Phenotypic characterisation of bone marrow-derived haematopoietic stem/progenitor cells from HIV-infected individuals

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Author contributions

CD and PM contributed to the study conception and design. Experimental work, data collection and analysis were performed by PM and CD. Clinical data and sample collection were performed by JJCP. MSP provided supervision, and reviewed and edited the manuscript. CD and MSP were responsible for fundraising. The first draft of the manuscript was written by PM; all authors contributed to subsequent drafts of the manuscript and approved the final version.

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Statements and Declarations:

The authors declare no conflicts of interest nor competing interests.

Dear Editor,

Human immunodeficiency virus (HIV-1) infection remains a significant global public health concern, particularly in sub-Saharan Africa where the majority of HIV-1 infections are concentrated [1]. People living with HIV (PLWH) often present with haematological abnormalities including alterations of the bone marrow (BM; dysplasia and cellularity changes) and most commonly cytopenias (anaemia, thrombocytopenia and neutropenia) [2]. From these clinical observations it is clear that HIV not only impacts the immune system but also the broader haematopoietic system. The underlying cause of HIV-mediated changes in the haematological system remains complex and multifactorial.

In the bone marrow (BM), haematopoietic stem cells (HSCs) are at the apex of the haematopoietic differentiation cascade and differentiate into several more committed progenitor sub-populations to give rise to a heterogenous population of haematopoietic stem and progenitor cells (HSPCs) [2]. The various HSPC sub-populations express different combinations of cell surface makers that allow for their identification using multiparametric flow cytometry. The impact of HIV-1 infection on the sub-population distribution of HSPCs in the BM, and its influence on haematopoietic reconstitution dynamics, are poorly investigated and remains unclear. We investigated HSPC sub-population distribution in the BM of HIV-1 infected and HIV-1 negative individuals.

BM aspirates from 10 HIV-1 negative and 15 HIV-1 infected individuals with varying CD4 count, viral load and antiretroviral (ARV) therapy status sent to the National Health Laboratory Service (NHLS), Tshwane Academic Division (TAD), for routine diagnostic testing, were used in this study. Malignant disease was ruled out before the BM aspirates were used for the study. Mononuclear cells were isolated by density gradient centrifugation. A total of 1 x 10^6 CD45+ BM-derived mononuclear cells were stained with the following panel of monoclonal antibodies: human lineage (Lin) cocktail-FITC (Biolegend, San Diego, USA), CD10-BV650 (clone: HI10a; BD Bioscience, San Jose, USA), CD34-APC-AF700 (clone: 581; Beckman Coulter, Miami, USA), CD38-ECD (clone: LS198-4-3; Beckman Coulter, Miami, USA), CD45RA-APC (clone: 2H4; Beckman Coulter, Miami, USA), CD49f-SB780 (clone: GoH3; eBioscience, San Diego, USA), CD90-BV510 (clone: 5E10; Biolegend, San Diego, USA), CD117-PE (clone: 104D2; Biolegend, San Diego, USA) and CD133/1-PE-Violet 770 (clone: REA753; Miltenyi Biotec, Bergisch Gladbach, Germany). Zombie Violet™ Fixable viability dye (Biolegend, San Diego, USA) was used to exclude dead cells. Cell suspensions were acquired on a CytoFLEX flow cytometer (Beckman Coulter, Miami, USA). Post-acquisition flow cytometric data analysis was performed using Kaluza Analysis software (version 2; Beckman Coulter, Miami, USA) (Figure 1a-c). All statistical analyses and graphs were generated using the GraphPad Prism software package (version 9.3.1; GraphPad Software Inc., San Diego, USA). Statistical significance between the HIV-positive and negative groups was tested by two-way ANOVA multiple comparisons test with p-values ≤ 0.05 considered to be statistically significant.

All aspirates had a cell viability of >85% (94.31 ± 4.52%). BM aspirates from HIV-infected patients had a significantly ($p<0.0001$) lower proportion of CD34+/CD38+/CD117-/CD133-/CD45RA+/CD10+/CD49f-/CD90- defined lymphoid progenitors compared to aspirates from HIV-negative patients (Figure 1d). Based on the expression of CD38, these cells are likely to be committed progenitors. Galy *et al* [3] demonstrated that Lin-/CD34+/CD45RA+/CD10+ progenitors, in particular those that express CD38 and HLA-DR but do not express significant levels of Thy-1 (CD90) nor c-kit (CD117), exhibit B, T, natural killer and dendritic cell differentiation potential. Based on their report, the CD34+/CD38+/CD117-/CD133-/CD45RA+/CD10+/CD49f-/CD90- sub-population in our study was defined as lymphoid progenitors. Ichii *et al* [4] suggested that CD10 expression is associated with B-cell differentiation potential with minimal T and natural killer cell potential. HIV-infected patients known to be receiving ARV treatment were observed to have a higher proportion of these lymphoid progenitors as they were above the mean value of the group (Figure 1d). However, the ARV treatment status of some of the HIV infected individuals was not indicated in the clinical reports, and without complete clinical records from all the patients in the HIV-infected group, a reliable interpretation regarding the impact of ARV treatment on the frequency of lymphoid progenitors in HIV-patients cannot be made. In contrast, BM aspirates from HIV-infected patients had a significantly ($p<0.0001$) higher proportion of CD34+/CD38+/CD117+/CD133-/CD45RA-/CD10-/CD49f-/CD90- cells, defined as myeloid progenitors, based on the expression of CD117 [5], when compared to aspirates from HIV-negative patients (Figure 1d). These results collectively suggest that the HSPC sub-population distribution in the BM of HIV-positive patients is myeloid-biased.

HIV infection is associated with accelerated epigenetic aging [6]. Notably, HIV-infection mirrors haematological aging with overlapping manifestations such as immune dysfunction and sustained immune inflammation and activation [7]. Similar to our observations in HIV-infected individuals, age-associated haematological changes are associated with a CD34+ HSPC bias toward myeloid differentiation with fewer lymphoid progenitors in the BM [8]. Participants in our study had a median age of 34 years (range 27–62). The haematological changes observed are thus unlikely to be age-related. The alterations observed in the HSPC pool in the BM of HIV-infected individuals in this study support the concept of HIV-induced haematopoietic aging.

HIV-1 infection also exhibits BM dysplastic features resembling myelodysplastic syndromes (MDS) [9]. A case study reported an elevated CD34+/CD117+/CD33+ myeloblast population in an HIV-infected individual with myelodysplasia [10]. The increase in myeloid progenitors observed in our study may reflect such MDS-like features in HIV-infected individuals, potentially driven by opportunistic infections, HIV-related medications, or direct effects of HIV on HSPCs.

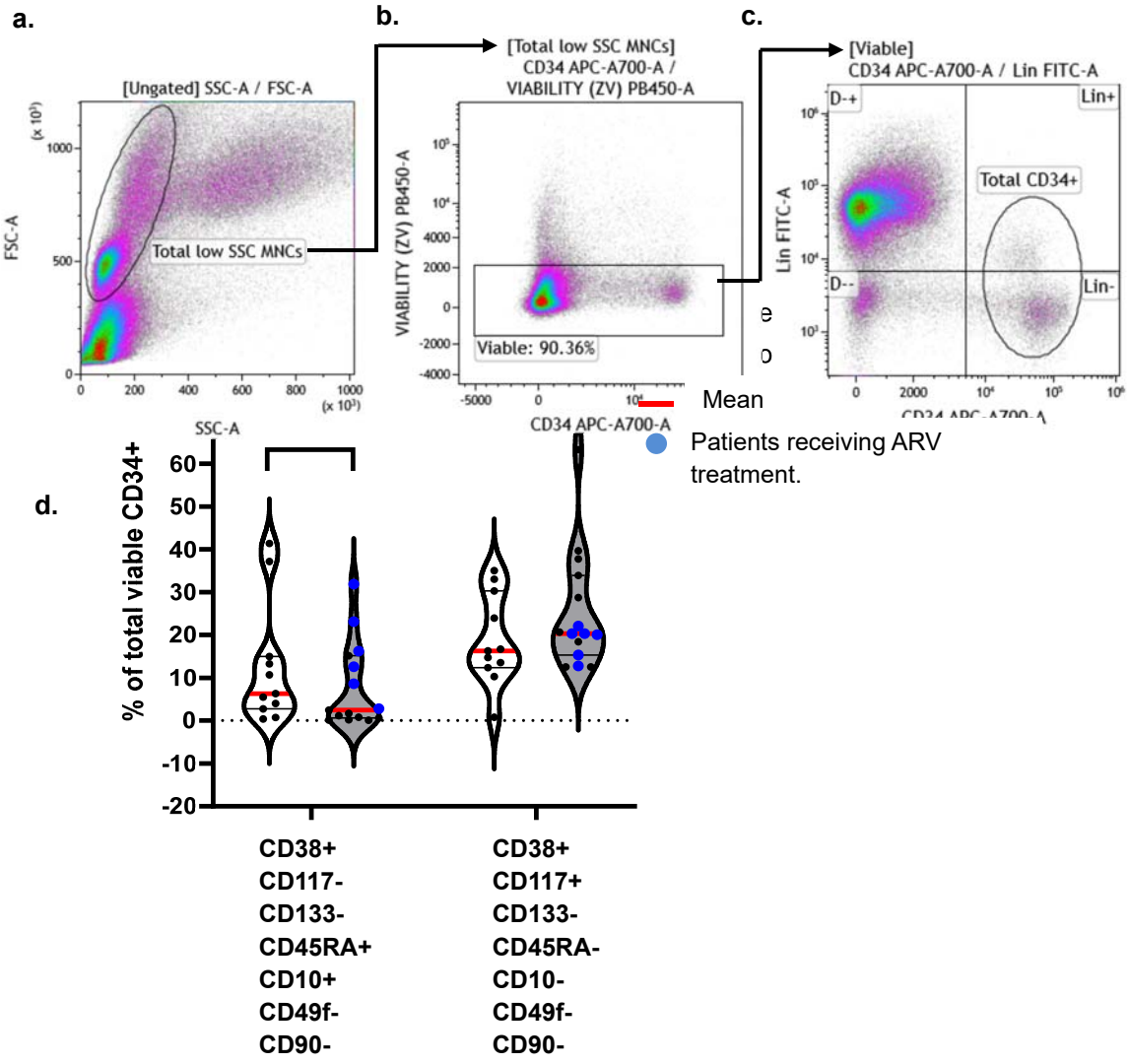

Fig. 1: Representative gating strategy and frequency of two HSPC sub-populations in the BM of HIV-infected and negative group. (a) A forward scatter (FSC) Logicle versus side scatter (SSC) Linear two-parameter density plot was used to select for total low SSC (channel 0-400) mononuclear cells (MNCs) as indicated by the "Total low SSC MNCs" region. (b) A Zombie Violet versus CD34-APC-A700 two-parameter density plot gated on "Total low SSC MNCs" was used to select for total viable cells that stain negative for Zombie Violet™ Fixable viability dye as indicated by the "Viable" region. (c) A Lin-FITC versus CD34-APC-A700 two-parameter density plot gated on "Viable" cells was used to select for total viable $CD34^{+}$ HSPCs of interest as indicated by the "Total CD34+" region. Quadrants indicate the negative/positive boundary (horizontal line) for Lin expression. (d) Violin plot displaying all data points with median value represented by red midline and HIV-infected patients receiving ARV treatment represented by blue dots. Statistical significance between HIV-positive and negative group was tested by two-way ANOVA multiple comparisons with ****p<0.0001

HIV-1 impairs haematopoiesis indirectly as a consequence of elevated levels of proinflammatory cytokines (IL-1, IL-6, TNF-α), some of which promote myeloid differentiation while inhibiting lymphopoiesis [11]. The HSPC differentiation imbalances observed could thus be the result in part of HIV-mediated changes in the cytokine milieu in the BM, warranting further studies on BM cytokine profiles in HIV-positive patients. Additionally, HIV-1 gp120-induced apoptosis of osteoblasts [12], that are essential for early lymphoid progenitor proliferation [13], may reduce the number of lymphoid progenitors in the BM of infected patients. HIV-1 can also directly interact with HSPCs, depleting them either through Fas-mediated apoptosis or via direct infection [2]. Further research is however needed to clarify the mechanisms involved and their effects on lymphoid progenitors.

This study has several limitations. The study relied on BM aspirates collected for diagnostic purposes rather than via prospective patient recruitment. Consequently, sample sizes between the two groups were not controlled and were unequal. Furthermore, incomplete clinical records limited access to key information such as CD4 counts, viral load, and ARV treatment status for some HIV-1 positive patients.

In conclusion, this study highlights the significant impact that HIV-1 has on haematopoiesis, particularly through its effects on HSPCs in the BM, which mirror aspects of haematological aging. The reduced number of lymphoid progenitors seen in HIV-infected individuals may contribute to impaired T-cell production, thereby exacerbating immune deficiency. While antiretroviral therapy can partially restore lymphopoiesis and CD4+ T-cell counts, some individuals experience limited immune reconstitution despite viral suppression. It is thus possible that extensive damage to HSPCs and lymphoid progenitors may hinder full immune recovery, thereby emphasizing the critical role that HSPCs play in the restoration and maintenance of immune function in HIV-1 infection.

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Declarations

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Conflicts of Interest/ Competing interests

The authors declare no conflicts of interest nor competing interests.

Ethics approval

Ethical clearance was received from the Research Ethics Committee (REC), Faulty of Health Sciences, University of Pretoria (Ethics ref no: 396/2021) to obtain BM aspirates as well as to conduct this project.

Consent to participate

A waiver was granted by the REC to obtain informed consent from patients from whom these samples were aspirated as only leftover sample intended to be discarded as biological waste following diagnostic tests were used for research purposes.

Consent for publication

All authors have read and approved this manuscript for publication.

Availability of data and material

Data is available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Author contributions

CD and PM contributed to the study conception and design. Experimental work, data collection and analysis were performed by PM and CD. Clinical data and sample collection were performed by JJCP. MSP provided supervision, and reviewed and edited the manuscript. CD and MSP were responsible for fundraising. The first draft of the manuscript was written by PM; all authors contributed to subsequent drafts of the manuscript and approved the final version.

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