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# Descriptive modification of inflammatory markers in HIV patients after cART initiation according to gender, smoking habit, CMV infection, BMI and serum lipids

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ABSTRACT

Persistent inflammation, despite anti-retroviral therapy (ART), is an independent predictor of mortality and comorbidities in HIV infection. Multiple factors, including lifestyle and chronic viral coinfections, may contribute. Several of these factors are also associated with a chronic inflammation in the general population. Little is known about the degree to which these factors influence inflammation in HIV infection, particularly within the first year of ART. The purpose of this study was to distinguish the effects of factors (gender, body mass index, cholesterol and triglyceride levels, smoke habit and cytomegalovirus seropositivity), known to contribute to inflammation, on inflammation biomarkers over the first year of ART in HIV-infected patients. Linear mixed model analysis revealed significant biomarker decreases [soluble CD14 (s-CD14), soluble CD163 (s-CD163) and D-dimer (DD)], or increases [C Reactive Protein (CRP) and interleukin-6 (IL-6)] over time in the whole cohort, differences in most categories (genders for IL-6, smoke habit for s-CD14, cytomegalovirus infection for s-CD163 and IL-6) and in some category × time interactions [gender for interleukin-7 (IL-7)], cytomegalovirus infection for s-CD14 and cholesterol levels for s-CD14 and Tumor Necrosis Factor α (TNF-α)]. This explorative longitudinal study suggests further investigations on targeting inflammation pathophysiology in HIV-infected patients on ART.

## 1. Introduction

Inflammation is a complex biological process that occurs in response to pathogens, like viruses, bacteria or parasites, as well as exposure to toxic agents or injuries. Inflammation plays a crucial role during HIV infection and inflammatory markers are independent predictors of non-AIDS events, including cardiovascular disease (CVD), cancer, osteoporosis, weakness, frailty and death [1–3].

Although levels of inflammatory markers tend to decrease with suppressive combination anti-retroviral therapy (cART), a chronic low-level of inflammation, with higher levels of several biomarkers of inflammation or immune activation in comparison with HIV-uninfected people, is observed in HIV-infected patients, even after several years of efficient cART [4,5]. Therefore, with longer lifespans, HIV-infected people become increasingly exposed to elevated inflammatory processes.

Many studies have investigated the role of inflammation in frailty of both HIV-infected and uninfected people [6–10]. Due to the strict relationship between inflammation and non-AIDS events during HIV infection, there is an urgent need for further investigations on therapies, aimed at reducing inflammation effects on HIV infection-related frailty.

Multiple factors likely contribute to ongoing inflammation during HIV infection despite cART, including lifestyle factors, microbial translocation, chronic hepatitis B and C or other viral coinfections and low-level HIV replication [11]. Like HIV infection, smoking, gender, overweight conditions or metabolic syndrome are also associated with a

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**Table 1**
Demographic and clinical characteristics of the treatment-naïve patients (n = 36) included in the study at initiating cART (T0).

| *Age* | |
|---|---|
| Mean (S.D.) | 42.00 (10.18) |
| Median | 40.50 |
| Range | 25 – 74 |
| *Gender n (%)* | |
| Male | 30 (83.3%) |
| Female | 6 (16.7%) |
| *Smoke habit n (%)* | |
| No | 21 (58.3%) |
| Yes | 12 (33.3%) |
| Former smokers | 3 (8.3%) |
| *CMV seropositivity (IgG) n (%)* | |
| n. (no records) | 6 |
| Negative | 3 (10.0%) |
| Positive | 27 (90.0%) |
| *BMI* | |
| Mean (S.D.) | 23.57 (3.96) |
| Median | 23.50 |
| Range | 16.70–33.40 |
| ≤ 25n (%) | 23 (63.9%) |
| > 25n (%) | 13 (36.1%) |
| *CHOL (mg/dL)* | |
| Mean (S.D.) | 162.97 (41.49) |
| Median | 162.00 |
| Range | 50.00 – 228.00 |
| n. (no records) | 2 |
| ≤ 200n (%) | 26 (76.5%) |
| > 200n (%) | 8 (23.5%) |
| *TG (mg/dL)* | |
| Mean (S.D.) | 113.76 (40.45) |
| Median | 120.00 |
| Range | 36.00 – 183.00 |
| n. (no records) | 2 |
| ≤ 150n (%) | 22 (64.7%) |
| > 150n (%) | 12 (35.3%) |
| *AIDS n (%)* | |
| Yes | 1 (2.78%) |
| No | 35 (97.22%) |
| *HIV RNA copies* | |
| Median | 22980.00 |
| Range | 313 – 1,127,000 |
| Geomean | 24531.30 |
| Bootstrap confidence interval | (12035.52; 48569.11) |
| *$CD4^+$ T cells (cell/uL)* | |
| Mean (S.D.) | 429.50 (249.24) |
| Median | 412.00 |
| Range | 12.00–980.00 |
| Geometric mean (95% bootstrap CI) | 315.36 (232.57, 456.34) |
| Geom. Mean (Geom. S.D.) | 315.36 (2.73) |
| *$CD8^+$ T cells (cell/uL)* | |
| Mean (S.D.) | 1266.86 (818.61) |
| Median | 1057.00 |
| Range | 213.00–4025.00 |
| Geometric mean (95% bootstrap CI) | 1037.61 (838.75, 1285.85) |
| Geom. Mean (Geom. S.D.) | 1037.61 (1.92) |
| *$CD4^+/CD8^+$* | |
| Mean (S.D.) | 0.39 (0.25) |
| Median | 0.36 |
| Range | 0.02–1.24 |
| Geometric mean (95% bootstrap CI) | 0.30 (0.25, 0.40) |
| Geom. Mean (Geom. S.D.) | 0.30 (2.19) |

Data are mean (standard deviation, S.D.), median and range (min–max). For HIV RNA copies, $CD4^+$ cells, $CD8^+$ cells and $CD4^+/CD8^+$ we computed the geometric mean (geomean) with corresponding bootstrap confidence interval. Categorical variables were described with counts and percentages. Categorical or dichotomized quantitative variables were coded as follows: gender (male/female), smoke habit (yes/no/former smoker), CMV IgG seropositivity (negative/positive), BMI (≤25/>25), CHOL (≤200/>200 mg/dL), TG (≤150 />150 mg/dL) and AIDS (yes/no). BMI = body mass index; CHOL = total cholesterol; CMV = cytomegalovirus; TG = triglycerids.

chronic, albeit low-grade, systemic inflammatory response in the general population [12]. Multiple studies regarding alternative interventions to manage the chronic inflammatory state in HIV patients on efficient cART have been published. Wooten at al. [13] examined the effect of healthy diet and exercise on inflammation in HIV-infected patients with undetectable HIV viremia and dyslipidemia. Rosuvastatin, a lipid-lowering drug, has proved to be effective in reducing inflammation markers and immune activation during HIV infection [14–16]. Smoking habit and overweight conditions, both also independently of HIV infection, influence immune/inflammation [17,18]. To complicate matters, cytomegalovirus (CMV) seems to act as a chronic modifier of cytokine action [19,20] and the presence of CMV infection has been associated with a variety of aging-related diseases in both HIV-positive and negative populations [10,21].

**Table 2**
Serum concentrations of inflammation and immune activation biomarkers at initiating cART (T0).

| *hs-CRP (ng/mL)* | |
|---|---|
| Mean (S.D.) | 24852.17 (38752.72) |
| Median | 11236.41 |
| Range | 841.31–190129.93 |
| Geometric mean (95% bootstrap CI) | 11814.45 (7792.01, 17996.36) |
| Geom. Mean (Geom. S.D.) | 11814.45 (3.40) |
| *DD (ng/mL)* | |
| n (missed) | 2 |
| Mean (S.D.) | 964.29 (971.71) |
| Median | 633.00 |
| Range | 21.00–3540.00 |
| Geometric mean (95% bootstrap CI) | 523.94 (342.75, 794.24) |
| Geom. Mean (Geom. S.D.) | 523.94 (3.53) |
| *s-CD14 (pg/mL)* | |
| Mean (S.D.) | 7888.62 (1759.40) |
| Median | 7443.78 |
| Range | 5382.99–12916.01 |
| Geometric mean (95% bootstrap CI) | 7714.49 (7177.89, 8231.80) |
| Geom. Mean (Geom. S.D.) | 7714.49 (1.23) |
| *s-CD163 (ng/mL)* | |
| Mean (S.D.) | 33.03 (13.60) |
| Median | 30.80 |
| Range | 13.68–71.71 |
| Geometric mean (95% bootstrap CI) | 30.52 (26.53, 35.17) |
| Geom. Mean (Geom. S.D.) | 30.52 (1.49) |
| *TNF-α(pg/mL)* | |
| n (missed) | 1 |
| Mean (S.D.) | 1.68 (0.97) |
| Median | 1.46 |
| Range | 0.04–3.97 |
| Geometric mean (95% bootstrap CI) | 1.44 (1.16, 1.84) |
| Geom. Mean (Geom. S.D.) | 1.44 (2.04) |
| *CCL5-RANTES (pg/mL)* | |
| Mean (S.D.) | 438.82 (338.65) |
| Median | 402.53 |
| Range | 50.48–1327.79 |
| Geometric mean (95% bootstrap CI) | 307.09 (228.40, 429.95) |
| Geom. Mean (Geom. S.D.) | 307.09 (2.53) |
| *IL-6 (pg/mL)* | |
| Mean (S.D.) | 1.85 (0.90) |
| Median | 1.65 |
| Range | 0.58–3.97 |
| Geometric mean (95% bootstrap CI) | 1.64 (1.39, 1.93) |
| Geom. Mean (Geom. S.D.) | 1.64 (1.63) |
| *IL-7 (pg/mL)* | |
| Mean (S.D.) | 7.36 (5.03) |
| Median | 7.35 |
| Range | 0.47–22.54 |
| Geometric mean (95% bootstrap CI) | 5.40 (4.02, 7.41) |
| Geom. Mean (Geom. S.D.) | 5.40 (2.46) |

Data are mean (standard deviation, S.D.), median, range (min–max), geometric mean (Geomean) with corresponding bootstrap confidence interval. CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α.

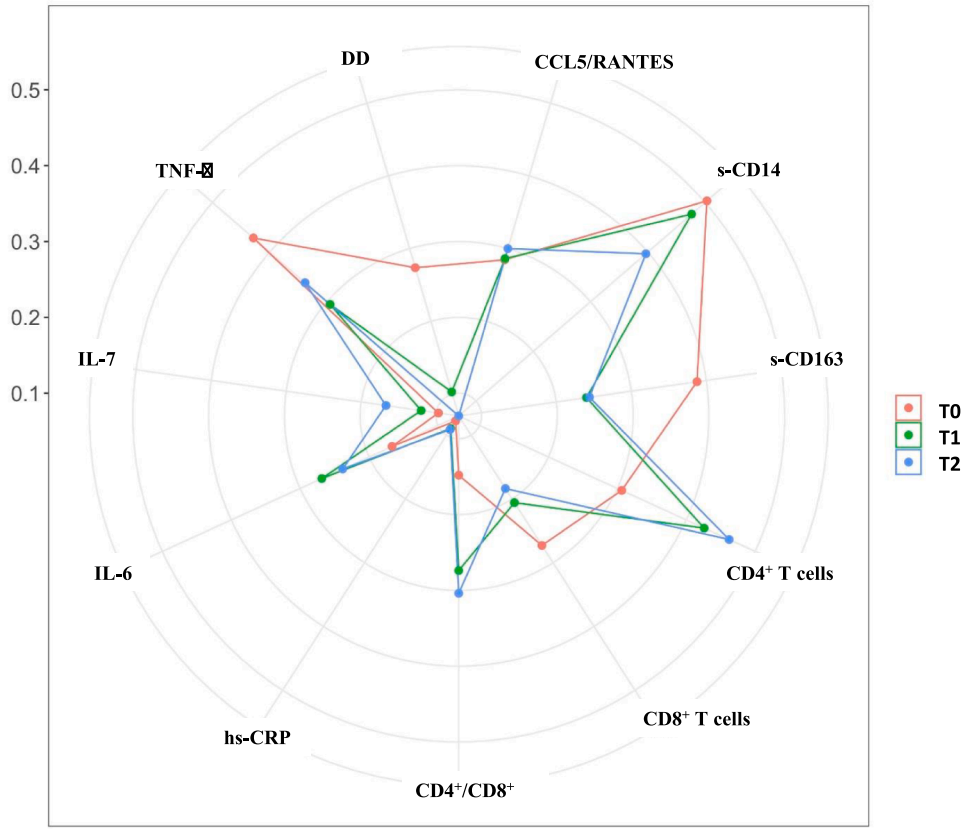


**Fig. 1.** . Representation of inflammatory and immunological marker evolution (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted, pg/mL; DD = D-dimer, ng/mL; hs-CRP = high sensitivity C Reactive Protein, ng/mL; IL-6 = interleukin-6, pg/mL; IL-7 = interleukin-7, pg/mL; s-CD14 = soluble CD14, pg/mL; s-CD163 = soluble CD163, ng/mL; TNF-α = Tumor necrosis factor α, pg/mL) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) are represented by radar chart. Results are reported as estimated average percentage (%) variations. Radar plots were constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time.

Little is known about the degree to which a potentially modifiable or an easily measurable condition, like Body Mass Index (BMI), smoke habit or CMV IgG seropositivity, influences changes in the levels of inflammatory markers, particularly within the first year after cART initiation. We found only one study, describing differences in the inflammatory/immune activation response to cART based on both the BMI prior to cART initiation and the BMI changes during the first year of cART [18].

In a previous study, our research group explored the impact of various cART regimens on several markers of immune activation and inflammation in HIV-infected patients during the first year of therapy and found that the integrase strand transfer inhibitor elvitegravir had the worst impact on some inflammatory biomarkers, particularly in the first 6 months of cART [22]. The current study analyzes in depth previous data, aiming to distinguish, by the use of Linear Mixed Model statistical analysis, the effects of factors known to generally contribute to inflammation (gender, body mass index, cholesterol and triglyceride levels, smoke habit and cytomegalovirus infection) on biomarkers of inflammation over the first year of cART in HIV-infected patients.

## 2. Methods

### 2.1. Study population

We conducted an observational retrospective cohort study, aimed to distinguish the effects of gender, smoke habit, CMV IgG seropositivity, BMI, total cholesterol (CHOL) and triglyceride (TG) levels on the trends of several markers of inflammation over the first year of cART in HIV-infected patients. Patients were recruited from the HIV-infected patients of the MASTER cohort, followed at the University Department of Infectious and Tropical Diseases, University of Brescia and ASST Spedali Civili di Brescia, Italy [23]. For this study, all treatment-naïve adults, initiating cART between March 2015 and February 2016, were screened for eligibility using the hospital database. Patients were included in the study if: (i) they were Caucasian; (ii) they initiated cART (T0) according to the current guidelines and maintained the regimen during at least 12 months, with HIV RNA < 37 copies/ml at 6 (T1) and 12 (T2) months after initiating cART; (iii) serum samples were available either at initiating cART (T0) and T1 and T2 and were stored at −80 °C. Excluding criteria were: co-infections with known hepatitis viruses, CVD, diabetes, uncontrolled hypertension, malignancy, other systemic inflammatory diseases (rheumautologic diseases, granulomatous diseases, inflammatory bowel diseases, vasculitis, autoinflammatory diseases) or AIDS-defining conditions, diagnosed within 30 days of cART initiation and during the first 12 months. BMI was calculated as the ratio of weight and height squared (kg/m$^2$). Smoke habit and CMV IgG seropositivity were retrieved from the clinical history.

### 2.2. Laboratory assessment

Serum samples were tested at baseline before starting cART (T0) and six months (T1) and twelve months (T2) after cART initiation, as previously described [22]. HIV RNA copies, CD4$^+$ and CD8$^+$ T cell count, CD4$^+$/CD8$^+$ ratio, TG and CHOL levels were retrieved from the clinical records. Levels of D-Dimer (DD) were measured using the Abbott Architect automated analyzer (Abbott Architect D-Dimer assay). Tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), interleukin-7 (IL-7), C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted (CCL5/RANTES), soluble CD14 (s-CD14), soluble CD163 (s-CD163), high sensitivity C Reactive Protein (hs-CRP) markers of immune activation and inflammation were determined using commercial ELISA kits (R&D System ELISA kit, Minneapolis, MN, USA), applying the manufacturer's protocols.

### 2.3. Statistical analysis

For each quantitative variable at baseline (T0), we computed the following descriptive statistics: mean, standard deviation, median and range. For highly skewed variables (HIV viremia, IL-7, hs-CRP, DD, CCL5/RANTES), we computed the geometric mean and corresponding bootstrap confidence interval. Categorical variables were described with counts and percentages. Categorical or dichotomized quantitative variables were coded as follows: gender (male/female), smoke (yes/no/former smoker), CMV IgG seropositivity (positive/negative), BMI (≤25/>25), CHOL (≤200/>200 mg/dl) and TG (≤150 mg/dl/>150 mg/dl). Inflammatory markers were modelled on log scale (due to highly positive skewed distributions) using Linear Mixed Model (LMM) [24], using subject as random factor (random intercepts model). Results are reported as estimated average relative variation, expressed as percentage ([A/B – 1]*100) and corresponding confidence intervals. Radar plots were constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. All the analyses were performed using R (version 3.6.1) and all tests were two-sided and assumed a significance level of 5%.

### 2.4. Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. All patients provided written informed consent to include their clinical and biological data in the study and for archiving a serum sample at baseline and every 6 months for scientific purposes. The MASTER study was approved by the Ethical Committee of the ASST Spedali Civili di Brescia (Coordinating Centre) and of all the participant Centers [23]. The present study was approved on December 20th 2016 (clinicaltrials.gov, Identifier NCT03280940).

## 3. Results

Thirty-six patients were recruited for this study. The cohort comprised thirty males (83.3%) and six females (16.6%) (mean age of 38.6 years, range 22–69 years), 12 smokers, 21 non-smokers and 3 former smokers. One third of the patients were overweight (BMI > 25, 36.1%) or had high TG levels (TG > 150 mg/dL, 35.3%) and one fourth had high CHOL levels (CHOL > 200 mg/dL, 23.5%). Most of them were CMV IgG seropositive (90%). Demographic and HIV infection-related characteristics at baseline (T0) are shown in Table 1.

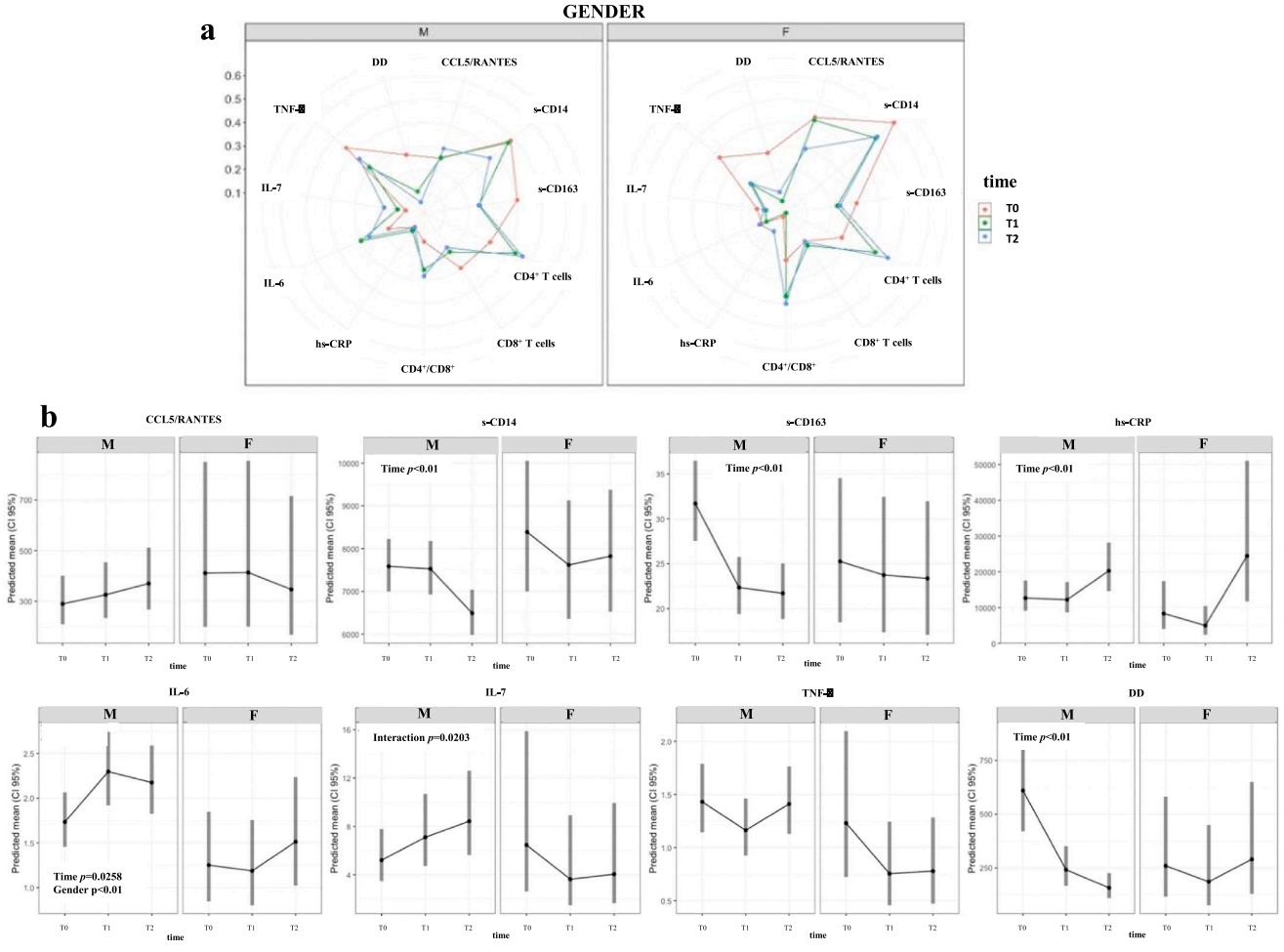


**Fig. 2.** . Trends of inflammatory and immunological markers (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) and according to gender (M = male; F = female). (**a**) The radar plot, representing inflammatory and immunological marker evolution, was constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. Results are reported as estimated average percentage (%) variation. (**b**) Graphic representation of trends of serum marker levels (CCL5/RANTES, pg/mL; DD, ng/mL; hs-CRP, ng/mL; IL-6, pg/mL; IL-7, pg/mL; s-CD14, pg/mL; s-CD163, ng/mL; TNF-α, pg/mL) over time and according to gender. Significant differences corresponding to Linear Mixed Model (LMM) analyses (see Supplemental Table 7) are reported for each marker.

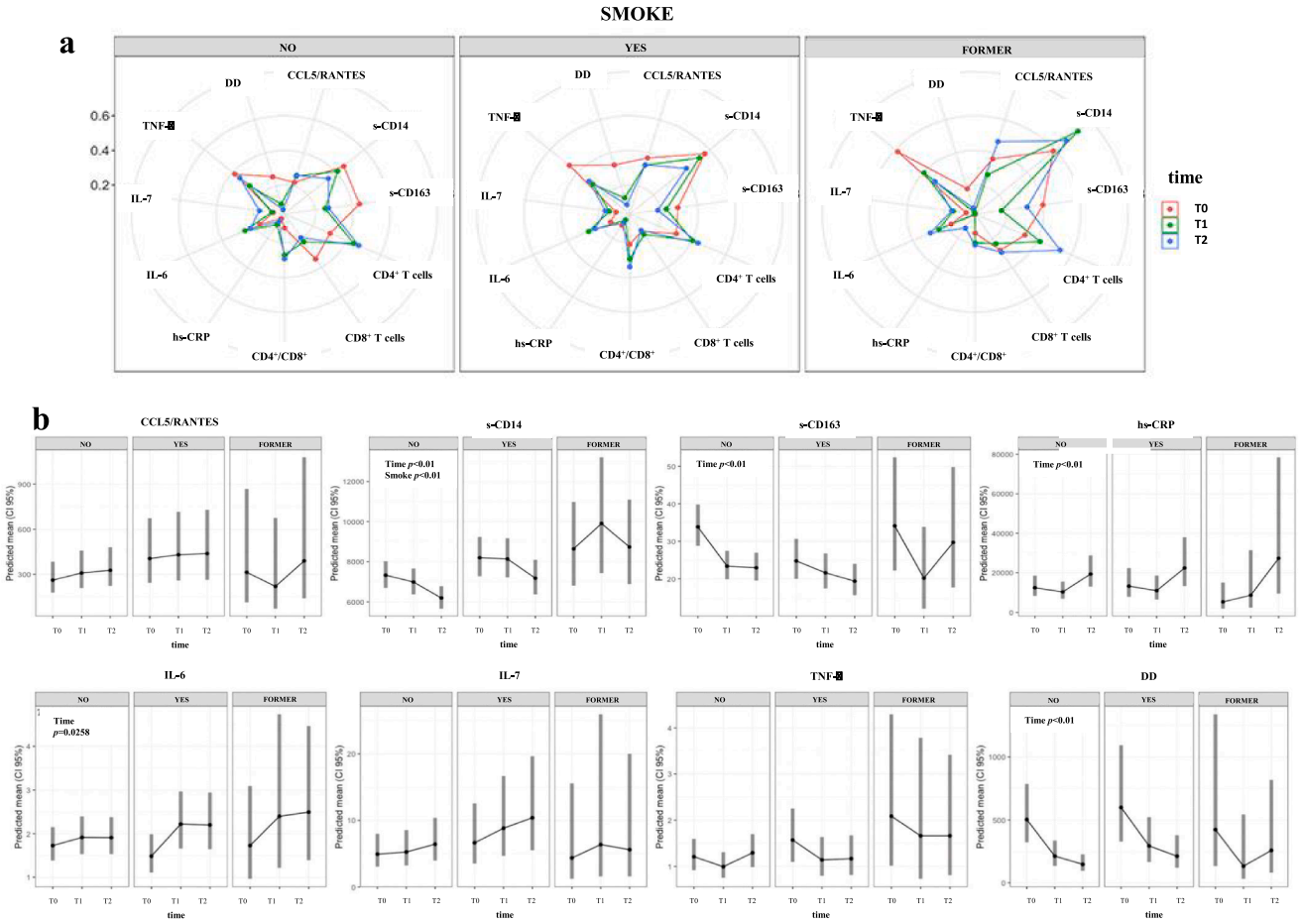


**Fig. 3.** . Trends of inflammatory and immunological markers (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) and according to smoke habit (No = no smokers; Yes = smokers; former = former smokers). (**a**) The radar plot, representing inflammatory and immunological marker evolution, was constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. Results are reported as estimated average percentage (%) variation. (**b**) Graphic representation of trends of serum marker levels (CCL5/RANTES, pg/mL; DD, ng/mL; hs-CRP, ng/mL; IL-6, pg/mL; IL-7, pg/mL; s-CD14, pg/mL; s-CD163, ng/mL; TNF-α, pg/mL) over time and according to smoke habit. Significant differences corresponding to Linear Mixed Model (LMM) analyses (see Supplemental Table 8) are reported for each marker.

Hs-CRP, DD, s-CD14, s-CD163, TNF-α, CCL5/RANTES, IL-6 and IL-7 levels at baseline (T0) are shown in Table 2 (separated according to gender, smoke habit, CMV IgG seropositivity, BMI, total cholesterol (CHOL) and triglyceride (TG) levels in Supplemental Tables 1–6). Trends of inflammatory and immunological markers from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) are represented by radar chart in Fig. 1. As expected, cART increased $CD4^+$ T cells $CD4^+/CD8^+$ ratio and decreased $CD8^+$ T cells. Heterogeneous modifications of inflammation markers during the first year of cART were observed in the whole cohort. As expected, therapy had an impact on inflammatory markers and the effect was more pronounced during the first 6 months after cART initiation (Figs. 2-7). Estimates from LMMs of the effect of one year (time) of effective cART on average percentage (%) variation of inflammatory markers are shown in Supplemental Tables 7–12. The statistically significant $p$ values for LMMs (time, category and category × time interaction) are also shown in Figs. 2-7. Significant decreases for s-CD14, s-CD163 and DD and increases for hs-CRP and IL-6 were observed in the whole cohort (time effect).

Trends of inflammatory changes were represented over time in each category (gender, smoking habit, CMV IgG seropositivity, BMI, TG and CHOL levels). Some differences emerged (Figs. 2-7 and Supplemental Tables 7–12). Genders significantly differed for IL-6 levels (significantly lower in females than in males, $p < 0.01$), smokers for s-CD14 (current and former smokers with higher levels than not smokers, $p < 0.01$), CMV uninfected and infected patients for s-CD163 and IL-6 levels (significantly higher levels of s-CD163, $p = 0.0214$, and significantly lower levels of IL-6, $p = 0.021$, in CMV uninfected patients). Further, overweight patients (BMI > 25) showed a trend to higher levels for IL-6 ($p = 0.0757$) and patients with high TG levels (TG > 150 mg/dL) a trend to higher levels for hs-CRP ($p = 0.0632$).

We did not observe significant differences in the trends of inflammation markers during the first 12 months of cART (category × time interaction), except for IL-7 between genders (with decrease in females and increase in males; gender × time interaction, $p = 0.0203$), for s-CD14 between CMV infected and uninfected patients (with a decrease in CMV infected patients and nearby stable values in CMV uninfected patients; CMV IgG seropositivity × time interaction, $p = 0.0317$) and for s-CD14 and TNF-α patients with normal or high CHOL levels (with a decrease of both markers in patients with CHOL ≤ 200 mg/dL and an increase in patients with CHOL > 200 mg/dL; high CHOL × time interaction, $p = 0.0203$ and $p = 0.0121$ respectively).

## 4. Discussion

This explorative and descriptive study aimed to identify differences and trends in the inflammatory profile in HIV-infected patients during

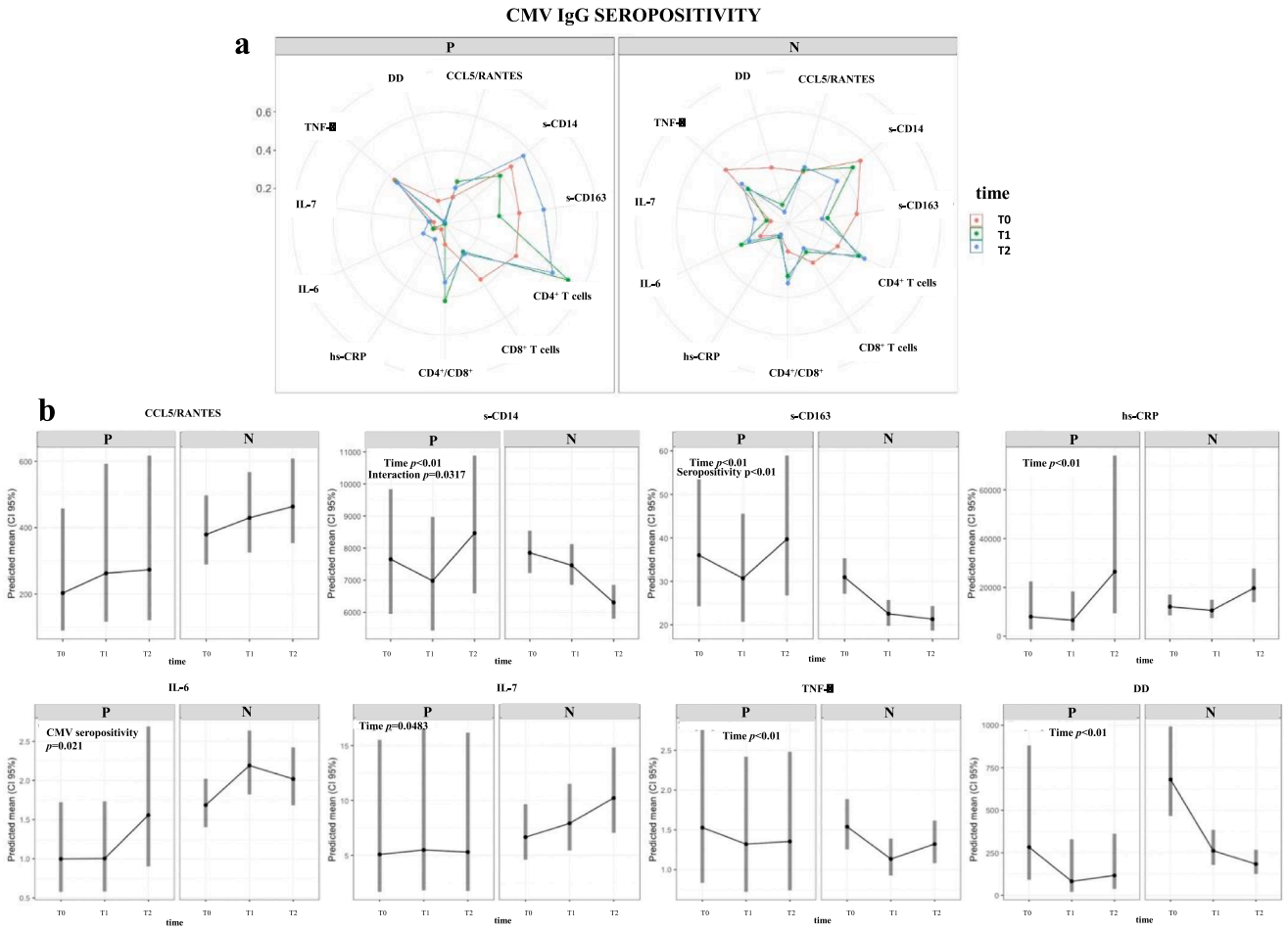


**Fig. 4.** . Trends of inflammatory and immunological markers (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) and according to cytomegalovirus (CMV) IgG seropositivity/negativity (P = positive; N = negative). (**a**) The radar plot, representing inflammatory and immunological marker evolution, was constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. Results are reported as estimated average percentage (%) variation. (**b**) Graphic representation of trends of serum marker levels (CCL5/RANTES, pg/mL; DD, ng/mL; hs-CRP, ng/mL; IL-6, pg/mL; IL-7, pg/mL; s-CD14, pg/mL; s-CD163, ng/mL; TNF-α, pg/mL) over time and according to CMV IgG seropositivity/seronegativity. Significant differences corresponding to Linear Mixed Model (LMM) analyses (see Supplemental Table 9) are reported for each marker.

the first year of cART, particularly with regard to gender, smoke habit, CMV infection, BMI, TG and CHOL levels, known to be potential contributors to inflammation [10–12,13–21].

In general, we observed a great heterogeneity in inflammatory marker levels among patients, that seemed not to be fully accounted for by differences among the studied categories. Notwithstanding, a decrease in most inflammatory markers was observed during the first 6 months of cART, but a significant decrease after 12 months of treatment was observed only for s-CD14, s-CD163 and DD, while IL-6 and hs-CRP significantly increased during therapy. A trend to lower levels of TNF-α ($p = 0.0578$), although characterized by a decrease in the first six months of therapy but a subsequent increase during the period from six to twelve months from cART initiation was also observed.

We also observed that levels of some serum inflammatory markers seemed to differ among categories. IL-6 was significantly lower in females than in males; smokers and former smokers had higher s-CD14 levels than non-smokers; CMV uninfected and infected patients differed for s-CD163 (higher in seronegative patients) and IL-6 (lower in seronegative patients) values; patients with BMI > 25 showed a trend to higher IL-6 levels and patients with high TG levels to higher hs-CRP values.

Whilst most inflammatory marker trends looked similar in all categories after cART initiation, significant differences in category × time interactions were observed for IL-7 and gender (increased in males in respect to females), s-CD14 and CMV infection (decreased in CMV-infected patients) and s-CD14 and TNF-α and CHOL levels (both increased in patients with CHOL > 200 mg/dL).

It has already been demonstrated that cART initiation has an impact on inflammatory markers, reaching a plateau phase following an initial decrease within the first year after cART-induced HIV suppression [25], but, even in patients with maintained total HIV suppression over a long time, some inflammatory markers remain higher than in general population [5]. Our results are consistent with previously established findings, regarding inflammation in HIV-infected patients on cART. However, our findings also suggest that, while inflammation seems globally improved with cART, there is a distinct constellation of persistent immune and inflammatory response activation according to non-HIV-correlated variables, some of which modifiable.

Gender-related differences in inflammatory markers have already been studied in HIV-infected and uninfected patients with discordant results. In the general population, premenopausal females seem to have higher CRP and lower IL-6 and TNF-α levels than males, presumably because of the inhibitory effect of estrogens on the expression of inflammatory markers [26]. In HIV-infected subjects [27], women show

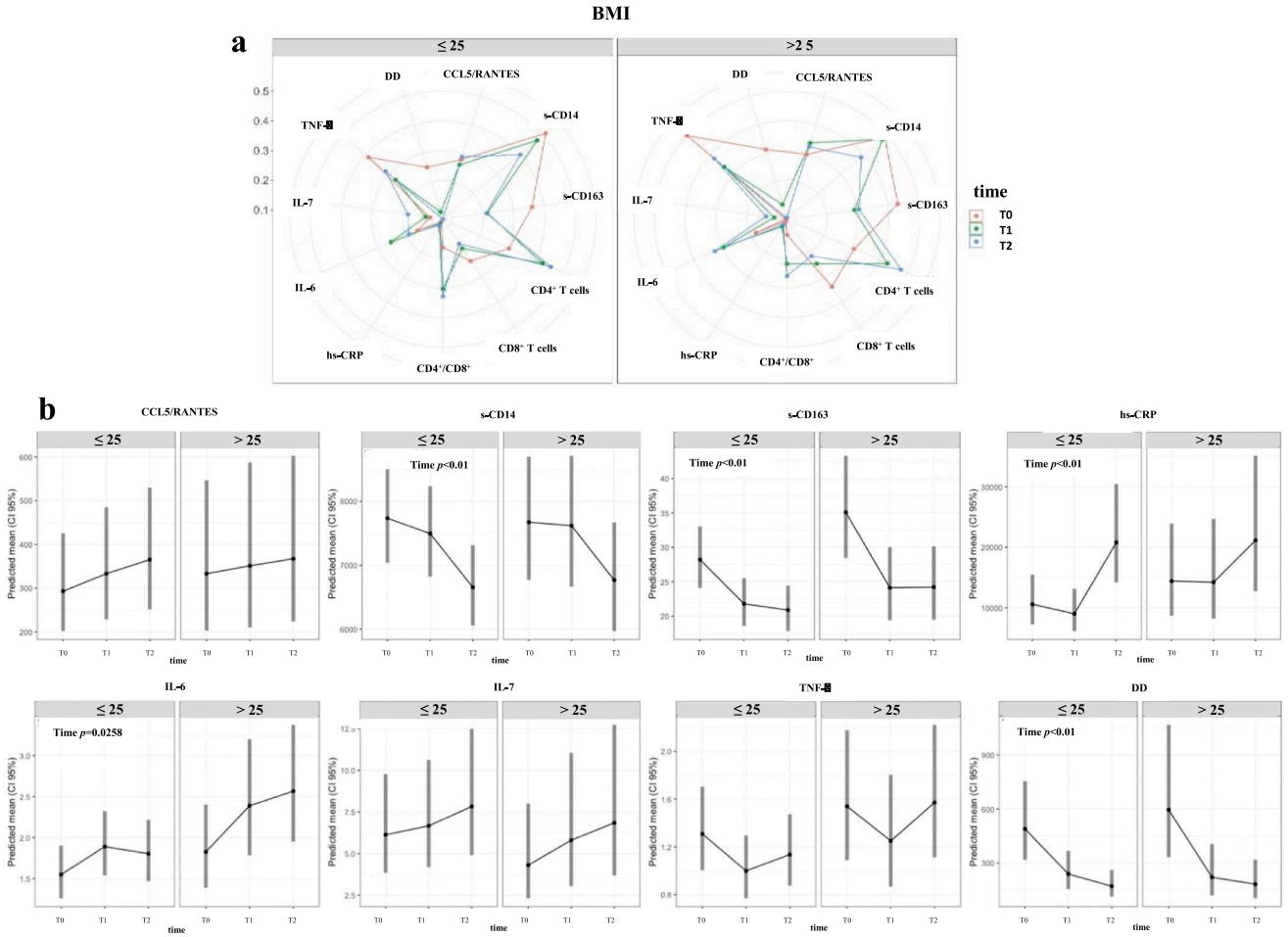


**Fig. 5.** . Trends of inflammatory and immunological markers (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) and according to BMI (≤25 or > 25). (**a**) The radar plot, representing inflammatory and immunological marker evolution, was constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. Results are reported as estimated average percentage (%) variation. (**b**) Graphic representation of trends of serum marker levels (CCL5/RANTES, pg/mL; DD, ng/mL; hs-CRP, ng/mL; IL-6, pg/mL; IL-7, pg/mL; s-CD14, pg/mL; s-CD163, ng/mL; TNF-α, pg/mL) over time and according to BMI. Significant differences corresponding to Linear Mixed Model (LMM) analyses (see Supplemental Table 10) are reported for each marker.

lower CRP, lipopolysaccharide and s-CD14 levels than men at baseline, however, after 12 months of cART, women have higher levels of IFN-γ and TNF-α compared with men, despite a higher $CD4^+$ T cell count. Furthermore, men experience a greater decrease in s-CD14 and CRP levels over time, suggesting that women experience a minor cART-related reduction in inflammation and immune activation [27].

In our study, most inflammatory marker trends after cART initiation were not different between genders, but we observed that IL-6 was lower in females and, spite of a trend to increase in both females and males, the increase was significantly higher in males ($p < 0.01$).

Surprisingly, we observed that, spite of levels of IL-7 did not significantly differ between males and females, their trends were opposite, with a decrease in females and an increase in males (gender × time interaction $p = 0.0203$). IL-7 is involved in T-cell homeostasis, promoting the differentiation, maturation and proliferation of T cells, the survival of naïve and memory cells and the immune cell reconstitution during lymphopenia. This is obtained through thymic and extrathymic differentiation and, when thymic functions physiologically decline with age, mainly through peripheral expansion [28–32]. An inverse correlation exists between endogenous IL-7 plasma levels and $CD4^+$ T cell count during HIV infection and IL-7 administration to HIV-infected patients receiving cART raises $CD4^+$ T cell count by the induction of their proliferation and the promotion of their survival [29,33–35]. However, IL-7 also support HIV persistence during cART, through the induction of viral production in a small number of productively infected cells and the proliferation of latently infected cells [36]. Our data show that, even if IL-7 levels did not significantly differ between males and females, their respective trends were statistically significant (gender × time interaction, $p = 0.0203$). However, our data are difficult to interpret, because $CD4^+$ and $CD8^+$ T cells and $CD4^+/CD8^+$ ratio trends were similar between the two genders (Supplemental Table 7) and, furtherly, women were under-represented in the studied cohort.

In the general population, body weight and adiposity correlate directly with serum CRP and IL-6 [37,38] and weight loss reduces their levels [39]. Conley et al. [40] found similar findings also in treated and virally suppressed HIV-infected people. Here, we did not observe statistically significant differences in inflammatory marker levels either between patients with BMI ≤ and > 25 or between patients with low and high TG or CHOL levels, however we noticed a trend to higher levels of IL-6 in overweight patients and hs-CRP in patients with high TG levels and a significantly different trend over time for s-CD14 and TNF-α, increasing in patients with hypercholesterolemia and decreasing in patients with normal CHOL values. Therefore, treatment strategies that target metabolic abnormalities, independent of their effects on HIV

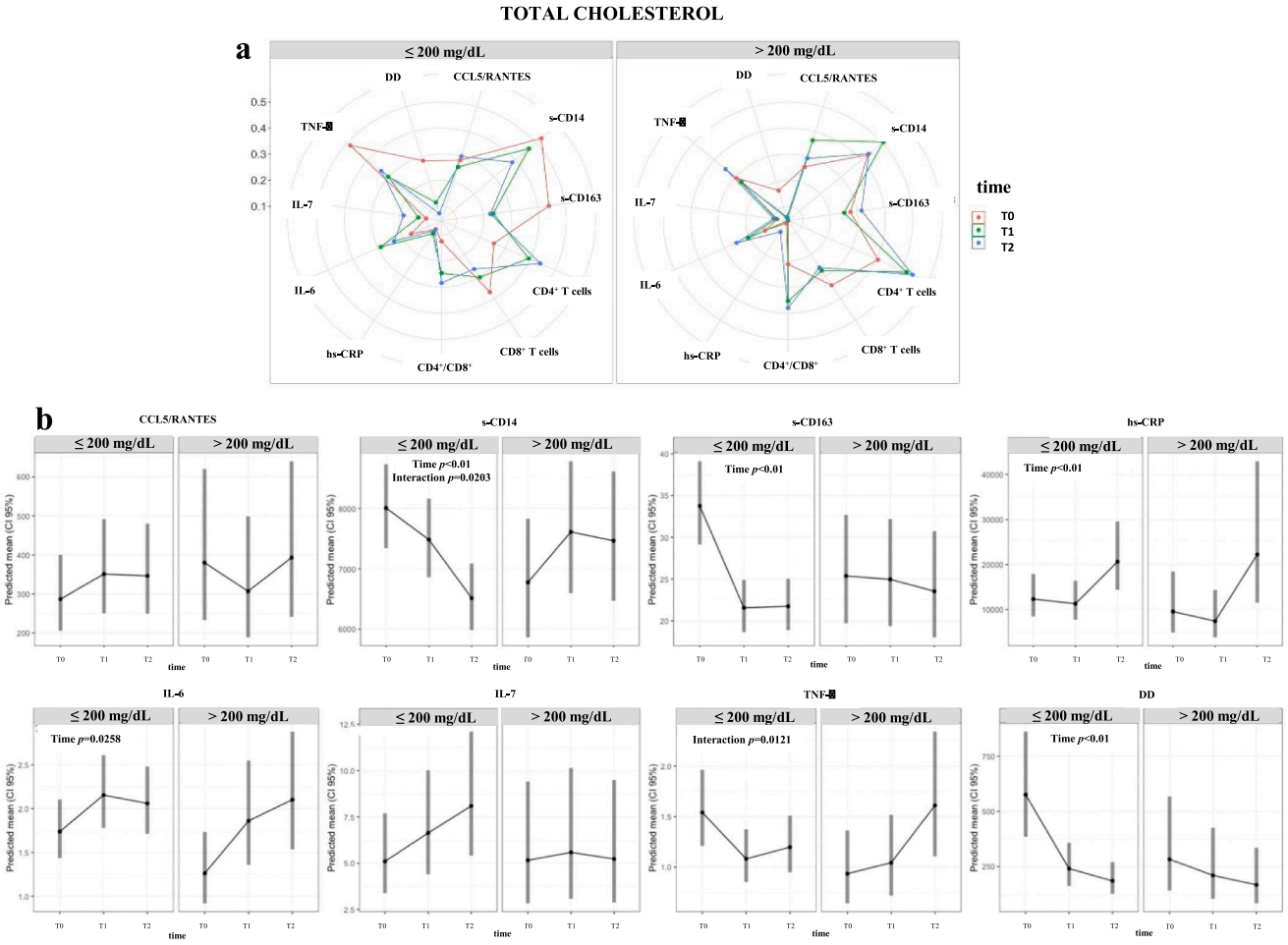


**Fig. 6.** . Trends of inflammatory and immunological markers (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) and according to total cholesterol (CHOL) levels (≤200 or > 200 mg/dL). (**a**) The radar plot, representing inflammatory and immunological marker evolution, was constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. Results are reported as estimated average percentage (%) variation. (**b**) Graphic representation of trends of serum marker levels (CCL5/RANTES, pg/mL; DD, ng/mL; hs-CRP, ng/mL; IL-6, pg/mL; IL-7, pg/mL; s-CD14, pg/mL; s-CD163, ng/mL; TNF-α, pg/mL) over time and according to CHOL levels. Significant differences corresponding to Linear Mixed Model (LMM) analyses (see Supplemental Table 11) are reported for each marker.

pathogenesis, may be an important component of reducing inflammation-associated complications among HIV-infected persons.

Finally, in this study we observed that smokers and former smokers had higher s-CD14 levels than non-smokers, but trend over time was similar among the groups. Castley et al. [41] noticed lower s-CD14 levels in non-smokers compared to smokers in a cohort of HIV-infected patients. More recently, emphysema and s-CD14 have been associated with a higher prevalence of lung nodules in HIV-infected patients [42]. Several studies in humans suggest that smoking may alter the periodontal, upper gastro-intestine (esophagus and stomach) and respiratory microbiomes and in some cases may shift the microbiome toward pathogenic bacteria [43]. Patients who have chronic HIV infection and are receiving suppressive cART display intestinal dysbiosis, associated with increased microbial translocation, contributing to systemic inflammation [44]. The impact of smoke in microbial translocation and inflammation through alterations in the intestinal microbiota in HIV-infected patients need further studies.

We acknowledge some limitations of our study, including the size of our single-center cohort and the low proportion of some categories of patients (CMV infected patients and females) that prevented us to generalize the results. Moreover, data on living setting (urban vs. rural), diet, physical activity were not collected. On the basis of data presented here, we are increasing the dataset to confirm these preliminary observations. Despite these limitations, the strength of our study, compared with other published studies evaluating inflammatory biomarkers, is the longitudinal design, with patients initiating the same class of cART (integrase inhibitors) and all patients with HIV suppression from the sixth month of cART.

Our results represent an important deepening of our previous study regarding those patients [22], that provide further data on changes of inflammatory markers during the first year of cART. In the current study, we focused our attention on potentially modifiable and measurable conditions that may influence changes in the levels of inflammatory markers.

In conclusion, due to the crucial role of inflammation in frailty and correlated non-AIDS events during HIV infection, there is an urgent need for further investigations on key factors that may contribute to the low-level chronic inflammation and immune activation state at the basis of HIV infection-related frailty. The identification of such key factors may help to shed light on its pathogenesis and to determine new therapeutic approaches in its prevention or mitigation.

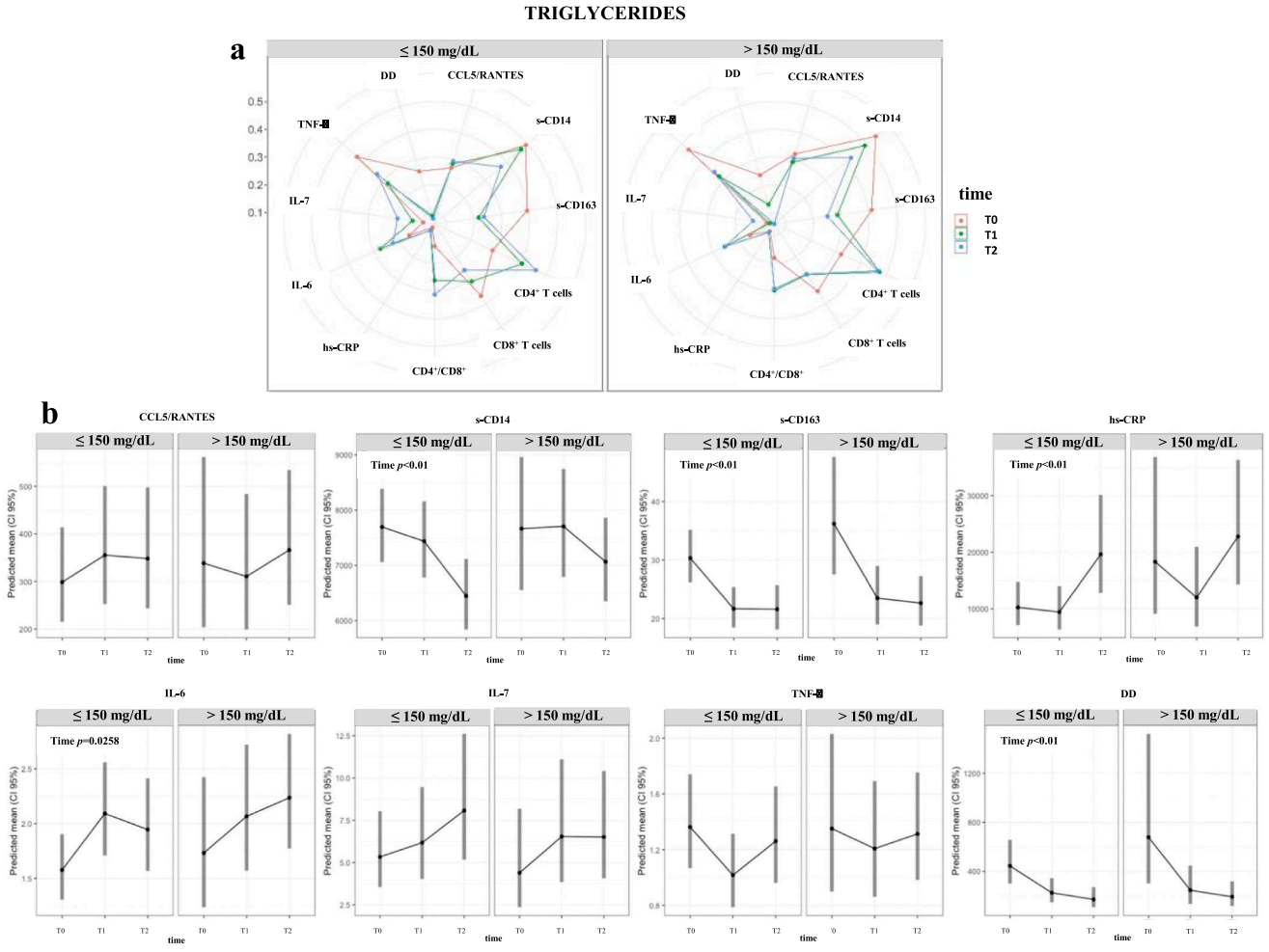


**Fig. 7.** . Trends of inflammatory and immunological markers (CCL5/RANTES = C-C motif chemokine ligand 5/Regulated upon activation normal T cell expressed and presumably secreted; DD = D-dimer; hs-CRP = high sensitivity C Reactive Protein; IL-6 = interleukin-6; IL-7 = interleukin-7; s-CD14 = soluble CD14; s-CD163 = soluble CD163; TNF-α = Tumor necrosis factor α) in serum from T0 (baseline) to T1 (after 6 months of therapy) and T2 (after 12 months of therapy) and according to triglyceride (TG) levels (≤150 or > 150 mg/dL). (**a**) The radar plot, representing inflammatory and immunological marker evolution, was constructed scaling all variables (in a range from 0% to 100%) and plotting averages grouped by time. Results are reported as estimated average percentage (%) variation. (**b**) Graphic representation of trends of serum marker levels (CCL5/RANTES, pg/mL; DD, ng/mL; hs-CRP, ng/mL; IL-6, pg/mL; IL-7, pg/mL; s-CD14, pg/mL; s-CD163, ng/mL; TNF-α, pg/mL) over time and according to TG levels. Significant differences corresponding to Linear Mixed Model (LMM) analyses (see Supplemental Table 12) are reported for each marker.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E. Q.R. received travel grants from Bristol-Myers Squibb, Gilead Sciences, ViiV Healthcare, Janssen-Cilag, Merck Sharp & Dohme and consultancy fees from Janssen-Cilag, ViiV Healthcare and Merck Sharp & Dohme; F. C. reports acting as principal investigator of several company- sponsored clinical trials in the field of HIV infection (ViiV Healthcare–GlaxoSmithKline, Gilead Sciences, and Janssen-Cilag). The remaining authors declare that they have no conflicts of interest.].

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**Data availability**

For ethical and legal restriction we cannot upload a minimal dataset. The data are available upon request, the interested researchers could contact directly Dr. Eugenia Quiros-Roldan (eugeniaquiros@yahoo.it).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2021.155547.

## References

[1] K. Angelidou, P.W. Hunt, A.L. Landay, C.C. Wilson, B. Rodriguez, S.G. Deeks, et al., Changes in Inflammation but Not in T-Cell Activation Precede Non-AIDS-Defining

Events in a Case-Control Study of Patients on Long-term Antiretroviral Therapy, J. Infect. Dis. 218 (2018) 239–248, https://doi.org/10.1093/infdis/jix666.
[2] A.R. Tenorio, Y. Zheng, R.J. Bosch, S. Krishnan, B. Rodriguez, P.W. Hunt, et al., Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment, J. Infect. Dis. 210 (2014) 1248–1259, https://doi.org/10.1093/infdis/jiu254.
[3] B. Grund, J.V. Baker, S.G. Deeks, J. Wolfson, D. Wentworth, A. Cozzi-Lepri, et al., Relevance of Interleukin-6 and D-Dimer for Serious Non-AIDS Morbidity and Death among HIV-Positive Adults on Suppressive Antiretroviral Therapy, PLoS ONE 11 (2016), e0155100, https://doi.org/10.1371/journal.pone.0155100.
[4] S. Hattab, M. Guiguet, G. Carcelain, S. Fourati, A. Guihot, B. Autran, et al., Soluble biomarkers of immune activation and inflammation in HIV infection: impact of 2 years of effective first-line combination antiretroviral therapy, HIV Med. 16 (2015) 553–562, https://doi.org/10.1111/hiv.12257.
[5] M.A. French, M.S. King, J.M. Tschampa, B.A. da Silva, A.L. Landay, 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. 200 (2009) 1212–1215, https://doi.org/10.1086/605890.
[6] X. Yao, H. Li, S.X. Leng, Inflammation and immune system alterations in frailty, Clin. Geriatr. Med. 27 (2011) 79–87, https://doi.org/10.1016/j.cger.2010.08.002.
[7] J. Walston, M.A. McBurnie, A. Newman, R.P. Tracy, W.J. Kop, C.H. Hirsch, et al., Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study, Arch. Intern. Med. 162 (2002) 2333–2341.
[8] A.S. Baranoski, A. Harris, D. Michaels, R. Miciek, T. Storer, P. Sebastiani, M. Montano, Relationship between poor physical function, inflammatory markers, and comorbidities in HIV-infected women on antiretroviral therapy, J. Womens Health 23 (2014) 69–76, https://doi.org/10.1089/jwh.2013.4367.
[9] Anzinger, J.J., Butterfield, T.R., Angelovich, T.A., Crowe, S.M., Palmer, C.S. (2014). Monocytes as regulators of inflammation and HIV-related comorbidities during cART. J. Immunol. Res., 2014, 569819 (2014). doi: 10.1155/2014/569819.
[10] J.B. Margolick, J.H. Bream, T.L. Nilles, H. Li, S.J. Langan, S. Deng, R. Wang, N. Wada, S.X. Leng, Relationship Between T-Cell Responses to CMV, Markers of Inflammation, and Frailty in HIV-uninfected and HIV-infected Men in the Multicenter AIDS Cohort Study, J. Infect. Dis. 218 (2018) 249–258, https://doi.org/10.1093/infdis/jiy005.
[11] K.M. Erlandson, T.B. Campbell, Inflammation in Chronic HIV Infection: What Can We Do? J. Infect. Dis. 212 (2015) 339–342, https://doi.org/10.1093/infdis/jiv007.
[12] Blaum C, Brunner FJ, Kröger F, Braetz J, Lorenz T, Goßling A, et al. Modifiable lifestyle risk factors and C-reactive protein in patients with coronary artery disease: Implications for an anti-inflammatory treatment target population. Eur.J.Prev. Cardiol. 2019 Nov 10:2047487319885458 (2019). doi: 10.1177/2047487319885458 [Epub ahead of print].
[13] J.S. Wooten, P. Nambi, B.K. Gillard, H.J. Pownall, I. Coraza, L.W. Scott, et al., Intensive lifestyle modification reduces Lp-PLA2 in dyslipidemic HIV/HAART patients, Med. Sci. Sports Exerc. 45 (2013) 1043–1050, https://doi.org/10.1249/MSS.0b013e3182843961.
[14] L. Calza, E. Vanino, C. Salvadori, R. Manfredi, V. Colangeli, A. Cascavilla, et al., Tenofovir/emtricitabine/efavirenz plus rosuvastatin decrease serum levels of inflammatory markers more than antiretroviral drugs alone in antiretroviral therapy-naive HIV-infected patients, HIV Clin. Trials 15 (2014) 1–13, https://doi.org/10.1310/hct1501-1.
[15] A.R. Eckard, et al., Effect of 24 weeks of statin therapy on systemic and vascular inflammation in HIV-infected subjects receiving antiretroviral therapy, J. Infect. Dis. 209 (2014) 1156–1164, https://doi.org/10.1093/infdis/jiu012.
[16] Funderburg, N.T., Jiang, Y., Debanne, S.M., Storer, N., Labbato, D., Clagett, B., et al. Rosuvastatin treatment reduces markers of monocyte activation in HIV-infected subjects on antiretroviral therapy. Clin. Infect. Dis., 58, 588-595 (2014). doi: 10.1093/cid/cit748.
[17] R. Valiathan, M.J. Miguez, B. Patel, K.L. Arheart, D. Asthana, Tobacco smoking increases immune activation and impairs T-cell function in HIV infected patients on antiretrovirals: a cross-sectional pilot study, PLoS ONE 9 (2014), e97698, https://doi.org/10.1371/journal.pone.0097698.
[18] V. Mave, K.M. Erlandson, N. Gupte, A. Balagopal, D.M. Asmuth, T.B. Campbell, et al., Inflammation and Change in Body Weight With Antiretroviral Therapy Initiation in a Multinational Cohort of HIV-Infected Adults, J. Infect. Dis. 214 (2016) 65–72, https://doi.org/10.1093/infdis/jiw096.
[19] M. Weltevrede, R. Eilers, H.E. de Melker, D. van Baarle, Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: A systematic review, Exp. Gerontol. 77 (2016) 87–95, https://doi.org/10.1016/j.exger.2016.02.005.
[20] S. Di Benedetto, M. Gaetjen, L. Müller, The Modulatory Effect of Gender and Cytomegalovirus-Seropositivity on Circulating Inflammatory Factors and Cognitive Performance in Elderly Individuals, Int. J. Mol. Sci. 20 (2019) pii: E990, https://doi.org/10.3390/ijms20040990.
[21] A. Garg, S. Gianella, M. Nakazawa, R. Trout, S.A. Spector, Association of Cytomegalovirus DNA and Immunologic Markers of Cardiovascular Disease. *Open Forum*, Infect. Dis. 6 (2019) ofz113, https://doi.org/10.1093/ofid/ofz113.
[22] E. Quiros-Roldan, F. Castelli, A. Bonito, M. Vezzoli, S. Calza, G. Biasiotto, I. Zanella, Inflammation in HIV Study Group. The impact of integrase inhibitor-based regimens on markers of inflammation among HIV naïve patients, Cytokine 126 (2020), 154884, https://doi.org/10.1016/j.cyto.2019.154884.
[23] C. Torti, E. Raffetti, F. Donato, F. Castelli, F. Maggiolo, G. Angarano, et al., Cohort Profile: Standardized management of antiretroviral therapy cohort (MASTER Cohort), Int. J. Epidemiol. 46 (2017), e12, https://doi.org/10.1093/ije/dyv192.
[24] N.M. Laird, J.H. Ware, Random-Effects Models for Longitudinal Data, Biometrics 38 (1982) 963–974.
[25] N.I. Wada, L.P. Jacobson, J.B. Margolick, E.C. Breen, B. Macatangay, S. Penugonda, et al., The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation, AIDS 29 (2015) 463–471, https://doi.org/10.1097/QAD.0000000000000545.
[26] A. Khera, D.K. McGuire, S.A. Murphy, H.G. Stanek, S.R. Das, W. Vongpatanasin, et al., Race and gender differences in C-reactive protein levels, J. Am. Coll. Cardiol. 46 (2005) 464–469.
[27] J.S. Mathad, N. Gupte, A. Balagopal, D. Asmuth, J. Hakim, B. Santos, et al., Sex-Related Differences in Inflammatory and Immune Activation Markers Before and After Combined Antiretroviral Therapy Initiation, J. Acquir. Immune Defic. Syndr. 73 (2016) 123–129, https://doi.org/10.1097/QAI.0000000000001095.
[28] M.V. Soares, N.J. Borthwick, M.K. Maini, G. Janossy, M. Salmon, A.N. Akbar, IL-7-dependent extrathymic expansion of CD45RA+ T cells enables preservation of a naive repertoire, J. Immunol. 161 (1998) 5909–5917.
[29] T.J. Fry, E. Connick, J. Falloon, M.M. Lederman, D.J. Liewehr, J. Spritzler, et al., A potential role for interleukin-7 in T-cell homeostasis, Blood 97 (2001) 2983–12890.
[30] C.D. Surh, J. Sprent, Homeostasis of naive and memory T cells, Immunity 29 (2008) 848–862, https://doi.org/10.1016/j.immuni.2008.11.002.
[31] V. Nguyen, A. Mendelsohn, J.W. Larrick, Interleukin-7 and Immunosenescence, J. Immunol. Res. 2017 (2017) 4807853, https://doi.org/10.1155/20a17/4807853.
[32] T.J. Fry, C.L. Mackall, The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance, J. Immunol. 174 (2005) 6571–6576.
[33] J.H. Colle, J.L. Moreau, A. Fontanet, O. Lambotte, M. Joussemet, S. Jacod, et al., Regulatory dysfunction of the interleukin-7 receptor in CD4 and CD8 lymphocytes from HIV-infected patients–effects of antiretroviral therapy, J. Acquir. Immune Defic. Syndr. 42 (2006) 277–285, https://doi.org/10.1097/01.qai.0000214823.11034.4e.
[34] G. Marchetti, A. Gori, A. Casabianca, M. Magnani, F. Franzetti, M. Clerici, et al., Comparative analysis of T-cell turnover and homeostatic parameters in HIV-infected patients with discordant immune-virological responses to HAART, AIDS 20 (2006) 1727–1736.
[35] S. Beq, J.F. Delfraissy, J. Theze, Interleukin-7 (IL-7): immune function, involvement in the pathogenesis of HIV infection and therapeutic potential, Europ. Cytokine Netw. 15 (2004) 279–289.
[36] C. Vandergeeten, R. Fromentin, S. DaFonseca, M.B. Lawani, I. Sereti, M. M. Lederman, et al., Interleukin-7 promotes HIV persistence during antiretroviral therapy, Blood 121 (2013) 4321–4329, https://doi.org/10.1182/blood-2012-11-465625.
[37] P.M. Ridker, C.H. Hennekens, J.E. Buring, N. Rifai, C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women, New Engl. J. Med. 342 (2000) 836–843.
[38] P.M. Ridker, N. Rifai, M.J. Stampfer, C.H. Hennekens, Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men, Circulation 101 (2000) 1767–1772.
[39] M. Dietrich, I. Jialal, The effect of weight loss on a stable biomarker of inflammation C-reactive protein, *Nutr. Rev.* 63 (2005) 22–28.
[40] L.J. Conley, T.J. Bush, A.W. Rupert, I. Sereti, P. Patel, J.T. Brooks, et al., Obesity is associated with greater inflammation and monocyte activation among HIV-infected adults receiving antiretroviral therapy, AIDS 29 (2015) 2201–2207, https://doi.org/10.1097/QAD.0000000000000817.
[41] A. Castley, L. Williams, I. James, G. Guelfi, C. Berry, D. Nolan, Plasma CXCL10, sCD163 and sCD14 Levels Have Distinct Associations with Antiretroviral Treatment and Cardiovascular Disease Risk Factors, PLoS ONE 11 (2016), e0158169, https://doi.org/10.1371/journal.pone.0158169.
[42] M. Triplette, K.M. Sigel, A. Morris, S. Shahrir, J.P. Wisnivesky, C.Y. Kong, et al., Emphysema and soluble CD14 are associated with pulmonary nodules in HIV-infected patients: implications for lung cancer screening, AIDS 31 (2017) 1715–1720, https://doi.org/10.1097/QAD.0000000000001529.
[43] Z. Savin, S. Kivity, H. Yonath, S. Yehuda, Smoking and the intestinal microbiome, Arch. Microbiol. 200 (2018) 677–684, https://doi.org/10.1007/s00203-018-1506-2.
[44] D.M. Dinh, G.E. Volpe, C. Duffalo, S. Bhalchandra, A.K. Tai, A.V. Kane, et al., Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection, J. Infect. Dis. 211 (2015) 19–27, https://doi.org/10.1093/infdis/jiu409.