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# Cytokines assets in PLWH in two-drug dolutegravir based or three-drug antiretroviral regimen

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## Abstract

To minimize the toxicity and impact of combined antiretroviral therapy (cART) on the lifestyle of people living with Human Immunodeficiency Virus (PLWH), scientific community evaluated the efficacy, safety and sustained virologic response of two drugs antiretroviral regimens, in particular dolutegravir (DTG). The effects of deintensification therapy on inflammatory settings are currently unknown in PLWH. Thus, our study explored the inflammatory state in virologically suppressed HIV individuals between patients in treatment with a DTG-containing dual therapy (2DR) versus triple regimen therapies (3DR). We enrolled a total of 116 subjects in 2DRs or 3DRs regimens, and the plasma levels of pro- and anti-inflammatory cytokines (in particular IL-1 $\beta$ , IL-10, IL-18, IL-33, IL-36 and IFN- $\gamma$ ) have been evaluated. CD4+ cell's median value was 729.0 cell/ $\mu$ L in the 3DR group and 771.5 cell/ $\mu$ L in 2DR group; the viral load was negative in all patients. Significant differences were found in levels of IL-18 (648.8 cell/ $\mu$ L in 3DR group vs. 475.0 cell/ $\mu$ L in 2DR group,  $p=0.034$ ) and IL-36 (281.7 cell/ $\mu$ L in 3DR group vs. 247.0 cell/ $\mu$ L in 2DR group,  $p=0.050$ ), and a correlation between IL-18 and IL-36 was found in 3DR group ( $\rho=0.266$ ,  $p=0.015$ ). This single-center retrospective pharmacological study confirms the absence of significant differences in IL-1 $\beta$ , IL-10, IL-33, and IFN- $\gamma$  levels between patients on two-drug antiretroviral regimens compared to patients on 3DR antiretroviral regimens. Patients in 2DR show greater control over IL-18 and IL-36 serum levels, cytokines related to an increased cardiovascular risk and development of age-related chronic diseases. Based on our results, we suggest that DTG-based 2DR antiretroviral regimens could be associated with better control of the chronic inflammation that characterizes the population living with HIV in effective ART.

**Keywords** HIV, DTG, 2DRs, 3DR, Inflammation

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## Introduction

The introduction of combined antiretroviral therapy (cART) significantly decreased the HIV-related morbidity and mortality among people living with HIV, and their life expectancy gradually became comparable to the general population [1]. With the increase in life expectancy among PLWH, the number of people over 50 years of age increases [2] which leads to an increase in age-related comorbidities. [3]. A plethora of mechanisms contribute to the pathogenesis of non-HIV-related diseases in HIV patients [3], and inflammation plays a central role [4]. Despite cART related viral suppression, inflammatory activation persists [5] inducing a proinflammatory and procoagulant condition [6–8], resulting in increased morbidity and mortality. The Strategies for Management of Antiretroviral Therapy (SMART) trial demonstrated the association between IL-6, D-dimer and persistent immune system activation underlying the onset of non-AIDS-related morbidity and mortality in PLWH receiving effective treatment [9]. Although the mechanisms underlying this phenomena are not yet fully known, the HIV reservoirs [10], microbial translocation [11], antiretroviral therapy, [12] were considered. To minimize the impact of cART, not only on PLWHs' lifestyle but also on a drug toxicity, the efficacy, safety and virologic performance (SVR) of antiretroviral dolutegravir (DTG) based regimens containing only two drugs [13] have been evaluated and appear to be a good therapeutic option [14, 15]. Proinflammatory cytokines of IL-1 superfamily, including IL-1 $\beta$ , IL-18, IL-33 and IL-36 showed several structural similarities and common signaling pathway which resulted in the activation of NF- $\kappa$ B and MAPK and then promoting the transcription of several inflammatory genes. The immunosuppressive cytokine IL-10, notoriously, was correlated with viral load and was reduced after successful antiretroviral therapy. Since the effects of therapeutic deintensification on inflammatory status, currently, are not well known in HIV. In this study, we compared the inflammatory state in virologically suppressed PLWH in DTG-based dual therapy (2DR) and patients with different triple therapy (3DR).

## Materials and methods

### Study design and patients

This was a cross sectional study conducted on outpatients who were being followed up at the Infectious Diseases Clinic, University "Gabriele d'Annunzio" SS. Annunziata in Chieti between 01 January 2022 and 01 March 2022.

We included patients who met all the following criteria: age > 18 years, HIV-1 infection for at least 60 months under stable (without any pharmacological therapy change over the last 48 weeks) and effective (HIV RNA undetectable in last 2 blood samples) cART.

The exclusion criteria were: any acute infection at enrollment or in last three months, non-steroidal anti-inflammatory drug (NSAID) therapy in the last seven days before blood sampling, concomitant therapy with corticosteroids, current pregnancy, cardiac ischemia or stroke in last 6 months.

### Plasma samples

To avoid a day-to-day variability in cytokine release determined by eating and physical activity, venous blood samples were collected using venous blood drawn from the antecubital vein, in the morning between 08:00 and 9.00 h. after overnight fasting, and the following biomarkers were measured: blood count with leukocyte formula, C-reactive protein (CRP), creatinine, blood urea nitrogen (BUN), eGFR, aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transpeptidase ( $\gamma$ GT), alkaline phosphatase, dehydrogenated lactate (LDH), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, cystatin-C, sodium, potassium, uric acid, 25-OH-vitamin D; plasma cystatin C was determined using the BN II system with the nephelometric technique. (BN II System - Siemens Healthcare Diagnostic, Inc).

CD4+ and CD8+ T cell counts were obtained by flow cytometry of lymphocyte subpopulations. Plasma viral load (HIV-RNA) was determined using the "Amplicor" method (Roche Molecular Diagnostics, Milan, Italy) with a detection limit of > 27 HIV RNA copies/mL of plasma.

All laboratory tests were performed at the Clinical Pathology of the University 'G. D'Annunzio' SS Annunziata Hospital of Chieti.

### Cytokines levels

The surplus of blood contained in plasma tubes was centrifuged at 4000 rpm for 5 min, then the plasma thus obtained was stored in 2 mL containers, cataloged and frozen at -20 °C until the analysis of cytokine levels, carried out within 90 days of storage. We have evaluated plasma concentration of proinflammatory Interleukins (IL)-1 $\beta$ , IL-10, IL-18, IL-33, IL-36, and of the anti-inflammatory cytokine IL10, and of Interferon (IFN)- $\gamma$  cby enzyme-linked immunosorbent assays (ELISA) using commercially available kits (Diaclone SAS; France; Cusabio, Houston, USA and Boster, CA, USA). All samples for a given assay, including standards, were analyzed in duplicate at the same time following the manufacturer's instructions. The lower detection limit of assay was  $\leq$  1 pg/mL for IL-18,  $\leq$  4,69 pg/mL for IL-1 $\beta$ ,  $\leq$  4,9 pg/mL for IL-10,  $\leq$  5 pg/mL for IFN $\gamma$ ,  $\leq$  12,2 9 pg/mL for IL-33,  $\leq$  19,5 9 pg/mL for IL-36. Standards and samples were analyzed in duplicate. Cytokine levels were calculated plotting the optical density (O.D.) of each sample against the standard curve. Values that differed from the mean

of duplicate by greater than 10% were not considered for further analysis. The variation coefficient of both inter-assay and intra-assay was <5%.

### Statistical analysis

Normality distribution for quantitative variables was assessed by the Shapiro-Wilk. Descriptive analysis was carried out using median and interquartile range (IQR) for the quantitative variables and percentage values for the qualitative ones. Test. Pearson's chi-square test or Fisher's exact test was used to evaluate the association between categorical variables while the non-parametric Wilcoxon rank-sum test for unpaired two-samples to evaluate the differences between continuous variables and outcome considered.

The relationships among cytokines were tested using Spearman's correlation coefficient ( $\rho$ ) in 3DR group and 2DR group. Statistical significance was set at the level of  $\leq 0.05$ . All analyses were performed using Stata software v17.1 (StataCorp, College Station, USA).

## Results

### Descriptive analysis of the sample

A total of 116 patients were enrolled: 89 (76.7%) were male and the median age was 51 years (IQR 43.0–59.0). The median years since the diagnosis of HIV infection was 15 years (IQR 8.5–24.0). Forty six patients (39.7%) were smokers, 44 (38.3%) had elevated blood pressure values, 6 (5.2%) had type II diabetes mellitus, depressive syndrome was present in 41 (35.3%) patients, lipodystrophy in 7 (6.0%), dyslipidemia in 83 (71.6%) and chronic renal failure in 12 (10.4%). Body Mass Index (BMI) at the time of study had a median of 25.6 kg/m<sup>2</sup> (IQR 23.3–27.9).

The risk factors for HIV infection were: unprotected homosexual sex in 41 (35.3%), unprotected heterosexual sex in 55 (47.4%), promiscuous use of syringes for intravenous drug intake in 17 (14.7%), unprotected bisexual sex in 2 (1.7%) and vertical transmission in 1 subject (0.9%).

During the study, 82 patients were in cART with 3 anti-retroviral drugs (3DR group) and 34 patients in DTG-based cART composed of two molecules (2DR group). The comparison analysis did not reveal statistically significant differences between groups for socio-demographic characteristics, specifically for gender (male 75.6% in 3DR group vs. 79.4% in 2DR group,  $p=0.659$ ) and median age (51.0 (IQR 43.0–60.0) in 3DR group vs. 50.5 (IQR 44.0–57.0) in 2DR group,  $p=0.673$ ).

CD4+T cells median value was 729.0 cell/uL (IQR 492.0–934.0) in 3DR group and 771.5 (IQR 589.0–956.0) in 2DR group, CD8+T cells were a median of 729.5 cell/uL (IQR 550.5–993.0) in 3DR group and a median of 737.5 cell/uL (IQR 603.0–1021.0) in 2DR group, CD4/

CD8 ratio has a median value of 0.9 (IQR 0.7–1.3) in 3DR group and a median value of 1 (IQR 0.7–1.5) in 2DR group. The viral load was undetectable in all patients.

### Analysis of hematochemical and viro-immunological parameters

The main hematochemical values measured in the sample during the study, were within normal ranges. Significant differences emerged in ALT [a median of 22.5 U/L (IQR 16.0–32.0)] in 3DR group vs. a median of [16 U/L (IQR 14.0–21.0) in 2DR group,  $p=0.011$ ] and  $\gamma$ GT levels [a median of 34 U/L (IQR 21.0–54.0)] in 3DR group vs. a median of 22.5 [(IQR 16.0–38.0) in 2DR group,  $p=0.018$ ]: despite the statistical significance revealed, the median values fall within the reference interval. No other significant differences in biochemical parameters were found (Table 1).

Serum levels of IFN $\gamma$  levels were 26.9 pg/ml (IQR 22.9–30.7) in 3DR group and of 27.1 pg/ml (IQR 24.1–31.9) in 2DR group. The IL-10 was 15.4 pg/dl (IQR 12.4–21.3) in the 3DR and of 16.7 pg/dl (IQR 13.4–22.7) in the 2DR; the IL-33 and IL-1 $\beta$  plasma levels were respectively 169.5 pg/ml (IQR 152.8–179.5) and 0.3 (IQR 0.2–0.4) in 3DR group, and 159.5 pg/ml (IQR 150.9–172.2) and 0.3 pg/dl (IQR 0.2–0.3) in 2DR group. Significant differences were found between the two groups in the levels of IL-18 (648.8 pg/ml in 3DR group vs. 475.0 pg/ml in 2DR group,  $p=0.034$ ) and IL-36 (281.7 pg/ml in 3DR group vs. 247.0 pg/ml in 2DR group,  $p=0.050$ ) (Fig. 1). For these cytokines found to be significant in comparison, the correlation between them in the two groups was calculated separately. Spearman rank correlation showed a positive correlation between IL-18 and IL-36 serum levels in 3DR group ( $\rho=0.266$ ,  $p=0.015$ ).

## Discussion

This study aims to evaluate plasma levels of pro- and anti-inflammatory cytokines in PLWH in 2DR and 3DR cART. Our results show that the anti-inflammatory cytokines profile measured in this study was not significantly different, while the pro-inflammatory cytokines IL-18 and IL-36 were significantly higher in PLWH receiving a 3DR anti-HIV therapy compared to those receiving a 2DR therapy.

IL-18 is a member of the IL-1 family of cytokines, produced constitutively by macrophages, endothelial cells, vascular smooth muscle cells and dendritic cells: this molecule has a potent proinflammatory activity that promotes the release of other cytokines, chemokines and cell adhesion molecules. High levels of IL-18 have been found in patients with metabolic syndrome [16] and have been correlated with the development of atherosclerotic plaques, plaque instability and acute cardiovascular events [17, 18], with cardiovascular risk score

**Table 1** Hematology and biochemical parameters in 3DR and 2DR groups

|                                   | 3DR (n=82)                      | 2DR (n=34)                      | P-value      |
|-----------------------------------|---------------------------------|---------------------------------|--------------|
| RBC (cells/mmc)                   | 4665000.0 (4360000.0-5050000.0) | 4590000.0 (4260666.0-4980000.0) | 0.817        |
| Hemoglobin (g/dl)                 | 14.6 (13.7–15.3)                | 15.1 (14.2–15.9)                | 0.077        |
| Platelets (cells/mmc)             | 201500.0 (175000.0-257000.0)    | 221000.0 (196000.0-252000.0)    | 0.205        |
| WBC (cells/mmc)                   | 6405.0 (5290.0-7740.0)          | 7025.0 (5980.0-7440.0)          | 0.378        |
| Neutrophils (cells/mmc)           | 3310.0 (2570.0-4570.0)          | 3345.0 (2710.0-4520.0)          | 0.636        |
| Lymphocytes (cells/mmc)           | 2090.0 (1690.0-2620.0)          | 2150.0 (1900.0-3000.0)          | 0.360        |
| Monocytes (cells/mmc)             | 530.0 (430.0-620.0)             | 555.0 (410.0-670.0)             | 0.524        |
| Eosinophils (cells/mmc)           | 165.0 (100.0-210.0)             | 140.0 (110.0-220.0)             | 0.897        |
| Basophils (cells/mmc)             | 40.0 (30.0–60.0)                | 40.0 (30.0–50.0)                | 0.949        |
| Creatinine (mg/dl)                | 1.0 (0.8–1.1)                   | 1.0 (0.9–1.2)                   | 0.098        |
| eGFR (ml/min/1,7 m <sup>2</sup> ) | 80.9 (71.9-101.5)               | 77.8 (72.0–89.0)                | 0.119        |
| Total Cholesterol (mg/dl)         | 187.0 (161.0-213.0)             | 184.5 (171.0-198.0)             | 0.791        |
| LDL-Cholesterol (mg/dl)           | 126.0 (101.0-152.0)             | 127.0 (115.0-138.0)             | 0.704        |
| Triglycerides (mg/dl)             | 107.5 (72.0-171.0)              | 100.0 (73.0-140.0)              | 0.507        |
| Uric acid (mg/dl)                 | 5.5 (4.6–6.2)                   | 5.5 (4.2–6.6)                   | 0.938        |
| AST (U/L)                         | 20.0 (17.0–25.0)                | 18.0 (16.0–25.0)                | 0.247        |
| ALT (U/L)                         | 22.5 (16.0–32.0)                | 16.0 (14.0–21.0)                | <b>0.011</b> |
| GGT (U/L)                         | 34.0 (21.0–54.0)                | 22.5 (16.0–38.0)                | <b>0.018</b> |
| Alkaline phosphatase (U/L)        | 66.5 (51.0–83.0)                | 62.0 (54.0–73.0)                | 0.381        |
| LDH (U/L)                         | 195.0 (176.0-220.0)             | 192.0 (171.0-215.0)             | 0.609        |
| Vitamin D (ng/ml)                 | 31.5 (23.2–36.1)                | 30.4 (24.8–44.4)                | 0.339        |
| CRP (mg/L)                        | 2.4 (1.0-5.5)                   | 1.2 (0.7–3.2)                   | 0.052        |
| Cistatina C (mg/L)                | 0.9 (0.8-1.0)                   | 0.9 (0.8–0.9)                   | 0.407        |
| HIV Infection                     |                                 |                                 |              |
| AIDS events                       | 12 (14.7%)                      | 3 (8.9%)                        | 0.548        |
| HIV duration                      | 15.5 (9–24)                     | 12.5 (7–24)                     | 0.291        |
| cART                              |                                 |                                 |              |
| INI                               | 20 (12.2%)                      | 0 (0.0%)                        | -            |
| PI                                | 26 (15.8%)                      | 0 (0.0%)                        |              |
| NNRTI                             | 36 (21.9%)                      | 0 (0.0%)                        |              |
| TAF/FTC                           | 64 (39.1%)                      | 0 (0.0%)                        |              |
| ABC/3TC                           | 18 (10.0%)                      | 0 (0.0%)                        |              |
| DTG/3TC                           | 0 (0.0%)                        | 23 (67.7%)                      |              |
| DTG/RPV                           | 0 (0.0%)                        | 11 (32.3%)                      |              |
| cART duration                     | 12.0 (8.0–17.0)                 | 12.0 (7.0–16.0)                 | 0.530        |
| Nadir CD4+(cells/mmc)             |                                 |                                 |              |
| >500                              | 14.0 (17.1%)                    | 6 (17.7%)                       | 0.957        |
| 200–500                           | 41 (50.0%)                      | 16 (47.1%)                      |              |
| <200                              | 27 (32.9%)                      | 12 (35.3%)                      |              |

Data are expressed as median and interquartile range (IQR)

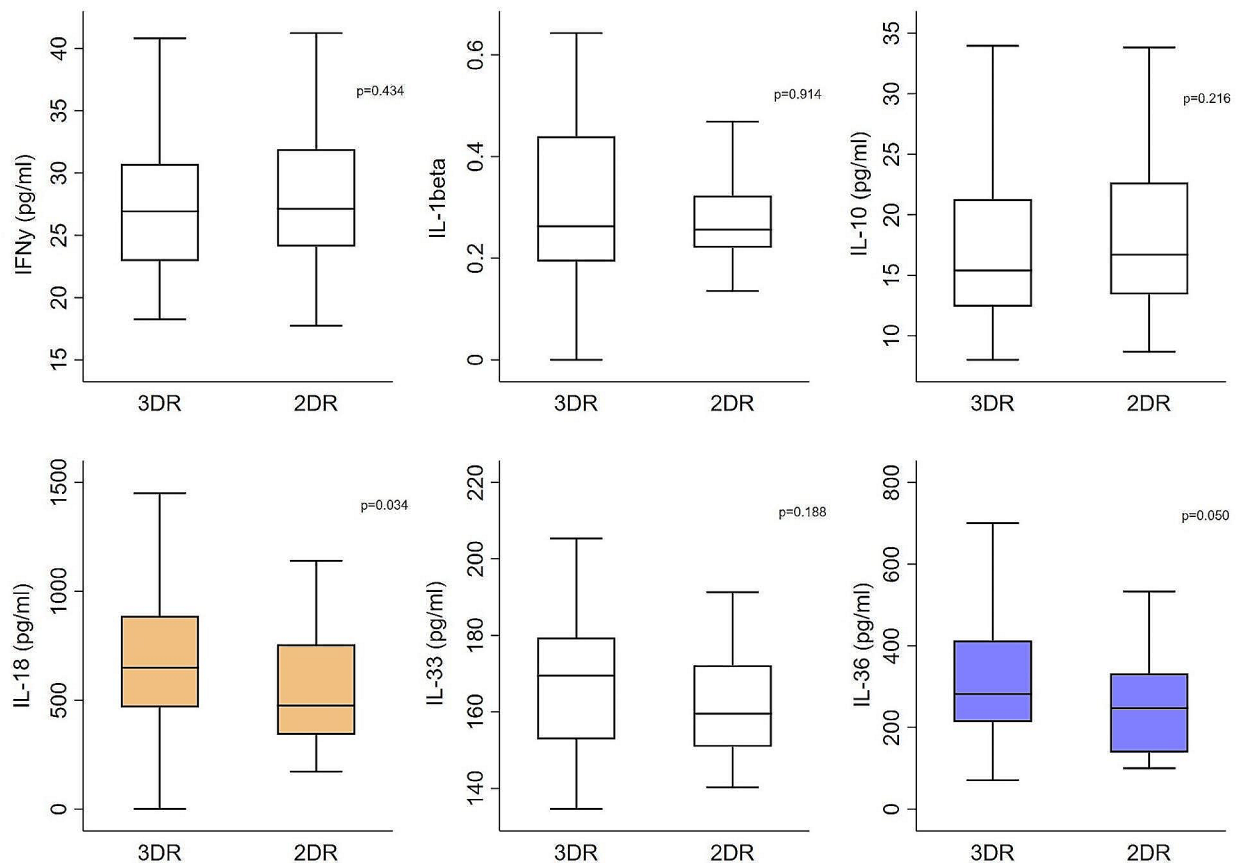
\*p-values are for Wilcoxon rank-sum test or Pearson chi-square test

RBC=Red blood cells; WBC=White blood cells; eGFR: Estimated Glomerular Filtration Rate, AST: aspartate transaminase, ALT: alanine transaminase; GGT: GammaGT; LDH: Lactate dehydrogenase; PCR: C-reactive protein; INI: Integrase inhibitors; PI: Protease inhibitors; NNRTI: Non-nucleoside reverse transcriptase inhibitors

(PROCAM, DAD, FRS, ASCVD) [19] and can become predictive of coronary events [20].

IL-36 has pro-inflammatory effects, and is expressed in numerous cells such as T cells, monocytes/macrophages, dendritic cells (DCs), keratinocytes, Langerhans cells, lung and gut cells. IL-36 plays an important role in immune cell activation, antigen presentation and inflammatory diseases [21–23], showing stimulatory effects on DCs and T-cells and interfacing innate

and adaptive immune responses in viral, bacterial and fungal infections [24]. Recently, IL-36 has aroused great interest because of its dysregulation in inflammatory diseases, in fact the serum levels of IL-36 were found to be significantly higher with coronary artery disease, correlated with TNF- $\alpha$ , IL-6 and IL-32 levels and coronary artery stenosis [25]. In ApoE knockout mice the IL-36 $\gamma$  can exert atherosclerosis-promoting effects by augmenting macrophage foam cell formation and uptake of



**Fig. 1** Box plots show the median levels of the cytokines in the two comparison groups. *P*-values are for Wilcoxon rank-sum test

oxidized low-density lipoproteins [26]. IL-36 signaling also induces the release of profibrotic mediators, suggesting a role in fibrotic disorders affecting kidneys, lung, and intestines [27]. To date, IL-36's involvement in PLWH is unknown, and in this study we showed that PLWH in 2DR regimens have significantly lower IL-18 and IL-36 levels than their counterparts in 3DR, potentially reducing the cardiovascular risk, the positive correlation between IL-18 and IL-36 found in the 3DR group shows a strong association with cardiovascular risk.

The levels of IL-1 $\beta$  and IL-33, cytokines with important roles in metabolic homeostasis, viral infection, inflammation and carcinogenesis, are not statistically significant different in the two groups, as well as the levels of the immunoregulatory IL-10 and immunostimulatory IFN- $\gamma$ .

Lower levels of IL-18 and IL-36 in the 2DR group could indicate a good control on the inflammatory state even in cART regimens composed by only two drugs. Thus, DTG-based cART dual therapies acting on inflammation state might be able to reduce, over time, the cardiovascular risk of the HIV patient which, nowadays, remains higher than the general population [28–30]. This study

has limitations, particularly due to the small sample size. As a result, the tests conducted can only be attributed to the specific comparisons made and have not allowed for the development of predictive models correlating the results with levels of inflammatory markers. Conducting future studies with a larger population will enable us to explore this relationship further.

## Conclusion

This single-center retrospective pharmacological study confirms the absence of significant differences in IL-1 $\beta$ , IL-10, IL-33, and IFN- $\gamma$  levels between patients on dolutegravir based two-drug antiretroviral regimens compared to patients on three-drug antiretroviral regimens.

Interestingly, the patients in dual therapies show greater control over IL-18 and IL-36 serum levels, cytokines related to an increased cardiovascular risk and chronic diseases related to ageing.

These data demonstrating that regimens featuring two antiretroviral agents, DTG based, could be associated with a good control on chronic inflammatory state in

PLWHs in cART, could be a further incentive for the clinician to approach the pharmacological simplifications of HIV regimen.

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#### Author contributions

UC and DGA wrote the main manuscript text, BP and DNM verified the analytical methods; FC and AL collected data; CE and RM carried out the experiment. FK conceived the original idea and JV supervised the project. All authors reviewed the manuscript.

#### Funding

None to declare.

#### Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

##### Ethics approval and consent to participate

No identification of patients is disclosed. Each patient received informed letters asking for approval. The study protocol was approved by the Ethics Committee at the University "G. d'Annunzio" Chieti-Pescara (Ethics Committee Project No. 16 of 05.09.2019). It was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Informed consent was obtained from all subjects enrolled in this study.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare no competing interests.

##### Conflict of interest

The authors have no relevant conflict of interest to disclose.

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#### References

- Rodger AJ, Lodwick R, Schechter M, Deeks S, Amin J, Gilson R, Paredes R, Bakowska E, Engsig FN, Phillips A, et al. Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. *AIDS*. 2013;27(6):973–9.
- Nasi M, De Biasi S, Gibellini L, Bianchini E, Pecorini S, Bacca V, Guaraldi G, Musini C, Pinti M, Cossarizza A. Ageing and inflammation in patients with HIV infection. *Clin Exp Immunol*. 2017;187(1):44–52.
- Sukumaran L, Kunisaki KM, Bakewell N, Winston A, Mallon PWG, Doyle N, Anderson J, Boffito M, Haddow L, Post FA, et al. Association between inflammatory biomarker profiles and cardiovascular risk in individuals with and without HIV. *AIDS*. 2023;37(4):595–603.
- Min AK, Fortune T, Rodriguez N, Hedge E, Swartz TH. Inflammasomes as mediators of inflammation in HIV-1 infection. *Transl Res*. 2023;252:1–8.
- Longenecker CT, Funderburg NT, Jiang Y, Debanne S, Storer N, Labbato DE, Lederman MM, McComsey GA. Markers of inflammation and CD8 T-cell activation, but not monocyte activation, are associated with subclinical carotid artery disease in HIV-infected individuals. *HIV Med*. 2013;14(6):385–90.
- French MA, King MS, Tschampa JM, da Silva BA, Landay AL. Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+T cells. *J Infect Dis*. 2009;200(8):1212–5.
- Wada NI, Jacobson LP, Margolick JB, Breen EC, Macatangay B, Penugonda S, Martinez-Maza O, Bream JH. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. *AIDS*. 2015;29(4):463–71.
- Lederman MM, Calabrese L, Funderburg NT, Claggett B, Medvik K, Bonilla H, Gripshover B, Salata RA, Taege A, Lisgaris M, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis*. 2011;204(8):1217–26.
- Kuller LH, Tracy R, Bellosso W, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, Neuhaus J, Nixon D, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med*. 2008;5(10):e203.
- Hunt PW, Hatano H, Sinclair E, Lee TH, Busch MP, Martin JN, McCune JM, Deeks SG. HIV-specific CD4+T cells may contribute to viral persistence in HIV controllers. *Clin Infect Dis*. 2011;52(5):681–7.
- Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, et al. CD4+T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med*. 2004;200(6):749–59.
- Ryom L, Boesecke C, Bracchi M, Ambrosioni J, Pozniak A, Arribas J, Behrens G, Mallon P, Puoti M, Rauch A, et al. Highlights of the 2017 European AIDS Clinical Society (EACS) guidelines for the treatment of adult HIV-positive persons version 9.0. *HIV Med*. 2018;19(5):309–15.
- Fabbiani M, Masini M, Rossetti B, Ciccullo A, Borghi V, Lagi F, Capetti A, Colafigli M, Panza F, Balain G et al. Efficacy and durability of Dolutegravir- or darunavir-based regimens in ART-Naive AIDS- or late-presenting HIV-Infected patients. *Viruses* 2023, 15(5).
- Llibre JM, Hung CC, Brinson C, Castelli F, Girard PM, Kahl LP, Blair EA, Angelis K, Wynne B, Vandermeulen K, et al. Efficacy, safety, and tolerability of dolutegravir-rilpivirine for the maintenance of virological suppression in adults with HIV-1: phase 3, randomised, non-inferiority SWORD-1 and SWORD-2 studies. *Lancet*. 2018;391(10123):839–49.
- Lagi F, Giacomelli A, Borghi V, Ciccullo A, Taramasso L, Madeddu G, D'Etto G, Giacometti A, Ducci F, De Vito A, et al. Efficacy and tolerability of dolutegravir/lamivudine versus dolutegravir/rilpivirine in switching from a three-drug regimen based on nonnucleoside reverse transcriptase inhibitors: a retrospective cohort study. *J Med Virol*. 2023;95(10):e29149.
- Zirlik A, Abdullah SM, Gerdes N, MacFarlane L, Schonbeck U, Khera A, McGuire DK, Vega GL, Grundy S, Libby P, et al. Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: results from the Dallas Heart Study. *Arterioscler Thromb Vasc Biol*. 2007;27(9):2043–9.
- Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, Humbert Y, Chvatchko Y, Tedgui A. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circ Res*. 2001;89(7):E41–45.
- Blankenberg S, Tiret L, Bickel C, Peetz D, Cambien F, Meyer J, Rupprecht HJ, AtheroGene I. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation*. 2002;106(1):24–30.
- Claudio U, Antonio A, Marcella R, Erica C, Jacopo V, Katia F. Association of inflammatory biomarkers and cardiovascular risk scores in an Italian cohort of HIV positive patient undergoing antiretroviral therapy. *Curr HIV Res* 2022.
- Blankenberg S, Luc G, Ducimetiere P, Arveiler D, Ferrieres J, Amouyel P, Evans A, Cambien F, Tiret L, Group PS. Interleukin-18 and the risk of coronary heart disease in European men: the prospective epidemiological study of myocardial infarction (PRIME). *Circulation*. 2003;108(20):2453–9.
- Yuan ZC, Xu WD, Liu XY, Liu XY, Huang AF, Su LC. Biology of IL-36 signaling and its role in systemic inflammatory diseases. *Front Immunol*. 2019;10:2532.
- Walsh PT, Fallon PG. The emergence of the IL-36 cytokine family as novel targets for inflammatory diseases. *Ann N Y Acad Sci*. 2018;1417(1):23–34.
- Cossarizza A, Cozzi-Lepri A, Mattioli M, Paolini A, Neroni A, De Biasi S, Tartaro DL, Borella R, Fidanza L, Gibellini L et al. Evaluating immunological and inflammatory changes of treatment-experienced people living with HIV switching from first-line triple cART regimens to DTG/3TC vs. B/F/TAF: the DEBATE trial. *Front Immunol* 2023, 14:1279390.
- Vigne S, Palmer G, Martin P, Lamacchia C, Strebel D, Rodriguez E, Olleros ML, Vesin D, Garcia I, Ronchi F, et al. IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+T cells. *Blood*. 2012;120(17):3478–87.
- Kazemian S, Ahmadi R, Rafiei A, Azadegan-Dehkordi F, Khaledifar A, Abdollahpour-Alitappeh M, Bagheri N. The serum levels of IL-36 in patients with coronary artery Disease and their correlation with the serum levels of IL-32, IL-6, TNF-alpha, and oxidative stress. *Int Arch Allergy Immunol*. 2022;183(10):1137–45.
- Zhang M, Liu J, Gao R, Hu Y, Lu L, Liu C, Ai L, Pan J, Tian L, Fan J. Interleukin-36gamma aggravates macrophage foam cell formation and atherosclerosis progression in ApoE knockout mice. *Cytokine*. 2021;146:155630.

27. Elias M, Zhao S, Le HT, Wang J, Neurath MF, Neufert C, Fiocchi C, Rieder F. IL-36 in chronic inflammation and fibrosis - bridging the gap? *J Clin Invest* 2021, 131(2).
28. Cahn P, Villanueva JA, Arribas J, Gatell J, Lama J, Norton M, Patterson P, Madero JS, Sued O, Figueroa MI, et al. Changes in lipid levels after 48 weeks of dual versus triple therapy observed in the GARDEL study. *J Int AIDS Soc*. 2014;17(4 Suppl 3):19554.
29. Mazzitelli M, Sasset L, Leoni D, Putaggio C, Cattelan AM. Real life use of dolutegravir doravirine dual regimen in experienced elderly PLWH with multiple comorbidities and on polypharmacy: a retrospective analysis. *Med (Baltim)*. 2021;100(52):e28488.
30. Tincati C, Mondatore D, Bai F, d'Arminio Monforte A, Marchetti G. Do combination antiretroviral therapy regimens for HIV infection feature diverse T-Cell phenotypes and inflammatory profiles? *Open Forum Infect Dis*. 2020;7(9):ofaa340.

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