Cellular Senescence Impacts Both the Innate and Adaptive Immune System in Frail Men with HIV.

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BACKGROUND

- Antiretroviral therapy (ART) has greatly improved the life expectancy of people living with HIV (PLWH), but comorbidity-free survival remains lower than those without HIV.
- Frailty is a syndrome that describes a decline in physical and cognitive health and is associated with a poorer quality of life and increased mortality.
- The cellular mechanisms driving frailty pathogenesis remain to be fully elucidated in HIV.

The aim of this study was to examine the relationship between cellular senescence and frailty in men with HIV.

METHODS

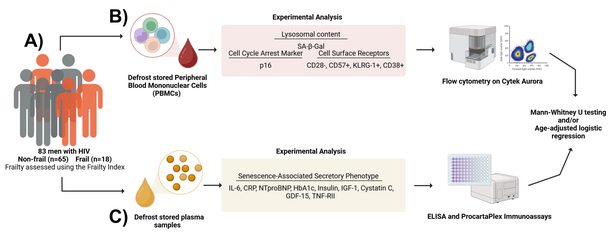


Figure 1. Schematic diagram of experimental methodology
Eighty-three men with HIV aged ≥50 years recruited from The Alfred Hospital (Melbourne, Australia) in 2015 were classified as non-frail (n=65) or frail (n=18) based on the Frailty Index (A). Flow cytometry was performed on stored Peripheral Blood Mononuclear Cells (PBMCs) using 16-colour panel identifying lymphocytes (natural killer cells, B cells, CD4+ & CD8+ T cells) and monocytes (classical, intermediate & non-classical), and assessing cell cycle arrest (p16), functional phenotype (CD28-, CD57+, KLRG-1+, CD38+) and senescence-associated β-galactosidase (SA-β-gal) activity (B). Soluble marker analysis was performed on stored plasma samples using Luminex and ELISA assays (C). Univariate analysis was performed using Mann-Whitney U testing and age-adjusted analysis was performed using logistic regression.

RESULTS

1. Clinical characteristics of the cohort.

	Total (n=83)	Non-frail (n=65)	Frail (n=18)	p value
Age, median (IQR)	60 (56-65)	59 (56-62)	62 (58-71)	**0.042**
Cigarette smoker[1], n (%)	49 (64)	36 (60)	13 (77)	0.213
Current CD4 + cell count	603 (352-799)	650 (361-788)	472 (312-801)	0.445
Nadir CD4 + cell count	157 (39-266)	168 (39-277)	112 (52-243)	0.505
Detectable HIV-viral load, n (%)	6 (7.2%)	5 (7.7%)	1 (5.5%)	1.000
Time since diagnosis of HIV[2]	245 (126-305)	241 (124-299)	259 (156-314)	0.761
Duration of ART[2]	198 (90-250)	190 (86-247)	206 (121-269)	0.426

[1]Cigarette smoker includes past and/or present smoking and data missing for n=6 participants (n=5 non-frail, n=1 frail).
[2]Shown as months.
All continuous variables are displayed as median (IQR). Mann-Whitney U testing was used to detect differences between continuous variables and Chi-Square testing was used to detect differences between categorical variables. Significant p values are in bold.

Frailty in HIV is associated with *enhanced* cellular senescence that broadly impacts the innate and adaptive immune system and potentially represents a novel target to improve healthy ageing in PLWH.

RESULTS CONT.

2. Cellular senescence is elevated in frail men with HIV.

- Median levels of the senescence marker SA-β-gal were between 1.4- and 1.6-fold higher across all adaptive (B cells & T cells) and innate (Monocytes & NK cells) immune cell populations in frail versus non-frail men (all p<0.05; Fig. 2B).
- Expression of the cell cycle arrest marker p16 was significantly elevated in frail men for all cell populations except classical monocytes (all p<0.05; Fig. 2C).
- In age-adjusted logistic regression, SA-β-gal and p16 cellular senescence remained significantly higher in all lymphocyte populations (p<0.05) and some monocyte populations (not shown).
- Frail men exhibited a higher median frequency of senescent CD8+/CD28- T cells (non-frail 48.7% [38.6-61.7] versus frail 59.8% [45.9-74.7], p=0.041; Fig. 2D).
- No significant differences were observed in CD4+ T cell senescence surface markers in frail men (Fig. 2E).

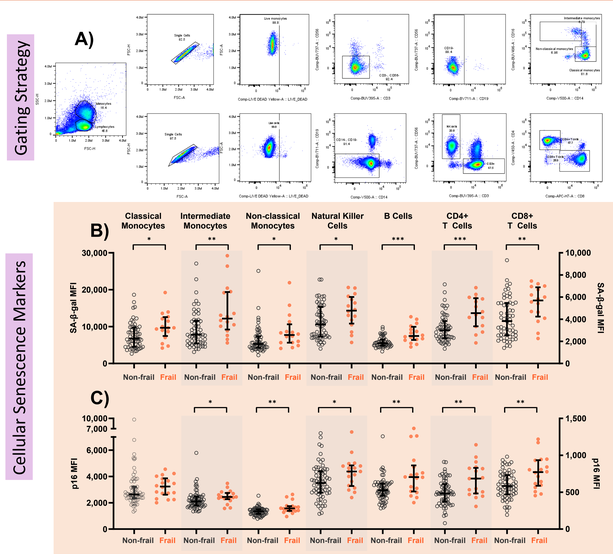


Figure 2. Cellular senescence in PBMCs from men with HIV.
Gating strategy for defining cellular lineages (A). SA-β-gal (B) and p16 (C) median fluorescence intensity (MFI) levels in cellular populations of non-frail (gray symbols) and frail (orange symbols) men with HIV. Monocytes use the left y-axis, NK, B and T cells use the right y-axis. The proportion of CD8+ (D) and CD4+ (E) T cells positive for the indicated surface markers. Graphs show median (IQR). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney U testing.

3. A soluble Senescence-Associated Secretory Phenotype (SASP) is elevated in frail men with HIV.

- Of the nine plasma soluble markers measured, five (IL-6, CRP, NTproBNP, HbA1c & Insulin) were significantly elevated in frail versus non-frail men (all p<0.05; Fig 3).
- Except for Insulin, all significantly elevated soluble markers were positively correlated, with the strongest relationship observed between IL-6 and CRP (rho 0.665, p<0.0001; not shown).

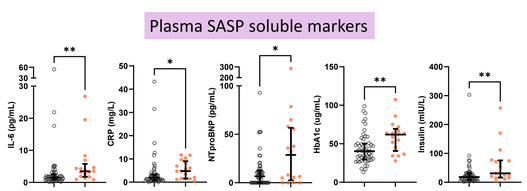


Figure 3. Plasma Soluble SASP levels in men with HIV.
The concentration of soluble SASP levels in plasma of non-frail (gray symbols) and frail (orange symbols) men with HIV (n=71). Graphs show median (IQR). *p<0.05, **p<0.01 by Mann-Whitney U testing. Abbreviations: IL-6, interleukin-6; CRP, c-reactive protein; NTproBNP, N-terminal pro-brain natriuretic peptide; HbA1C, Hemoglobin A1c.

RESULTS CONT.

4. IL-6 is correlated with all cellular senescence markers measured.

- When assessing the relationship between cellular and soluble senescence markers, IL-6 was positively correlated with all cellular senescence markers on all measured cell types (rho range: 0.238-0.404, all p<0.05, Fig. 4A&B).

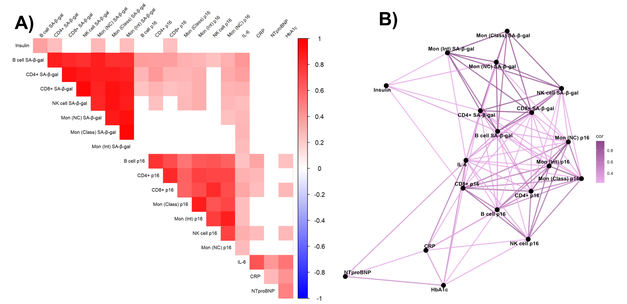


Figure 4. Correlations between soluble SASP and cellular senescence markers.
Spearman's Rank correlation coefficients between soluble SASP factors and cellular senescence makers are displayed as a heatmap (A) and a network plot (B). For the heatmap, the color ranges from blue (rho= -1) to red (rho=1), with white indicating insignificant associations. For the network plot, only significant correlations are shown, with the color ranging from light purple (rho=0.3) to dark purple (rho=0.9). The plots were generated using R version 2024.12.1 and the corrplot package. Abbreviations: SA-β-gal, senescence-associated β-galactosidase; Mon, monocyte; Class, classical; NC, non-classical; Int, intermediate.

CONCLUSION

We have shown that frailty in men with HIV is associated with chronic inflammation and enhanced immune cell senescence that broadly impacts the innate and adaptive immune system, potentially indicating heightened immune ageing. These cellular changes, combined with increased systemic inflammation, may contribute to frailty pathogenesis and represent a potential therapeutic target to improve healthy ageing in PLWH.

PLAIN LANGUAGE SUMMARY

Increased immune ageing is associated with frailty in men with HIV. Chronic inflammation may drive this increased immune aging and represents a therapeutic target for improving the quality of life of PWLH.

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