0%		30.7%	
Never	67.5%	30.8%		31.8%	
Viral load (copies/ml)			<0.001[4]	All <50 copies/ml	
≤1,000 median age [IQR]		17.6% (43.0 [39.5-44.0])			
1,001-20,000		25.5% (42.5 [34.8-55.5])			
20,001-100,000		31.4% (38.5 [36.3-44.8])			
>100,000		25.5% (38.0 [29.8-48.3])			
CD4 T cells (cells/µl)[1]					
Median (IQR)		416 (319-527)	<0.001[4]	619 (402-846)	
Range		4-1092		129-1433	
Missing data (n)		2		6	
Nadir CD4 (cells/µl)[1]					
Median (IQR)		267 (64-395)	NS[4]	203 (80-292)	
Range		1-688		8-593	
Missing data (n)		14		15	
Receiving cART n (%)		10 (19.2%)		86 (97.7%)	
Duration of cART (years)[1]					
Median (IQR)				4.8 (2.3-18.9)	
Range				0.2-22	
Missing data (n)				10	

[1] Participants with missing data are not included in the values listed.

[2] As compared to HIV- controls by Mann-Whitney test.

[3] As compared to HIV- controls by Chi-squared test.

[4] As compared to Virologically Suppressed HIV+ participants by Mann-Whitney test.

Table 2. Effect of HIV infection on biomarkers of monocyte activation in addition to age-related changes

		Viremic HIV+ (V)			Virologically Suppressed HIV+ (VS)			V vs VS
	n	**P value Slope[1]**	**Coefficient (95% CI)**	**P value vs HIV-[2]**	**P value Slope[1]**	**Coefficient (95% CI)**	**P value vs HIV-[2]**	**P value[3]**
Monocyte subsets								
Classical ($CD14^{++}CD16^{-}$)	264	NS	-3.96 (-7.13, -0.79)	**0.015**	NS	0.11 (-1.84, 2.05)	0.914	**0.013**
Intermediate ($CD14^{++}CD16^{+}$)	264	NS	2.68 (1.01, 4.35)	**0.002**	NS	0.84 (-0.29, 1.97)	0.143	**0.049**
Non-classical ($CD14^{+}CD16^{++}$)	264	**0.049**	2.12 (-0.23, 4.47)[4]	0.077[4]	**0.020**	-1.05[4] (-2.13, 0.02)	0.055[4]	0.108
Total $CD16^{+}$	264	NS	3.86 (0.60, 7.13)	**0.021**	NS	-0.38 (-2.37, 1.61)	0.707	**0.012**
Soluble factors								
CXCL-10	204	NS	204 (153, 255)	**<0.001**	NS	66.5 (30.5, 103)	**<0.001**	**<0.001**
sCD14	246	NS	432 (189, 675)	**0.001**	NS	602 (421, 783)	**<0.001**	0.208
sCD163	246	NS	683 (513, 854)	**<0.001**	NS	324 (159, 490)	**<0.001**	**<0.001**
Neopterin	246	**0.012**	11.0 (6.83, 15.1)[4]	**<0.001**[4]	NS	2.87 (1.36, 4.37)	**<0.001**	**0.031**
LPS	220	NS	5.71 (-4.51, 15.9)	0.272	NS	9.06 (-0.07, 18.2)	0.052	0.491

Linear regression analysis of changes in biomarker levels with age due to viremic (V) or virologically suppressed (VS) HIV infection adjusting for sex and smoking. The coefficient indicates the effect of HIV status on biomarker level in addition to the effect of age.

[1] P value <0.05 indicates the rate of change of the parameter with age (ie slope) in HIV+ individuals is significantly different to controls.

[2] P value reports direct effect of HIV status compared to HIV- controls, indicating the y intercept of the regression curve was significantly different to HIV- controls.

[3] P value indicates whether there was a significant difference in the y intercept between V and VS HIV+ individuals, as determined by student's t test.

[4] Where the slope was significantly different to HIV- individuals, an interaction term was introduced. The resulting equation was solved for the median age of the entire cohort, and the coefficient and p value for that value shown.

Table 3: Correlation of soluble and cellular monocyte activation biomarkers[#].

		CXCL10	Neopterin	sCD163	sCD14
Soluble markers					
CXCL10	204	-			
Neopterin	246	***0.547******	-		
sCD163	246	***0.409******	***0.375******	-	
sCD14	246	0.090	***0.221******	-0.022	-
LPS	220	***0.175****	0.078	***0.336******	-0.085
Monocyte subsets and phenotype					
Classical monocytes					
% Classical subset	237	***-0.273******	-0.095	-0.114	0.013
CD38 (MFI)	221	***0.162****	0.114	0.093	0.016
CD11b (MFI)	225	***0.346******	***0.188*****	***0.276******	0.101
Tissue factor (MFI)	78	***0.319*****	***0.412******	***0.399******	0.163
Intermediate monocytes					
% Intermediate subset	237	***0.356******	***0.162****	***0.158****$^{}$	-0.001
CD38 (MFI)	219	***0.161****	0.137	-0.021	0.014
CD11b (MFI)	223	***0.300******	***0.186*****	***0.234******	0.079
Tissue factor (MFI)	68	***0.422******	***0.334*****	***0.318*****	0.229
Non-classical					
% Non-classical subset	237	0.132	0.013	-0.095	0.001

[#]Determined by Spearman's correlation; Rho values shown.

Statistically significant correlations are shown in bold italic. * p value < 0.05, ** p value < 0.01, *** p value < 0.001.

MFI=Mean fluorescence intensity.