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ORIGINAL ARTICLE

# Intestinal Barrier Impairment and Immune Activation in HIV-Infected Advanced Late Presenters are Not Dependent on CD4 Recovery

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**Abstract**
Damage of the mucosal barrier in HIV infection, microbial translocation, and immune activation can persist even in patients on successful antiretroviral therapy (ART) especially advanced late presenters. The aim of this study was to find factors that determine immune activation and bacterial translocation in HIV-infected advanced late presenters on suppressive ART. Forty-three late presenters (CD4 < 200 cells/μl prior to ART) on successful ART (more than 2 years of ART) with optimal and suboptimal CD4 recovery were enrolled into this study. The serum concentrations of intestinal fatty acid-binding peptide (I-FABP), zonulin-1, programmed cell death-1 protein (PCDP-1), and soluble (s)CD14 were measured using the ELISA test. We found higher serum levels of I-FABP and sCD14 in successfully antiretroviral-treated advanced late presenters compared to healthy subjects ($p < 0.0001$ and $p = 0.0004$). The serum concentration of PCDP-1 and zonulin-1 in HIV-infected patients did not differ from healthy controls. The levels of microbial translocation and immune activation markers were not associated with the degree of CD4 recovery. A serum concentration of I-FABP above 2.03 ng/ml was independently associated with a shorter ART (OR 0.78; $p = 0.03$). Older age was related to serum levels of sCD14 above 2.35 μg/ml (OR 1.1; $p = 0.01$). Higher serum levels of I-FABP and sCD14 in successfully antiretroviral-treated advanced late presenters compared to healthy subjects suggest an incomplete reconstruction of the intestinal barrier and sustained immune activation despite good CD4 recovery. It was not the CD4 level, but the length of the suppressive ART that was found to be associated with the restoration of the intestinal barrier.

**Keywords** HIV infection · Advanced late presenters · Microbial translocation and immune activation

## Introduction

Nowadays, the prognosis in HIV-infected population has significantly improved by the introduction of antiretroviral therapy (ART). It is currently believed that HIV-infected patients who start the antiretroviral treatment early at high CD4 count levels are living almost as long as the uninfected population.

However, in patients diagnosed late in the course of HIV infection and subsequently late antiretroviral treated, a longer time period is needed to provide optimal immune reconstitution. Moreover, in some cases, the CD4 T-cell immune recovery is unsatisfactory even after long-term treatment.

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It is not surprising that late HIV diagnosis is associated with increased morbidity, mortality and worse treatment outcomes (Fisher 2008; Nakagawa et al. 2012). According to the European Late Presenter Consensus, late presentation is understood as a diagnosis of HIV with a CD4 count below 350 cells/μl and advanced HIV disease is defined as a diagnosis with a CD4 cell count below 200 cells/μl. The patients presenting with an AIDS-defining disease are considered as late presenters or advanced late presenters based on CD4 cell count (Antinori et al. 2011). Late presenters remain at an increased risk for the development of AIDS- and non-AIDS-defining diseases (Mocroft et al. 2010; Sackoff et al. 2006). The patients diagnosed late have a tenfold increased risk of death within 1 year of HIV diagnosis compared to those diagnosed promptly (3.8 vs. 0.35%) (Health Protection Agency 2012). The increased prevalence of non-AIDS-defining conditions seems to be associated with chronic immune activation and inflammation (Anzinger et al. 2014). Damage of the mucosal barrier during HIV infection and

translocation of the bacteria from the gastrointestinal tract have been proposed as a major causes of immune activation. Many studies have shown that immune activation can persist even in successfully antiretroviral-treated HIV-infected patients and is associated with premature aging of HIV-infected patients (Kaplan et al. 2011; Martin et al. 2013; Tenorio et al. 2014). It is worth noting that late presentation predisposes the patient to longer exposure to bacterial translocation and immune activation. Unfortunately, approximately 20% of patients may experience immune non-response even after suppressive ART, and this is more commonly seen in late presenters (Gaardbo et al. 2012). A poorer virological and immunological response during ART are observed more often in late presenters, and this promotes greater persistence of bacterial translocation and immune activation (Waters et al. 2011).

There are several markers of the impairment of the gut barrier, microbial translocation, and immune activation. Intestinal fatty acid-binding protein (I-FABP), released by necrotic enterocytes, and zonulin-1, which regulates the tight junctions between enterocytes, are useful markers for the detection of damage to the intestinal mucosa. Soluble CD14 (sCD14), a co-receptor for lipopolysaccharide (LPS), is released from monocytes upon activation. Programmed cell death 1 protein (PCDP-1) regulates lymphocyte activation but its sustained expression is characteristic of T-cell exhaustion. Increased levels of sCD14, I-FABP, and PCDP-1 in serum have been reported in HIV-infected antiretroviral treated and untreated patients compared to healthy subjects (Cockerham et al. 2014; Lederman et al. 2011; Tincati et al. 2016). Elevated markers of immune activation have been reported also in late presenters receiving ART (Bai et al. 2012; Hunt et al. 2003). However, data on advanced late presenters and the impact of $CD4^+$ T-cell restoration during ART on gut barrier, bacterial translocation, and immune activation are sparse.

The aim of this study was to assess the plasma concentrations of markers related to enterocyte damage, microbial translocation and immune activation in HIV-infected advanced late presenter patients and their association with the degree of CD4 cell count recovery during antiretroviral treatment. The marker concentrations were further compared to those observed in healthy subjects. We also analyzed other factors that determine immune activation and bacterial translocation in advanced late presenters on suppressive ART.

## Materials and Methods

### Study Group

The study group consisted of 32 (74.41%) males and 11 (25.59%) females. Nineteen patients with CD4 levels above 500 cells/μl during antiretroviral treatment were enrolled into one subgroup, while 24 subjects with CD4 levels below 350 cells/μl were grouped into the other subgroup.

Dominant route of HIV transmission was homo/bisexual contacts (24 patients: 55.81%); heterosexual intercourse and intravenous drug use were the causes of HIV infection in 9 patients (20.93%) and in 10 patients (23.25%), respectively.

At the time of diagnosis, 14 patients (32.56%) were classified as Stage A according to CDC criteria, 17 as Stage B (39.53%) and 12 as Stage C (27.91%). The median age of the HIV-infected patients was 40 years. The characteristics of the patients are summarized in Table 1.

### Methods

To answer these questions, we selected and analyzed two groups of HIV-infected advanced late presenters on ART with either complete or partial CD4 count recovery. The participation in this study was proposed to all HIV-diagnosed advanced late presenters who met the inclusion criteria attending a Lodz center between 1999 and 2014. However, only the consecutive patients who had provided written informed consent and who had provided a blood sample

**Table 1** Characteristics of the study group

| | HIV-infected patients | | | |
|---|---|---|---|---|
| | Median | Mean ± SD | Min–max | LQ–UQ |
| Age | 40 | 42.60 ± 10.58 | 26–65 | 15–50 |
| Time from HIV diagnosis (western blot) | 6 | 8.23 ± 6.03 | 2–25 | 3–12 |
| Duration of ART | 6 | 6.7 ± 4.1 | 2–18 | 3–10 |
| CD4 at the moment of ART introduction | 109 | 107.84 ± 64.40 | 1–199 | 59–167 |
| Viral load before ART introduction | 122,500 | 275,825.5 ± 449,522 | 3720–1,640,000 | 38,750–215,000 |
| CD4 during ART | 339 | 544.23 ± 359.54 | 202–1470 | 258–797 |
| CD4% during ART | 26 | 25.48 ± 10.44 | 8.2–46.4 | 15.9–34.3 |
| CD4/CD8 ratio during ART | 0.62 | 0.72 ± 0.47 | 0.21–2.34 | 0.38–0.91 |

*LQ–UQ* lower–upper quartiles

were included in the study. We compared the plasma concentrations of the above-mentioned markers in these subgroups as well as in HIV non-infected patients.

Inclusion criteria were as follows: (1) CD4 cell count below 200 cells/μl before the introduction of ART; (2) standard triple ART; (3) minimum 2 years of ART; (4) viral load < 50 copies/ml in all results within the last year; and (5) present CD4 cell count: (a) CD4 > 500 cells/μl, (b) CD4 < 350 cells/μl.

The exclusion criteria were as follows: (1) HBV/HCV co-infection; (2) age younger than 18 years; (3) prisoners, active drug users; (4) diagnosis of other chronic diseases; (5) treatment with drugs other than antiretrovirals; and (6) pregnancy.

The control group consisted of 20 healthy volunteers, age and sex matched.

We evaluated the following markers: (1) I-FABP and zonulin-1 as markers of the impairment of the gut barrier; (2) PCDP-1 as a marker of immune activation; and (3) sCD14 as a marker of monocyte activation by LPS (bacterial LPS) and concurrent microbial translocation.

Plasma concentrations of the markers associated with the impairment of the gut barrier (I-FABP and zonulin), lymphocyte activation (PCDP1-marker of T-cell activation) and microbial translocation and monocyte activation (sCD14) were measured using enzyme-linked immunosorbent assays.

Three ml of venous blood was collected, the sample was allowed to clot and centrifuged at 10,000×*g* for 2 min at 4 °C to remove particulate matter. All coded serum samples were stored at − 80 °C until analysis. Prior to quantitation, a frozen aliquot 500 μl of each serum sample and all reagents were stabilized at room temperature.

For detection of protein levels in the serum of the patients and controls, specific commercially available ELISA kits were used: I-FABP, sCD14 from R&D Systems (Minneapolis, USA) and PDCD-1, zonulin-1 from SunRedBio (Shanghai, China).

Washing steps were carried out with the Stat-Matic Plate Washer II (Sigma-Aldrich, USA).

The absorbance was read by a Multifunctional Microplate Reader VICTOR™ X4 (Perkin Elmer, USA). All ELISA results were analyzed with WorkOut 2.5 Software, standard curves were fitted with a four-parameter logistic model and the concentrations of samples were calculated from the proper standard curve.

In the HIV-infected patients, CD4 cell counts within 3 months were analyzed. CD4 cell counts were determined using flow cytometry. HIV-RNA levels were measured using the RT-PCR method (Cobas AmpliPrep/Cobas TaqMan HIV-1 Test, Roche Diagnostics, Switzerland).

Informed written consent was obtained from all participants prior to enrollment. This study was approved by the ethical committee of the Faculty of Medicine, Medical University of Lodz. The procedures followed were in accordance with the Helsinki Declaration of 1975.

### Statistical Analysis

The data distributions were assessed for normality. The average values and standard deviations of quantitative traits were calculated for parameters with normal distributions. Variables that were not normally distributed were expressed by the median (lower–upper quartiles). The Mann–Whitney *U* test for nonparametric continuous variables was used to compare the differences between two study groups. To compare categorical variables between groups, the Chi-square distribution test was used. Both univariate and multivariate logistic regressions were applied to determine whether serum I-FABP ≥ 2.03 (more than 2SD away from the healthy group's mean value) and sCD14 ≥ 2.35 (more than 2SD away from the healthy group's mean value) were associated with selected predictors.

## Results

### Analysis of I-FABP and Zonulin-1: Dependence on CD4 Recovery and Comparison to the Control Group

In HIV-infected patients, serum level of I-FABP was significantly higher than in the healthy controls. Significant concentration differences were observed not only between healthy subjects and all HIV-infected patients ($p < 0.0001$), but also in the comparison of healthy individuals to HIV-infected patients with CD4 > 500 cells/μl ($p = 0.0006$) and to HIV-infected patients with CD4 < 350 cells/μl ($p = 0.0016$; Tables 2, 3). I-FABP levels did not differ significantly between patients with CD4 > 500 cells/μl and CD4 < 350 cells/μl during antiretroviral treatment (Table 3).

The mean level of zonulin-1 serum in the antiretroviral-treated group was 32.65 ng/ml (SD 63.00 ng/ml) and did not differ from levels observed in healthy controls. There was no significant difference in the zonulin-1 serum level depending on the degree of CD4 recovery (Tables 2, 3).

### The Chance of I-FABP ≥ 2.03 ng/ml (More than 2SD Away from the Healthy Group's Mean Value)

The concentrations of I-FABP in healthy subjects were normally distributed with the mean value of 1.08 ng/ml (SD: 0.47 ng/ml). This value ± 2SD was determined as a cut-point in multivariate logistic regression.

Univariate analysis found the risk of I-FABP ≥ 2.03 ng/ml to be associated with a shorter duration of ART (borderline significance $p = 0.053$). We performed also multivariate

**Table 2** Serum levels of I-FABP, sCD14, PCPD-1, and zonulin in HIV-infected advanced late presenter patients and healthy controls

| | Healthy subjects | | | | HIV-infected antiretroviral-treated patients | | | | *p* |
|---|---|---|---|---|---|---|---|---|---|
| | Median | Mean ± SD | Min–min | LQ–UQ | Median | Mean ± SD | Min–max | LQ–UQ | |
| Age | 38 | 40.45 ± 8.70 | 26–65 | 32.5–51 | 40 | 42.60 ± 10.58 | 26–69 | 35–50 | > 0.05 |
| I-FABP (ng/ml) | 1.05 | 1.08 ± 0.47 | 0.35–2.27 | 0.68–1.35 | 2.28 | 2.53 ± 1.46 | 0.54–6.81 | 1.42–3.35 | < 0.0001 |
| sCD14 (μg/ml) | 1.46 | 1.66 ± 0.34 | 1.06–2.4 | 1.37–1.89 | 2.06 | 2.12 ± 0.49 | 1.43–3.48 | 1.77–2.49 | = 0.0004 |
| PCDP-1 (ng/ml) | 10.24 | 45.38 ± 62.44 | 2.23–228.44 | 4.82–74.71 | 5.34 | 23.24 ± 42.10 | 0.25–220.45 | 4.09–22.72 | > 0.05 |
| Zonulin-1 (ng/ml) | 10.26 | 22.37 ± 31.82 | 1.86–112.2 | 5.88–17.26 | 7.67 | 32.65 ± 63.00 | 0.68–317.85 | 5.945–14.525 | > 0.05 |

**Table 3** Serum levels of I-FABP, sCD14, PCPD-1, and zonulin in HIV-infected advanced late presenter patients depending on CD4 T-cell recovery

| | HIV-infected antiretroviral-treated patients with CD4 > 500 cells/μl | | | | HIV-infected antiretroviral-treated patients with CD4 < 350 cells/μl | | | | *p* |
|---|---|---|---|---|---|---|---|---|---|
| | Median | Mean ± SD | Min–max | LQ–UQ | Median | Mean ± SD | Min–max | LQ–UQ | |
| FABP (ng/ml) | 2.54 | 2.44 ± 1.08 | 0.54–4.23 | 1.48–3.35 | 2.05 | 2.59 ± 1.73 | 0.58–6.81 | 1.34–3.49 | > 0.05 |
| sCD14 (μg/ml) | 1.92 | 2.04 ± 0.44 | 1.43–3.24 | 1.78–2.14 | 2.12 | 2.18 ± 0.54 | 1.45–3.48 | 1.76–2.54 | > 0.05 |
| PCDP-1 (ng/ml) | 7.31 | 23.40 ± 37.83 | 3.14–130.09 | 4.12–10.84 | 4.47 | 23.11 ± 46.00 | 0.25–220.45 | 4–27.61 | > 0.05 |
| Zonulin (ng/ml) | 9.01 | 27.76 ± 52.29 | 3.32–171.63 | 6.14–12.19 | 6.61 | 36.29 ± 70.83 | 0.68–317.85 | 5.91–37.85 | > 0.05 |
| Age | 40 | 41.84 ± 10.06 | 26–64 | 35–52 | 40 | 43.20 ± 11.16 | 28–69 | 36.5–47.5 | > 0.05 |
| Duration of ART | 8 | 8.53 ± 4.25 | 2–18 | 5–11 | 7 | 7.01 ± 3.46 | 2–15 | 4–9.5 | > 0.05 |
| CD4 at the moment of ART introduction | 114 | 118 ± 54.85 | 15–190 | 80–167 | 101 | 99.79 ± 71.64 | 1–199 | 31–169.5 | > 0.05 |
| Viral load before ART introduction | 108,950 | 485,065 ± 717,683 | 3720–1,640,000 | 25,950–83,500 | 122,500 | 171,276 ± 185,651 | 6450–678,000 | 43,500–96,000 | > 0.05 |
| CD4 during ART | 802 | 884.37 ± 282.85 | 564–1470 | 663–1124 | 269 | 274.96 ± 47.11 | 202–350 | 328.5–315.5 | < 0.0001 |
| CD4% during ART | 33.4 | 33.27 ± 7.72 | 18.2–46.4 | 28.6–38.1 | 16.1 | 19.31 ± 7.77 | 8.2–35.9 | 14.1–23.25 | < 0.0001 |
| CD4/CD8 ratio during ART | 0.86 | 0.98 ± 0.53 | 0.37–2.34 | 0.68–1.19 | 0.41 | 0.52 ± 0.28 | 0.21–1.18 | 0.34–0.59 | 0.0006 |

analysis including not only duration of ART, but also other factors associated with the impairment of the gut barrier and immune activation well-known from medical literature as age, gender, degree of CD4 recovery (number of CD4 cells and CD4/CD8 ratio) (Bruno et al. 2017; Griesbeck et al. 2016; Jain et al. 2013; Muenchhoff et al. 2018; Somsouk et al. 2015; Tincati et al. 2016; Wang and Kotler 2014).

Multivariate logistic regression also showed that shorter durations of antiretroviral treatment were independently associated with higher levels of I-FABP [odds ratio (OR) 0.78; $p = 0.03$; Table 4]. Gender, age, and degree of CD4 recovery (number of CD4 cells and CD4/CD8 ratio) had no impact on the prevalence of I-FABP ≥ 2.03 ng/ml.

## Analysis of sCD14 and Pcdp-1: Dependence on CD4 Recovery and Comparison to the Control Group

The mean sCD14 serum concentration in antiretroviral-treated HIV late presenters was 2.12 μg/ml (SD 0.49 μg/ml) and was significantly higher than concentration observed in healthy controls ($p = 0.0004$).

**Table 4** Univariate and multivariate analyses of chance of I-FABP ≥ 2.03 ng/ml (healthy group's mean value ± 2SD) in HIV-infected advanced late presenter patients ($n = 24$)

| Characteristics | OR | 95% CI | $p$ |
|---|---|---|---|
| Univariate analysis of chance of I-FABP ≥ 2.03 ng/ml (healthy group's mean value ± 2SD) | | | |
| Gender: women | 1.06 | 0.27–4.19 | > 0.05 |
| Age | 0.99 | 0.94–1.05 | > 0.05 |
| Duration of ART | 0.84 | 0.71–1.00 | = 0.053 |
| Good or poor CD4 T-cell recovery[a] | 0.73 | 0.22–2.44 | > 0.05 |
| CD4/CD8 ratio during ART | 1.53 | 0.40–5.82 | > 0.05 |
| Multivariate analysis of chance of I-FABP ≥ 2.03 ng/ml (healthy group's mean value ± 2SD) | | | |
| Gender: women | 1.36 | 0.28–6.49 | > 0.05 |
| Age | 1.02 | 0.95–1.09 | > 0.05 |
| Duration of ART | 0.78 | 0.63–0.98 | = 0.03 |
| Good or poor CD4 T-cell recovery[a] | 0.22 | 0.04–1.17 | > 0.05 |
| CD4/CD8 ratio during ART | 1.93 | 0.46–7.98 | > 0.05 |

*OR* Odds ratio

[a]Good CD4T cell recovery means CD4 > 500 cells/μl during ART, poor CD4 T-cell recovery means CD4 < 350 cell/μl during ART

**Table 5** Univariate and multivariate analyses of chance of sCD14 ≥ 2.35 μg/ml (healthy group's mean value ± 2SD) in HIV-infected advanced late presenter patients (n = 14)

| Characteristics | OR | 95% CI | $p$ |
|---|---|---|---|
| Univariate analysis of chance of sCD14 ≥ 2.35 μg/ml (healthy group's mean value ± 2SD) | | | |
| Gender: women | 1.71 | 0.40–7.43 | > 0.05 |
| Age | 1.09 | 1.02–1.17 | = 0.015 |
| Duration of ART | 1.03 | 0.87–1.21 | > 0.05 |
| Good or poor CD4 T-cell recovery[a] | 1.88 | 0.47–7.54 | > 0.05 |
| CD4/CD8 ratio during ART | 1.03 | 0.25–4.30 | > 0.05 |
| Multivariate analysis of chance of sCD14 ≥ 2.35 μg/ml (healthy group's mean value ± 2SD) | | | |
| Gender: women | 2.18 | 0.41–11.41 | > 0.05 |
| Age | 1.1 | 1.02–1.19 | = 0.01 |
| Duration of ART | 0.99 | 0.81–1.20 | > 0.05 |
| Good or poor CD4 T-cell recovery[a] | 1.79 | 0.33–9.76 | > 0.05 |
| CD4/CD8 ratio during ART | 1.45 | 0.26–7.96 | > 0.05 |

[a]Good CD4 T-cell recovery means CD4 > 500 cells/μl during ART, poor CD4 T-cell recovery means CD4 < 350 cell/μl during ART

Significant concentration differences were observed also in the comparison of healthy individuals to HIV-infected patients with CD4 > 500 cells/μl ($p = 0.0006$) and to HIV-infected patients with CD4 < 350 cells/μl ($p < 0.0001$; Tables 2, 3).

The sCD14 concentration did not differ between antiretroviral-treated patients with good and poor CD4 recovery (Table 3).

The mean PCDP-1 serum concentration in antiretroviral-treated HIV-infected patients was 23.24 ng/ml (SD 42.10 ng/ml) and did not differ from results obtained in healthy controls. There were no significant differences in PCDP-1 concentrations in relation with the degree of CD4 recovery (Tables 2, 3).

### The Chance of sCD14 ≥ 2.35 μg/ml (More than 2SD Away from the Healthy Group's Mean Value)

The concentrations of sCD14 in the healthy subjects were normally distributed with a mean value of 1.66 μg/ml (SD 0.24 μg/ml). This value ± 2SD was determined as a cut-point in multivariate logistic regression.

Univariate and multivariate analyses confirmed that only older age was independently associated with higher serum levels of sCD14 (OR 1.09; $p = 0.015$ and OR 1.1; $p = 0.01$).

The degree of CD4 T-cell recovery (number of CD4 cells and CD4/CD8 ratio), duration of ART and gender had no significant impact on the serum levels of sCD14 (Table 5).

## Discussion

The damage to the tissue of the mucosal barrier and subsequent microbial translocation in HIV-infected patients may contribute to persistent immune activation and inflammation. The depletion of CD4 T cells in gut-associated lymphoid tissue (GALT) and the apoptosis of enterocytes are observed not only during acute HIV infection, but also in later stages of the disease (Brenchley et al. 2004; Mehandru et al. 2007). Successful antiretroviral treatment can improve the function of GALT; however, the number of $CD4^+$ T cells in the gastrointestinal tract does not recover even after years of treatment (Mehandru et al. 2006). Many studies report that late presenters who start ART at lower CD4 counts experience poorer CD4 recovery and lower rates of virologic suppression compared to patients who initiate treatment at a higher CD4 count due to longer exposure to HIV-associated inflammation and immune activation (Kelley et al. 2009). The results of the Strategies for Management of Antiretroviral Therapy trial highlighted a link between inflammatory processes and the incidence of non-AIDS diseases, including cardiovascular pathologies and neoplasms (Kuller et al. 2008; Sigel et al. 2016). The increased incidence of non-AIDS diseases in antiretroviral-treated patients is also associated with persistent microbial translocation and chronic immune activation (Deeks et al. 2013).

I-FABP is a good marker indicating impairment of the gut epithelial barrier (Funaoka et al. 2010). In our study, we reported significantly higher serum levels of I-FABP in antiretroviral-treated HIV-infected individuals compared to healthy controls; this could indicate persistent damage of

the intestinal mucosal tissue in this group of patients. We showed that in advanced late presenters, I-FABP levels were not associated with the degree of CD4 T-cell recovery. Our observations are in accordance with results obtained by other authors who also reported that when ART was started late (that is when CD4 T-cell count falls below 350 cells/µl), the reconstitution of the intestinal mucosa was very poor, even after years of therapy (Guadalupe et al. 2003; Sun et al. 2007). However, we found the duration of the antiretroviral treatment had a positive impact on the restoration of the epithelial barrier structure in advanced late presenter patients. A lower serum concentration of I-FABP was independently associated with longer duration of ART, suggesting that long-term suppression of HIV replication could decrease viral-associated damage to the intestinal mucosa. We speculate that in advanced late presenters, damage to the gut epithelial barrier and difficulties in reconstructing the intestinal mucosal tissue are associated with persistent immune activation, which is reported even in patients with successful virologic response and great CD4 T-cell recovery.

In terms of immune activation, increased levels of sCD14 were found in advanced late presenters undergoing suppressive antiretroviral treatment compared to age matched healthy controls. This suggests an intensification of immune activation in HIV-infected patients with late diagnosis, even with good CD4 T-cell recovery. Importantly, sCD14 level is an independent predictor of mortality in HIV infection (Sandler et al. 2011). Similar to our results, Méndez-Lagares et al. (2013) found increased plasma levels of sCD14 in HIV-infected patients experiencing suppressive ART. In our study, the duration of ART, degree of CD4 T-cell recovery, and gender were not associated with serum levels of sCD14. In the group of advanced late presenters, ART does not completely reverse the immune activation, which in turn may lead to the development of non-AIDS-related diseases. Therefore, patients who started antiretroviral treatment late, especially shortly after ART initiation, should be monitored more closely.

Importantly, only older age was independently associated with higher serum levels of sCD14. Notably, many studies have also shown that aging is associated with chronic immune activation (Appay and Kelleher 2016; Hearps et al. 2012). Excessive immune activation and persistent inflammation characterized both HIV infection and progressive aging contribute to the development of non-HIV-associated cancers, neurocognitive, cardiovascular, liver, and kidney diseases (d'Ettorre et al. 2011; Nasi et al. 2014; Tarancon-Diez et al. 2017).

Therefore, there is an increased risk of non-AIDS-defining illnesses in older people regardless of CD4 T-cell recovery. As a result, late presenters should be advised early to start primary prevention of cardiovascular disease including statin therapy.

Long-term stimulation of the immune system in the course of HIV infection causes exhaustion of the effector functions of T lymphocytes. High expression of co-inhibitory molecule programmed death-1 on virus-specific T cells in the HIV infection is a marker of exhausted T cells (Breton et al. 2013; Day et al. 2006). In a study by Cockerham et al. (2014), it has been observed that increased PCDP-1 serum concentration during early HIV infection declined over first 12 months of ART; after several years of treatment levels of PCDP-1 were comparable to levels seen in the HIV-uninfected controls. This may explain why mean levels of PCDP-1 reported in our study did not significantly differ between antiretroviral-treated HIV-infected patients and healthy controls. Cockerham et al. (2014) reported significantly higher PCDP-1 expression on CD4 and CD8 in immunologic non-responders than in patients with good CD4 T-cell recovery. However, we did not confirm this association between serum levels of PCDP-1 and the degree of CD4 T-cell reconstitution. The possible explanation for the above-mentioned difference is that our patients were advanced late presenters with possible high levels of PCDP-1 expression before the introduction of antiretroviral treatment. Recent studies showed that the level of PCDP-1 expression in HIV-specific CD8 cells correlates with disease severity, as measured by viral load and declining CD4 cell counts (Trautmann et al. 2006).

The limitations of our study include the use of a small group and lack of assessment of markers associated with enterocyte damage, microbial translocation, and the immune activation before initiating ART. However, our group was carefully selected to rule out the influence of various parameters on the evaluated markers.

In conclusion, higher serum levels of I-FABP and sCD14 in successfully antiretroviral-treated advanced late presenters as compared to healthy subjects indicate to an incomplete reconstruction of the small intestine mucosal barrier and sustained immune activation despite good CD4 cell recovery. Therefore, we propose closer monitoring of patients who late started antiretroviral treatment. The residual immune activation observed in advanced late presenters may be associated with an increased risk of the development of non-AIDS-defining events, even in those with good CD4 cell recovery. It is not CD4 level, but the length of the suppressive antiretroviral treatment, is associated with the restoration of the epithelial barrier structure.

Early introduction of the ART may protect small intestine mucosal barrier and have positive impact on the activation of the immune system.

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