

Inflammatory Phenotypes Predict Changes in Arterial Stiffness following ART Initiation

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Summary: Adults starting ART with advanced immunosuppression can be phenotyped into three inflammatory clusters. One cluster was defined by high T-cell PD1 expression and another by a hyper-inflamed biological profile. Changes in arterial stiffness on ART varied according to inflammatory phenotype.

Abstract

Background: Inflammation drives vascular dysfunction in HIV, but in low-income settings causes of inflammation are multiple, and include infectious and environmental factors. We hypothesised that patients with advanced immunosuppression could be stratified into inflammatory phenotypes that predicted changes in vascular dysfunction on ART.

Methods: We recruited Malawian adults with CD4<100 cells/ul two weeks after starting ART in the REALITY trial (NCT01825031). Carotid femoral pulse wave velocity (cfPWV) measured arterial stiffness 2, 12, 24 and 42 weeks post-ART initiation. Plasma inflammation markers were measured by electrochemiluminescence at weeks 2 and 42. Hierarchical clustering on principal components identified inflammatory clusters.

Results: 211 HIV-positive participants grouped into three clusters of inflammatory marker profiles representing 51 (24%) (cluster-1), 153 (73%) (cluster-2) and 7 (3%) (cluster-3) individuals. Cluster-1 showed markedly higher CD4 and CD8 T-cell expression of HLADR and PD1 vs cluster-2 and cluster-3 (all $p<0.0001$). Although small, individuals in cluster-3 had significantly higher levels of cytokines reflecting inflammation (IL6, IFN γ , IP10, IL1RA, IL10), chemotaxis (IL8), systemic and vascular inflammation (CRP, ICAM1, VCAM1) and SAA (all $p<0.001$). In mixed-effects models, cfPWV changes over time were similar for cluster-2 vs cluster-1 (relative-fold-change 0.99 (95% CI 0.86-1.14, $p=0.91$), but greater in cluster-3 vs cluster-1 (relative-fold-change 1.45 (95% CI 1.01-2.09, $p=0.045$).

Conclusions: Two inflammatory clusters were identified: one defined by high T-cell PD1 expression and another by a hyper-inflamed profile and increases in cfPWV on ART. Further clinical characterisation of these inflammatory phenotypes could help target vascular dysfunction interventions to those at highest risk.

Keywords: Inflammatory phenotype, Arterial stiffness, Sub-Saharan Africa

Introduction

Chronic inflammation persists in people living with HIV (PLWH) despite long-term suppressive antiretroviral therapy (ART), contributing to vascular dysfunction and non-communicable diseases [1-3]. Low income sub-Saharan Africa (SSA) faces an epidemiological transition in cardiovascular disease (CVD), as urbanisation increases traditional cardiovascular risk factors [4, 5]. Hypertension is already highly prevalent; diabetes, dyslipidaemia and obesity are increasing [6-8]. Already, 80% of haemorrhagic strokes in people under 65 years occur in low and middle income countries [9].

Continued increases in non-communicable disease in low-income SSA are of major public health concern and the focus of sustainable development goal 3.4 [9, 10]. Life expectancy is increasing amongst PLWH in the region and the effects of persistent chronic inflammation superimposed upon an evolving background of cardiovascular risk in an ageing cohort are unknown [11, 12].

In high-income settings, drivers such as microbial translocation, persistent HIV replication and latent co-infections have been implicated in chronic inflammation [13-15]. Precise pathways remain unclear, with some individuals being affected more than others, and become even more complex in low-income settings where additional drivers of inflammation are multiple. Recurrent acute or sub-acute infections such as TB, CMV, malaria, helminths and invasive bacterial disease underpin an exhausted immune system [16-18]. Cotrimoxazole decreases immune activation, suggesting that modifying infection-related drivers may help reduce chronic inflammation [19]. Late presentation with low CD4 is still common in SSA, and is associated with lymph node fibrosis and poor immune reconstitution on ART [20-22]. Immune activation has also been linked to poor ART adherence [23], food insecurity [24] and host genetics [25] in SSA cohorts.

A pragmatic approach to this complex process might be to identify common inflammatory phenotypes that identify those at highest risk from the complications of chronic inflammation, and hence target limited resources for risk reduction. This approach has been used successfully to guide treatment in other diseases characterised by chronic inflammation, such as asthma and systemic lupus erythematosus (SLE) [26, 27].

Arterial stiffness has been used as a measure of vascular dysfunction in HIV studies as well as other chronic inflammatory conditions [28-33]. cfPWV is a gold standard measurement of arterial stiffness and has been validated as a physiological biomarker of cardiovascular events and mortality [29, 30]. We have previously shown that arterial stiffness, as measured by carotid femoral pulse wave velocity (cfPWV), is increased in PLWH during the first 3 months of ART and in people experiencing unstructured treatment interruptions [3, 15].

We aimed to investigate whether PLWH starting ART with advanced immune suppression could be stratified into inflammatory phenotypes, and whether these phenotypes predicted changes in arterial stiffness following ART initiation.

Methods

ART-naïve adults with a new HIV diagnosis and CD4 <100 cells/uL were recruited prospectively from the ART clinic and voluntary HIV testing clinic at Queen Elizabeth Central Hospital, Blantyre, Malawi, along with HIV negative adults with no evidence of infection within the previous 3 months (full details of recruitment in [3]; most HIV-positive patients were recruited from the REALITY trial [NCT01825031]). In brief, participants underwent a detailed clinical assessment and blood draw for plasma storage at 2 (enrolment) and 42 weeks post ART initiation. The enrolment visit for HIV-positive participants was 2 weeks after ART initiation to minimise visit burden in this unwell group. cfPWV was assessed on all participants at enrolment, and 10, 22 and 40 weeks later. All participants provided informed written consent and ethical approval was granted by the College of Medicine Research and Ethics Committee (COMREC), University of Malawi (P.09/13/1464) and the University of Liverpool Research and Ethics Committee (UoL000996).

Surface immunophenotyping of T-cells was performed on fresh PBMCs using flow cytometry as previously described [3]. T-cell activation, exhaustion and senescence was defined as CD38/HLADR, PD1 and CD57 expression, respectively. Monocytes were defined as classical (CD14⁺⁺CD16⁻), intermediate (CD14⁺⁺CD16⁺) or nonclassical (CD14⁺CD16⁺). Stored plasma was tested for 22 cytokines: Proinflammatory Panel-1 (IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL12p70, IL-13,

TNF- α), Vascular Injury Panel-2 (SAA, CRP, VCAM-1, ICAM-1), Chemokine Panel-1 (MIP-1 β , IP-10, MCP-1), Angiogenesis -Panel-1 (VEGF-A, bFGF) and single analyte assays for IL1R antagonist and IL-7, all from Meso Scale Discovery (MSD, Rockland, MD, USA). Assays were performed following the manufacturer's instructions and recommended dilutions for human plasma. Soluble CD163 was measured in plasma diluted at 1:20 using DuoSet antibodies (R&D Systems, Minneapolis, MN, USA) on MSD Multiarray plates. CMV viral loads were quantified by DNA PCR in a subset of participants with available plasma as described previously[34]. A negative CMV PCR was defined as <100 copies/mL and corresponded with values less than 3 cycle thresholds above background fluorescence.

Statistical analysis

Categorical and continuous variables were compared using chi-squared and Kruskal-Wallis or Wilcoxon ranksum tests, respectively. We used principal component analysis (PCA) to reduce the dimensionality/eliminating redundancy across all 22 biomarkers., Briefly, PCA enables reduction of a larger number of variables into a smaller number of summary components, while still retaining as much of the variance in the original variables as possible. Prior to PCA, the biomarkers were log-transformed (for approximate normality) and scaled to ensure biomarkers with larger intrinsic variation did not dominate the PCA. Unsupervised hierarchical clustering was then performed on the principal components (PCs), using Ward's minimum variance method and squared Euclidean distance as the distance measure, and determining the optimal number of clusters using the Silhouette method [35]. For PCA, we used a single imputation for absolute values of missing biomarker data (ranging from 0.5-20%) for patients with any biomarker values using the SVDImpute algorithm (which imputes based on eigenvectors, analogous to PCA) within the R impute package [36].

We used logistic regression to investigate predictors of CMV positivity adjusting for clinical risk factors, using multivariable fractional polynomials to account for non-linear effects of biomarkers. We then examined the association between changes in cfPWV (\log_{10} transformed for normality) over time and inflammatory clusters using linear mixed models with patient-specific random intercepts.

We adjusted the model for baseline cfPWV values, confounders (age and sex) and mediators (blood pressure and haemoglobin) as previously identified [3].

Analysis was undertaken using Stata 13.1 (Statacorp, College Station, USA) and R V3.5.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

In total 279 HIV-positive participants were enrolled. The median age was 36 years (IQR 31–43), nadir CD4 count 41 cells/ μ L (18–62) and HIV viral load 110,000 copies/mL (4000–290,000). The median(IQR) blood pressure was 120/73mmHg (108/68–128/80) and total cholesterol 3.6 mmol/L (3.0–4.4). 110 HIV-negative participants had comparable age and blood pressure (median[IQR] 35 years [31–41] and 128/75mmHg [114/68–134/82]). All but one HIV patient received first line ART of tenofovir-lamivudine-efavirenz, with the exception receiving zidovudine-lamivudine-nevirapine. 45 (16%) HIV-positive participants were diagnosed with an acute co-infection at study enrolment, including 30 with TB, 4 with cryptococcal meningitis and 4 with malaria. CMV PCR was positive for 61 (32%) of 193 tested HIV-positive and 1 (2%) of 59 HIV-negative participants at enrolment, with median(IQR) CMV viral load 928 copies/mL (412–3052) in those positive. CMV PCR became undetectable for all except one HIV-positive participant after 42 weeks of ART. The median(IQR) cfPWV for the HIV-positive participants was 7.3 m/s (6.5–8.2).

15 participants died. Of 14 PLWH who died, five were due to TB, three cryptococcal meningitis, one pneumonia, one Kaposi sarcoma, one gastroenteritis and three of unknown cause. One HIV-negative participant died from a cerebrovascular accident on a background of hypertension and obesity.

Inflammatory biomarkers

Inflammatory biomarker data was available for 211 (76%) HIV-positive and 62 (56%) HIV-negative participants. At enrolment, almost all markers were significantly elevated in those with HIV compared to those without, particularly anti-inflammatory cytokines (IL10 and IL1Ra: 2.9 and 1.9 fold higher); T-cell activation (IFN γ and IL7: 2.3 and 4.9 fold higher); and angiogenesis (bFGF and VEGF: 4.9 and 2.6 fold higher) (Supplementary Table 1). All inflammatory biomarkers except IL13 decreased in

HIV-positive participants during the following 42 weeks on ART. However levels of TNF α , IL7, MCP1, IP10, VEGF, bFGF, ICAM and CRP remained significantly elevated compared to HIV-negative patients at the final study visit (all $p < 0.001$, Supplementary Table 2).

Whilst many cytokines were univariably associated with CMV positivity, only associations with VCAM and MIP1 β , and baseline CD4, persisted in multivariable models after adjustment for age and sex ($p = 0.04$ and 0.01 respectively, Supplementary Table 3).

Amongst HIV-positive participants, individual biomarkers were not associated with cfPWV at enrolment on univariate analysis ($p > 0.05$), with the exception of IL13 (spearman rho -0.21 , $p = 0.01$, Supplementary Table 4).

Characterisation of immune phenotypes during ART initiation

Unsupervised hierarchical clustering of the 22 inflammatory biomarkers was performed for the 211 HIV-positive participants with any biomarker data. Three clusters were identified representing 51 (24%) (cluster-1), 153 (73%) (cluster-2) and 7 (3%) (cluster-3) participants (Figure 1). Figure 2 and Supplementary Table 5 compare the inflammatory biomarkers across clusters.

Although cluster-3 was small, participants had markedly increased pro-inflammatory cytokines compared to cluster-1 and cluster-2. Concentrations of IL6, CRP and IFN γ were between 10 and 50 fold higher (all $p < 0.0001$, Supplementary Table 5) and levels of serum amyloid was over 100 fold higher ($p = 0.0003$). For participants in cluster-1, CRP and serum amyloid were increased compared to cluster-2 (both $p = 0.02$) but to a lesser extent than the levels seen in cluster-3 (Supplementary Table 5).

Table 1 shows clinical characteristics and cell surface immune activation markers of patients in each cluster, and supplementary Table 6 shows the relevant characteristics for those without available biomarker data. There was no evidence of differences in age, blood pressure, diagnosis of acute co-infection or CMV positivity between clusters ($p = 0.77$, 0.47 , 0.72 and 0.90 , respectively). There was a trend towards lower nadir CD4 count in cluster-3 compared to cluster-2 [CD4 17 cells/cells/ μ L (11–

44) versus 41 (19–63), $p=0.08$], but no evidence of overall difference across the three groups ($p=0.44$).

Participants in cluster-1 were characterised by higher proportions of PD-1 expressing CD4 and CD8 T-cells compared to cluster-2 and cluster-3 [Table 1, CD4: median 69% versus 39% and 33%, respectively; CD8: 54% versus 33% and 42%, respectively, both $p<0.0001$] as well as a lower proportion of intermediate monocytes [7.7 versus 10.3 versus 12.8 respectively, $p=0.004$]. Lower proportions of activated CD4 and CD8 T-cells were found in cluster-2 [CD4: 68% versus 86% in cluster-1 and 69% in cluster-3; CD8: 72% versus 84% vs 83% respectively, $p<0.0001$]. However, all 14 deaths in HIV-positive participants were in cluster-2 ($p=0.097$).

Relationship between Immune phenotype at ART initiation and arterial stiffness

Median (IQR) cfPWV at baseline for clusters 1, 2 and 3 was 7.5m/s (7.4–7.6), 7.3m/s (7.2–7.4) and 6.6m/s (6.3–6.8) respectively ($p=0.04$). When adjusted for *a priori* identified confounders and mediators in multivariate analysis, baseline cfPWV was lower in cluster-2 and cluster-3 compared to cluster-1 [cluster-2: fold change -9% (95% CI -17% to 1%), $p=0.08$; cluster-3: fold change -17% (95% CI -53% to -15%), $p=0.002$, Table 2]. The effect of inflammatory cluster on baseline cfPWV was not attenuated when CMV positivity was added to this model [cluster-2: fold change -10% (95% CI -19% to 0%), $p=0.04$; cluster-3: fold change -37% (95% CI -56% to -11%), $p=0.008$].

Between week 2 and week 42, change in cfPWV for clusters 1, 2 and 3 was -0.5m/s (95% CI -1.0 to +0.1), -0.4m/s (95% CI -1.0 to +0.2) and +0.1m/s (95% CI -0.6 to +1.7) respectively (Figure 3).

Mixed effects models were constructed to assess the effect of inflammatory biomarker cluster on cfPWV slope over 42 weeks when adjusted for *a priori* identified confounders and mediators (Table 3). Compared with the rate of change in cluster-1, fold change for cluster-2 was 0.99 (95% CI 0.86 to 1.14, $p=0.91$) and for cluster-3 was 1.45 (95% CI 1.01 to 2.09, $p=0.045$).

Discussion

Patients living with HIV infection and presenting with advanced immune suppression can be classified according to distinct inflammatory phenotypes. We identified two inflammatory phenotypes: one characterised by T-cell activation and, in particular, high levels of PD-1 expression, and another smaller subgroup with markedly elevated inflammatory cytokines and a higher proportion of intermediate monocytes. These phenotypes tended to experience less favourable trends in arterial stiffness on ART compared to the third phenotype which had decreased T-cell activation and decreased cfPWV on ART.

Three pathways in particular were of interest in cluster-3. Biomarkers of angiogenesis, bFGF and VEGF have previously been linked to aberrant fibrosis, a possible mechanism for the development of non-communicable complications on ART [37]. High IFN γ and IL7 levels indicate a role for lymphocyte recruitment in this cluster. This is consistent with recent data showing production of IFN γ from cytotoxic T lymphocytes in HIV infection occurs due to delayed killing of resistant HIV-infected macrophages, which potentiate the inflammation cycle [38]. Anti-inflammatory cytokines were also upregulated in this cluster, with high levels of IL10 and IL1Ra. However, all deaths - which were predominantly due to infections - were in cluster-2 (the largest subgroup) where relatively lower levels of inflammation were seen. Taken together, we could hypothesise a survival benefit for those able to produce and regulate a more marked inflammatory response [39], with endothelial damage the cost in the longer term.

In contrast, cluster-1 was not defined by marked increases in inflammatory biomarkers, but was associated with higher proportions of activated T-cells, especially those expressing PD-1. PD-1 expressing T-cells have been previously shown to be independently associated with changes in cfPWV in this cohort [3]. Given that cell surface markers were not added to the cluster model (only the plasma biomarker data), this finding is interesting and the inflammatory biomarker signature warrants further investigation. We did not find any evidence that CMV viraemia explains inflammation related arterial stiffness in this cluster, although it was associated with plasma markers

of endothelial damage overall. However, our sample size was not large enough to detect a small effect of CMV on arterial stiffness.

Few studies to date have specifically dealt with inflammation in people with CD4 <100cells/ μ L who are commencing ART. As well as a low CD4 count predisposing to co-infections which may trigger inflammation, the inflammatory response itself might potentiate CD4 T-cell loss. Shive and colleagues previously demonstrated that pro-inflammatory cytokines driving CD4 T-cell cycling led to increased cell turnover and diminished responsiveness to IL7 [40]. As more patients presenting with low CD4 counts survive past the initial few months of ART, characterising inflammatory phenotypes at ART initiation may provide insights into the heterogeneity of chronic inflammation in the longer term [41, 42] and identify potential areas for intervention.

Previous studies from SSA have investigated the relationship between inflammation and vascular dysfunction. In a South African cohort, markers of endothelial damage including VCAM and ICAM were raised in people with HIV compared to negative controls and levels of VCAM were associated with CD4 count [43]. Siedner and colleagues demonstrated that changes in sCD14 and IL6 during the first 6 months of ART predicted a lower carotid intima thickness measurements after 7 years of ART [44]. Decreases in CRP and TNF α correlated with improvements in vascular function in a small cohort of malnourished patients in Southern Africa. Our data adds to current knowledge, suggesting that inflammatory phenotype at ART initiation may determine different trajectories of vascular function over time.

Sherzer and colleagues investigated cross sectional biomarker clustering for patients with HIV on ART in USA, in the context of cardiac failure [45]. Using a panel of 8 inflammatory and heart failure markers, they identified three clusters, one of which was also a pro-inflammatory phenotype and was associated with higher mortality. Banchereau and colleagues used inflammatory biomarkers and transcriptomics to stratify SLE phenotypes according to underlying mechanistic pathways and identify clinically applicable biomarkers and treatments for each group [26].

Study strengths include plasma biomarker data in a large, well clinically characterised cohort of patients with HIV infection, recruited close to ART initiation. Previous studies have concentrated on cross-sectional analysis; here we assessed changes in plasma biomarkers, cell surface markers and arterial stiffness during the first 42 weeks of ART. In addition, we also included a HIV-negative cohort for comparison at both time points.

Limitations include the fact that the number of participants in cluster-3 was very small, restricting inference about their characteristics. However, these patients may represent those at highest risk of inflammation driven complications of HIV and those most likely to benefit from targeted interventions. Although we did not find any differences that suggest this group of patients were experiencing greater rates of acute co-infection or sepsis, this remains a possibility given the low numbers. However, cfPWV remained lower in this group than the rest of the cohort even at week 10, making it unlikely that sepsis alone was an explanation for the lower arterial stiffness observed at enrolment.

Our cohort had advanced immune suppression with CD4 <100 cells/ μ L at ART initiation, restricting generalisability to the HIV population as a whole. Indeed inflammatory phenotypes may differ depending on HIV stage, as well as treatment status, and further work will be needed for those with higher CD4 counts and those established on ART. Although arterial stiffness represents one mechanism through which clinical cardiovascular disease may occur, large clinically validated studies will also be required in the future.

Reproducible inflammatory phenotypes could inform clinical trials seeking to reverse chronic inflammation. To date, trials in this area of interventions such as statins, valganciclovir, dietary supplements, metformin and anti-inflammatory immunomodulatory agents have shown only modest effects [46-50]. One possibility is that the effects of interventions may have been limited by the heterogeneity of the patient populations, and would be enhanced when targeted towards a particular inflammatory phenotype.

This study identifies potential clinically relevant inflammatory phenotypes in people living with HIV. In addition, we present novel data on the possible association between inflammatory phenotypes and change in vascular dysfunction on ART. Further elucidation of the clinical implications as well as drivers of inflammatory phenotypes might help identify therapeutic targets to modulate inflammation driven cardiovascular disease in people living with HIV.

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Tables

Table 1. Clinical characteristics of Inflammatory clusters

| | | Cluster 1 (n=51) | | Cluster 2 (n=153) | | Cluster 3 (n=7) | | p value |
|--------------------------|------------------------------------|---------------------|------------------|---------------------|------------------|---------------------|------------------|---------|
| | | Median or frequency | IQR or % | Median or frequency | IQR or % | Median or frequency | IQR or % | |
| Clinical characteristics | Age (years) | 38 | 31 – 44 | 36 | 31 – 43 | 40 | 31 - 43 | 0.77 |
| | Systolic BP (mmHg) | 124 | 109 – 132 | 119 | 108 – 128 | 128 | 106 – 129 | 0.47 |
| | Diastolic BP | 73 | 68 – 78 | 72 | 68 – 80 | 78 | 72 – 89 | 0.26 |
| | Heart rate (bpm) | 80 | 72 – 99 | 82 | 72 – 98 | 74 | 64 – 87 | 0.34 |
| | Temperature (°C) | 36.4 | 36.0 – 36.8 | 36.4 | 36.0 – 36.9 | 36.5 | 36.0 – 36.9 | 0.88 |
| | Haemoglobin (g/dL) | 11.5 | 10.1 – 12.6 | 11.4 | 9.9 – 13.0 | 12.7 | 11.9 – 13.0 | 0.51 |
| | Creatinine (µmol/L) | 62 | 52 – 72 | 66 | 56 – 79 | 60 | 51 – 65 | 0.029 |
| | Platelets (x10 ⁹ /L) | 215 | 172 – 325 | 240 | 189 – 332 | 216 | 144 – 435 | 0.34 |
| | Nadir CD4 (cells/mm ³) | 42 | 19 – 65 | 41 | 19 – 62 | 17 | 11 – 44 | 0.44 |
| | HIV viral load (copies/mL) | 117,000 | 46,000 – 249,000 | 111,000 | 39,000 – 295,000 | 210,000 | 86,000 – 601,000 | 0.51 |

| | | | | | | | | |
|--|------------------------------------|-----|------------|------|------------|------|------------|---------|
| | Acute co-infection | 11 | 22% | 45 | 29% | 1 | 14% | 0.72 |
| | CMV PCR positive | 16 | 33% | 43 | 28% | 2 | 40% | 0.90 |
| | Death | 0 | 0% | 14 | 9% | 0 | 0% | 0.097 |
| Arterial stiffness | Week 2 cfPWV (m/s) | 7.5 | 7.4 – 7.6 | 7.3 | 7.2 – 7.4 | 6.6 | 6.3 – 6.8 | 0.041 |
| | Week 12 PWV (m/s) | 7.4 | 6.9 – 7.6 | 7.3 | 6.5 – 7.6 | 6.9 | 6.5 – 7.5 | 0.30 |
| | Week 24 cfPWV (m/s) | 7.3 | 6.5 – 7.5 | 7.1 | 6.5 - 7.4 | 6.8 | 6.3 - 7.5 | 0.76 |
| | Week 44 cfPWV (m/s) | 7.4 | 7.0 – 7.9 | 7.1 | 7.0 – 7.9 | 7.5 | 6.6 – 8.1 | 0.75 |
| Immune cell surface phenotype | %CD4 Activation | 86 | 76 – 90 | 68 | 54 – 76 | 69 | 50 – 96 | <0.0001 |
| | %CD4 Exhaustion | 69 | 63 – 76 | 39 | 25 – 52 | 33 | 17 – 49 | <0.0001 |
| | %CD4 Senescence | 18 | 9 – 29 | 14 | 8– 22 | 17 | 17 – 18 | 0.42 |
| | %CD8 Activation | 84 | 76– 90 | 72 | 59 – 86 | 83 | 65 – 96 | <0.0001 |
| | %CD8 Exhaustion | 54 | 44 – 61 | 33 | 23 – 42 | 42 | 37 – 48 | <0.0001 |
| | %CD8 Senescence | 51 | 43– 59 | 54 | 44 – 66 | 54 | 51 – 63 | 0.45 |
| | %Intermediate Monocytes | 7.7 | 4.6 – 11.9 | 10.3 | 6.7 – 13.8 | 12.8 | 9.8 – 14.9 | 0.004 |

Table 2. Linear regression model showing the adjusted association between inflammatory cluster and arterial stiffness at 2 weeks post ART initiation

| | Fold change in cfPWV at baseline (m/s) | 95% Confidence Intervals | | P value |
|---|--|--------------------------|------|---------|
| | | | | |
| Cluster 2* | 0.91 | 0.83 | 1.01 | 0.08 |
| Cluster 3* | 0.63 | 0.47 | 0.85 | 0.002 |
| Age (years) (per 10 years older) | 1.18 | 1.12 | 1.23 | <0.0001 |
| Female sex | 1.00 | 0.91 | 1.10 | 0.96 |
| Systolic BP (mmHg) (per 10 mmHg higher) | 1.03 | 1.00 | 1.06 | 0.07 |
| Diastolic BP (mmHg) (per 10 mmHg higher) | 1.09 | 1.03 | 1.15 | 0.002 |
| Haemoglobin (g/dL) (per g/dL higher) | 1.01 | 0.99 | 1.04 | 0.23 |

*In comparison to cluster 1. cfPWV: carotid femoral Pulse Wave Velocity

Table 3. Mixed model analysis showing the effect of inflammatory biomarker cluster on cfPWV slope over 42 weeks post ART initiation

| | Fold change in cfPWV | 95% Confidence Interval | | p value |
|--|-----------------------------|--------------------------------|------|----------------|
| Average effect of Time on cfPWV at 42 weeks post ART | 0.94 | 0.90 | 0.98 | 0.007 |
| Average effect of Cluster on cfPWV at 42 weeks post ART | | | | |
| Cluster 2 versus Cluster 1 | 0.95 | 0.85 | 1.05 | 0.30 |
| Cluster 3 versus Cluster 1 | 0.89 | 0.69 | 1.14 | 0.35 |
| Adjusted effect of cluster on rate of change in cfPWV over 42 weeks ART^a | | | | |
| Cluster 2 versus Cluster 1 | 0.99 | 0.86 | 1.14 | 0.91 |
| Cluster 3 versus Cluster 1 | 1.45 | 1.01 | 2.09 | 0.045 |
| Enrolment Age (per 10 year higher) | 1.23 | 1.18 | 1.27 | <0.0001 |
| Enrolment Diastolic BP (per 10 mmHg higher) | 1.10 | 1.06 | 1.15 | <0.0001 |
| Female sex (vs male) | 0.97 | 0.90 | 1.04 | 0.36 |
| Enrolment Haemoglobin (g/dL) (per g/dL higher) | 1.01 | 1.00 | 1.03 | 0.11 |

Note: Adjusted for baseline cfPWV. ^aModel showing effect of interaction between cluster and time on cfPWV over 42 weeks post ART initiation, adjusted for average effect of age, blood pressure, sex and haemoglobin.

Figure legends

Figure 1. Identification of 3 distinct inflammatory biomarker clusters for 211 patients with HIV infection

Note: clusters plotted against the first two principal components (percentage total variation explained)

Figure 2. Heat map of 22 inflammatory biomarkers according to inflammatory cluster

Figure 3. Change in cfPWV over 42 weeks of ART according to inflammatory cluster

Figure 1

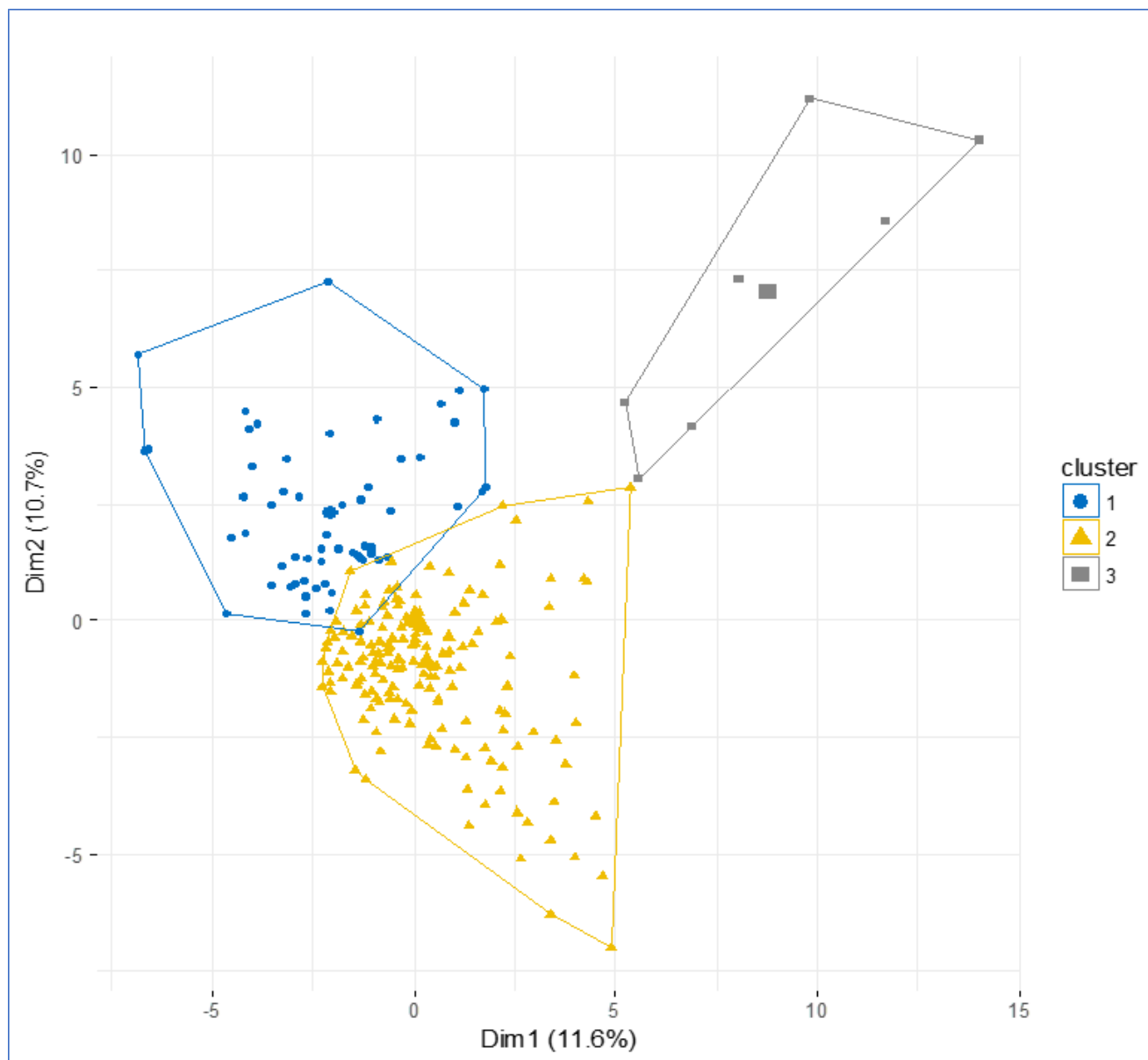


Figure 2

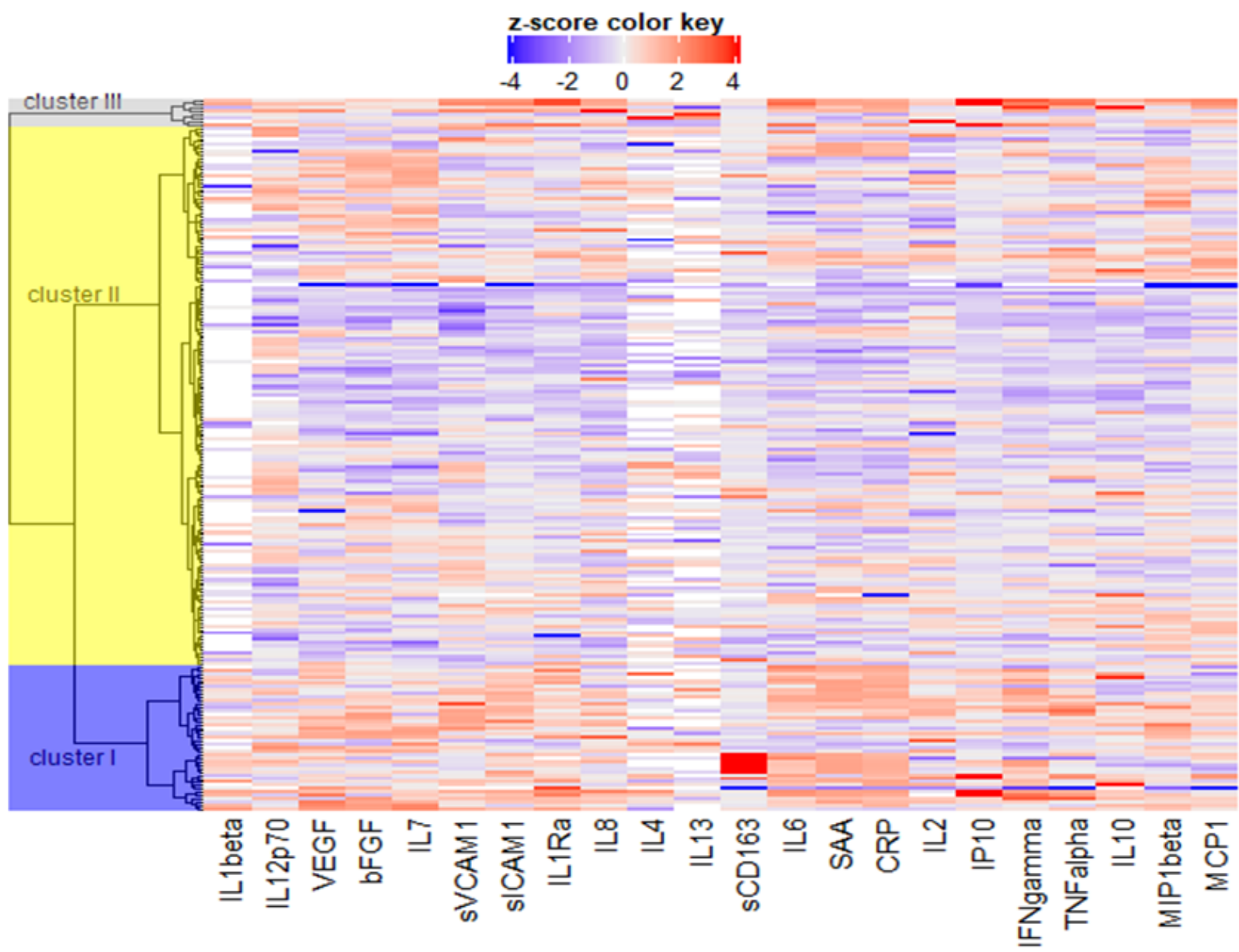


Figure 3

