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Article

Asymptomatic HIV-Infected Patients Present Blood Parameters Changed, According to Use of Therapy and the CD4+ T Cells Count

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In HIV-patients, the imbalance in immunological, hematological and biochemical factors can contribute to the progression to AIDS and non-AIDS comorbidities, even during combined antiretroviral therapy (cART). In this cross-sectional study, we aimed to analyze some of these parameters in 138 different asymptomatic HIV-infected patients, doing multiple comparisons between the groups, which are dichotomized in the presence / absence of cART and type of immune response (immunological responders [iR,>500cells/mL] or non-responders [iNR,<500cells/mL]). Were analyzed cytokines and other routine laboratory parameters. Our results showed high creatine phosphokinase and low IL-10 levels in cART-patients. They also presented metabolic alterations, including elevations in total cholesterol and triglycerides, particularly in those iNR. In ART-iR an increased alanine aminotransferase was observed. Those NAÏVE-iNR presented high LDL-cholesterol, C-reactive protein and lactate dehydrogenase values. The long-term non-progressors (LTNP) showed the best laboratory results. In conclusion, many blood parameters were changed in HIV-patients, especially in those under cART. To identify LTNP individuals could be important to discussions their early therapeutic onset.

Keywords: HIV/AIDS. Antiretroviral therapy. Metabolic dysfunctions. Biochemical exams. Hematological exams. Metabolic alterations.

INTRODUCTION

In an attempt to delay the progression of immunodeficiency and to restore immunity, is increasingly more common (Palella *et al.*, 1998) the early initiation of combined antiretroviral therapy (cART), which is able to inhibit HIV replication and to reduce plasma viral load (VL) to undetectable levels (DHHS, 2012). The efficacy of the therapy has been demonstrated worldwide by the decline in AIDS-related morbidity and mortality (Palella *et al.*, 1998; WHO, 2009; MS, 2018). However, despite effective treatment and the discovery of new drugs, AIDS continues to be a severe public health problem (MS, 2018).

Furthermore, persistent chronic inflammation and immune activation are potential determinants of morbidity and mortality due to non-AIDS-associated illnesses (Hunt *et al.*, 2003), even during cART, which contributes partially to the decline in these inflammatory parameters (Fauci *et al.*, 1996). For example, the homeostasis disturbance as a result of the HIV infection itself or due the use of cART can lead to cytokines profile changes (Tasca, Calvi, Souza, 2012) and the development of hematological, metabolic, and biochemical disorders such as anemia (Oliveira, Oliveira, Souza, 2011), diabetes (Hadigan *et al.*, 2001), dyslipidemia, and an increased risk of cardiovascular diseases (Venkataramana, 2013). Therefore, patient survival also depends on the stabilization of these parameters. Besides that, it is important the search for more alternate and cost-effective markers to be helpful to monitoring HIV-patients in poor and developing countries.

The objective of this study was to evaluate some of these parameters in asymptomatic HIV-infected patients, comparing cART naïve-patients and individuals receiving cART, and those with adequate and partial/incomplete immunological response. Our intention was to provide a

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rational basis for studying different groups in combination with more effective and long-term anti-inflammatory interventions, in order to improve the quality and life expectancy of this population.

MATERIAL AND METHODS

Study design and patients

This prospective observational study was conducted in 2012-2015 on a HIV-cohort (age > 18 and < 60 years) at the Specialized Outpatient Service of Infectology "Domingos Alves Meira", Botucatu Medical School, UNESP, Brazil. The exclusion criteria considered pregnant women, patients with diabetes mellitus, patients co-infected with hepatitis, syphilis, tuberculosis or HTLV, and patients who have or had cancer, cardiovascular or autoimmune diseases. After application of these exclusion criteria, only 138 patients remained in the study. The minimum number of participants by group calculated previously was 30 in each one.

The subjects were divided in five groups, according their therapeutic and immunological status: two groups of patients on cART and presenting undetectable plasma VL (HIV RNA < 40 copies/mL was defined as undetectable) for five years or more: one group of immunological responders (ART-iR, n=36, i.e. ≥500 cells/mL CD4+ T count) and the other of non-responders (ART-iNR, n=30, i.e, ≤500 cells/mL); two groups of naïve-patients recently HIV-diagnosed, one group CD4+ T count ≥500 cells/mL (NAÏVE-iR, n=30) and the other group being ≤500 cells/mL (NAÏVE-iNR, n=30); finally the fifth group, the long-term nonprogressors (LTNP, n=12). To be included in this last group we considered who had HIV-diagnosis for more than five years, cART-naive and with CD4+ T cells always ≥500 cells/mL, similarly described by Pantaleo *et al.* in 1995. The study flow chart is shown in Appendix 1.

Unfortunately we got only 12 LTNP-patients, due to some difficult aspects on their recruitment as the low incidence of this population among HIV+ patients (WHO, 2009), the transfer of some patients to other services or loss of follow up, and mainly due to indication by Brazil's Health Ministry in the early initiation of cART, since December 2014 - cART started to be offered to all HIV-individuals, regardless of CD4+ T values.

The study was approved by the local Ethics Committee (CEP at FMB/UNESP nº 4104-2011).

Measurement of serum cytokines

We collected 10 mL of blood (EDTA tube) from each patient after an overnight fast. Plasmatic levels of interleukins 6, 8 and 10 (IL-6, IL-8, IL-10) were performed at the Laboratory of Tropical Disease, using the CBA (Cytometric Beads Array) technique and analyzed via flow cytometry using a FACS Calibur (Becton, Dickinson and Company), Cell Quest Software and FCAP Array Software (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Results below the limits of detection were classified as zero based on the study of Fahley in 1998, who interpreted these low circulating cytokine levels as almost nonexistent. However, these three cytokines were chosen because, in addition to being related to the inflammatory state of individuals, they are found in a relatively larger plasma quantity compared to other cytokines which are usually available in our laboratory, using the above specified technique.

Information obtained from the medical records and by interview

Social demographic data, clinical results (CD4+ T count, VL quantification, cART regimen) and hematological, metabolic and biochemical (creatinine, C-reactive protein [CRP], creatine phosphokinase [CPK], lactate dehydrogenase [LDH], and the liver enzymes alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) parameters were available from electronic medical records.

Besides, the metabolic, biochemical and hematological results were divided into the following categories: normal value, above the normal limit, and below the normal limit. These changes in exams also were analyzed adjusting to gender and age. In addition, we measured all these parameters over a period of less than two months from sample collection to cytokine analysis.

Statistical analysis

To data distribution evaluation we performed the Shapiro-Wilk test.

The continuous data, expressed as the mean (ME) and standard deviation (SD), were compared among groups using One-Way ANOVA followed by the Tukey-Kramer post-hoc test if the distribution was symmetric, or Gamma Distribution if the variables showed slight asymmetry. For accounting variables, were applied Negative Binomial Regression and Poisson Regression depending on the parametric and non-parametric distribution.

For these continuous variables, in order to extract possible confounding factors / interference in the results, the statistical rates were adjusted for age and gender. For these, each specific variables were added to the model as a covariate. Such procedure was performed in the statistical program and is usual in analyzing data from heterogeneous populations.

Categorical variables are reported as frequency and percentage (%). Associations among them were evaluated using contingency tables and the chi-square or Fisher's exact test. We calculated the z-ratio for verify the significance of the difference between five independent proportions. Besides that, Pearson's correlation test was calculated for quantitative data and Spearman's correlation test for continuous and categorical variables.

We conducted all analyses with the research support group "EAP-FMB/UNESP" using the SAS for Windows v.9.2 and Statistic for Windows v.10.0 programs. The significance level was set at 5% or the corresponding *p*-value.

RESULTS

The average age was 37.2 (±9.1) and most participants were men (57.3%). The groups were homogenous to age, gender, alcohol addiction, skin color and other HIV-infection related characteristics, such as, usage of non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI) regimens, as well their duration. Only the "condom usage" variable was different among the groups ($p<0.001$), the cART-patients related a lower frequency of condom use in all sexual relations than NAÏVE-patients. The general socio-behavioral, clinical and epidemiological characteristics of the five groups are showed in Appendix 2. The time of HIV-diagnoses (7.4±6.6) was different only between NAÏVE-iR/iNR and the other three groups ($p=0.021$).

As it was already expected, the means of CD4+ T cells were different among the iNR versus iR. The CD4+ T nadir was also different, with highest values showed in LTNP and NAÏVE-iR. The lowest CD8+ T count was present in the cART groups and we found the higher VL value in the NAÏVE-iNR group.

For the hematological exam, we observed some differences among the groups especially to lymphocytes (LTNP > iNR groups) and eosinophils (LTNP showed the lowest value). The leucocytes of NAÏVE-iNR were lower than the means of NAÏVE-iR. These immunovirological and hematological parameters are shown in the Table I. No differences among the groups to red blood cells, platelets, neutrophils, monocytes, and basophils means.

In relation to metabolic exams, the comparison among all groups revealed some differences, especially in the ART-iNR group, which presented the highest means of total cholesterol, elevated levels of LDL, HDL and triglycerides. In the ART-iR, we observed an increase of triglycerides and HDL, compared to NAÏVE groups. In addition, the LTNP group presented the lowest means of LDL (Figure 1).

TABLE I - Immunovirological, hematological and biochemical exams of HIV-infected groups

Variables	NAIVE-iNR(1)	NAIVE-iR(2)	LTNP(3)	ART-iR(4)	ART-iNR(5)	*p value* *(five groups)*
CD4+ T (cells/mL)	335.5 ± 145.2*2-4	691.3 ± 120.9	862.1 ± 360.1	841.1 ± 243.9	345.5 ± 136.9*2-4	<0.001
CD8+ T (cells/mL)	1018.2 ± 852.4	1444.9 ± 751.0*1,4,5	1261.3 ± 672.6	932.5 ± 289.6	886.8 ± 553.3	<0.010
CD4+ T nadir (cells/mL)	316.5 ± 143.7*2	568.7 ± 122.9	526.5 ± 244.5	294.4 ± 162.2*1,2	98.1 ± 92.4*all	<0.010
Viral load (copies [x10^3] /mL)	413 ± 1188*2,3	41 ± 65	36 ± 79	undetectable	undetectable	<0.001
Platelets (cells/mL)	232.0 ± 98.7	266.9 ± 70.5	219.7 ± 61.3	272.4 ± 72.9	241.4 ± 53.4	ns
Red blood cells (cells/mL)	5.1 ± 0.5	5.1 ± 0.3	5.1 ± 0.4	4.1 ± 0.6	4.7 ± 0.5	ns
Leucocytes (cells/mL)	5.2 ± 1.4*2	7.1 ± 2.0	6.3 ± 1.1	6.9 ± 2.3	5.8 ± 1.9	<0.050
Neutrophils (%)	54.2 ± 14.8	50.9 ± 11.5	52.0 ± 11.1	53.2 ± 11.2	56.1 ± 9.7	ns
Lymphocytes (%)	34.4 ± 13.1	36.8 ± 10.8	39.2 ± 8.6*1,5	35.5 ± 8.7	33.1 ± 8.1	<0.050
Monocytes (%)	7.4 ± 2.0	6.6 ± 1.7	7.1 ± 2.4	6.7 ± 1.7	7.1 ± 1.9	ns
Eosinophils (%)	4.4 ± 5.2	4.6 ± 6.8	0.9 ± 0.3*all	3.0 ± 1.8*1,2	2.9 ± 2.9*1,2	<0.010
Basophils (%)	0.8 ± 0.3	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.4	0.8 ± 0.4	ns
CPK (U/L)	111.7 ± 100.8	101.7 ± 52.7	60.7 ± 24.4	183.7 ± 231.9*1,2,3	153.7 ± 210.4*3	<0.050
LDH (U/L)	503.5 ±83.7*5	483.4 ± 122.9	443.2 ± 54.1	448.3 ± 86.2	436.7 ± 89,1	<0.050
CRP (mg/dL)	0.9 ± 0.4*all	0.7 ± 0.6	0.5 ± 0.0*5	0.7 ± 0.3	0.7 ± 0.4	<0.050
Total Proteins (g/dL)	8.5 ± 0.6	8.6 ± 0.7	8.1 ± 0.9	7.7 ± 0.8*1,2	8.0 ± 0.5	<0.010
Creatinine (mg/dL)	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	ns
AST (U/L)	35.8 ± 20.6	33.0 ± 12.8	27.5 ± 8.9	33.6 ± 11.7	28.8 ± 11.0	ns
ALT (U/L)	44.6 ± 36.9	40.5 ± 31.0	38.5 ± 12.3	43.0 ± 21.5*5	33.6 ± 12.8	<0.050

* significant difference between the specified groups. Binomial Negative was used for the CD4+ and CD8+ T cells, Nadir, Viral Load and Patelets; Gamma test was used for CPK, CRP, ALT and AST; Anova and Tukey-Kramer were used for LDH, Total Proteins and Creatinine. Poisson test was used to the others. Ns: no significance. ART: antiretroviral therapy; Naïve: ART-naïve; iR: immunological responder; iNR: immunological non-responder; LTNP: long-term nonprogressors. Ns: no significance. CPK: creatine phosphokinase; LDH: lactate dehydrogenase; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

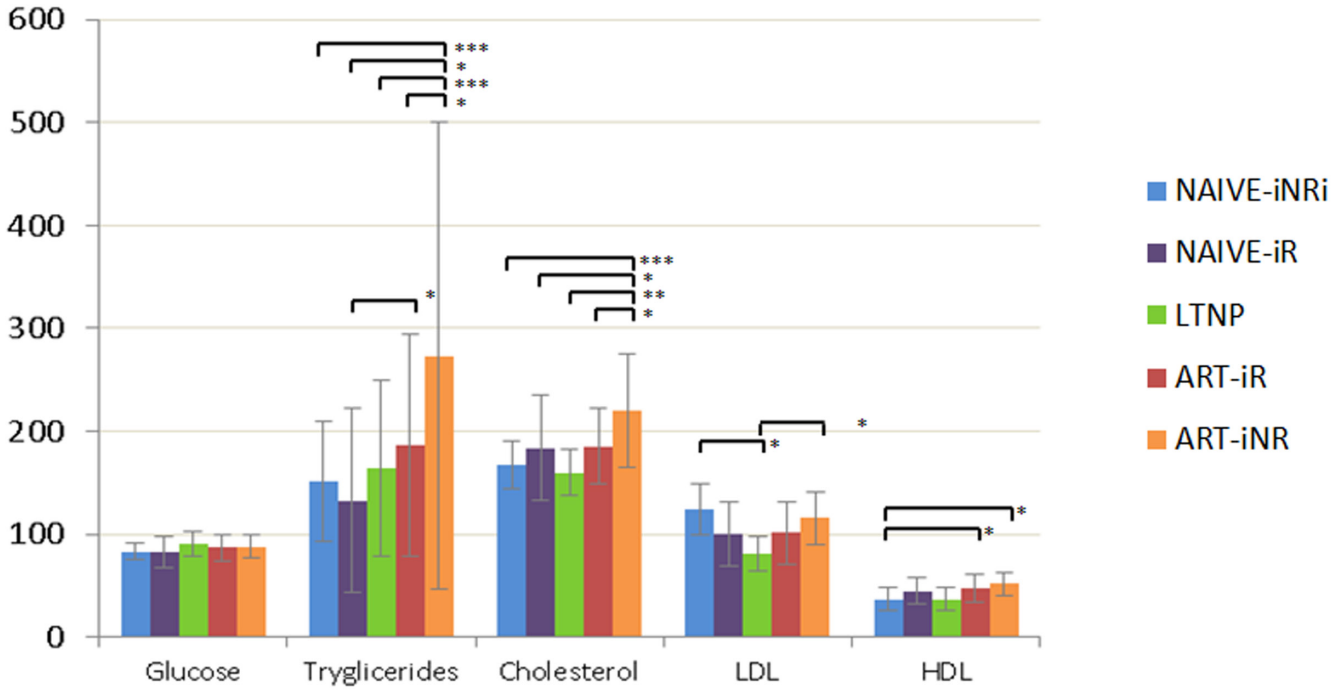

FIGURE 1 - Metabolic variables means in the five HIV-infected groups. Significant difference between the specified groups: * ≤0,05, ** ≤0,005, *** ≤0,0001. Gamma test was used for tryglicerides and Anova and Tukey-Kramer were used for the others. ART: antiretroviral therapy; Naïve: ART-naïve; iR: immunological responder; iNR: immunological non-responder; LTNP: long-term nonprogressors.

Some of the biochemical exams showed differences among the groups, such as total proteins (NAÏVE groups > ART-iR), LDH (NAÏVE-iNR > ART-iNR), CPK (cART groups presented the highest value) and CRP (increased in the iNR groups), highlighting the last two variables that presented the lowest values in LTNP, as demonstrated in the Table I.

So far, the averages presented above showed some differences that we have to analyze routinely in the patients and under clinical aspect. Besides that, we think that more important than some average values are the "abnormal results" presented by the patients' exams. Therefore, the results are matched with the reference values - those accepted like "normal / optimal results" - and they are categorized. Our analysis showed higher frequency of changed values (above or below of this limit was considered as normal range).

When we compared these changes among the groups, three variables were different: eosinophil, total cholesterol and HDL (Figure 2). The frequency of altered eosinophil occurred in naïve-individuals (more LTNP individuals presented eosinopenia and, the other naïve-patients showed more cases of eosinophilia). All LTNP presented normal values of total cholesterol, unlike the cART-patients, whose showed high frequency of abnormal values. Finally, HDL was decreased mainly in NAÏVE-iNR group. These results are shown in Figure 3.

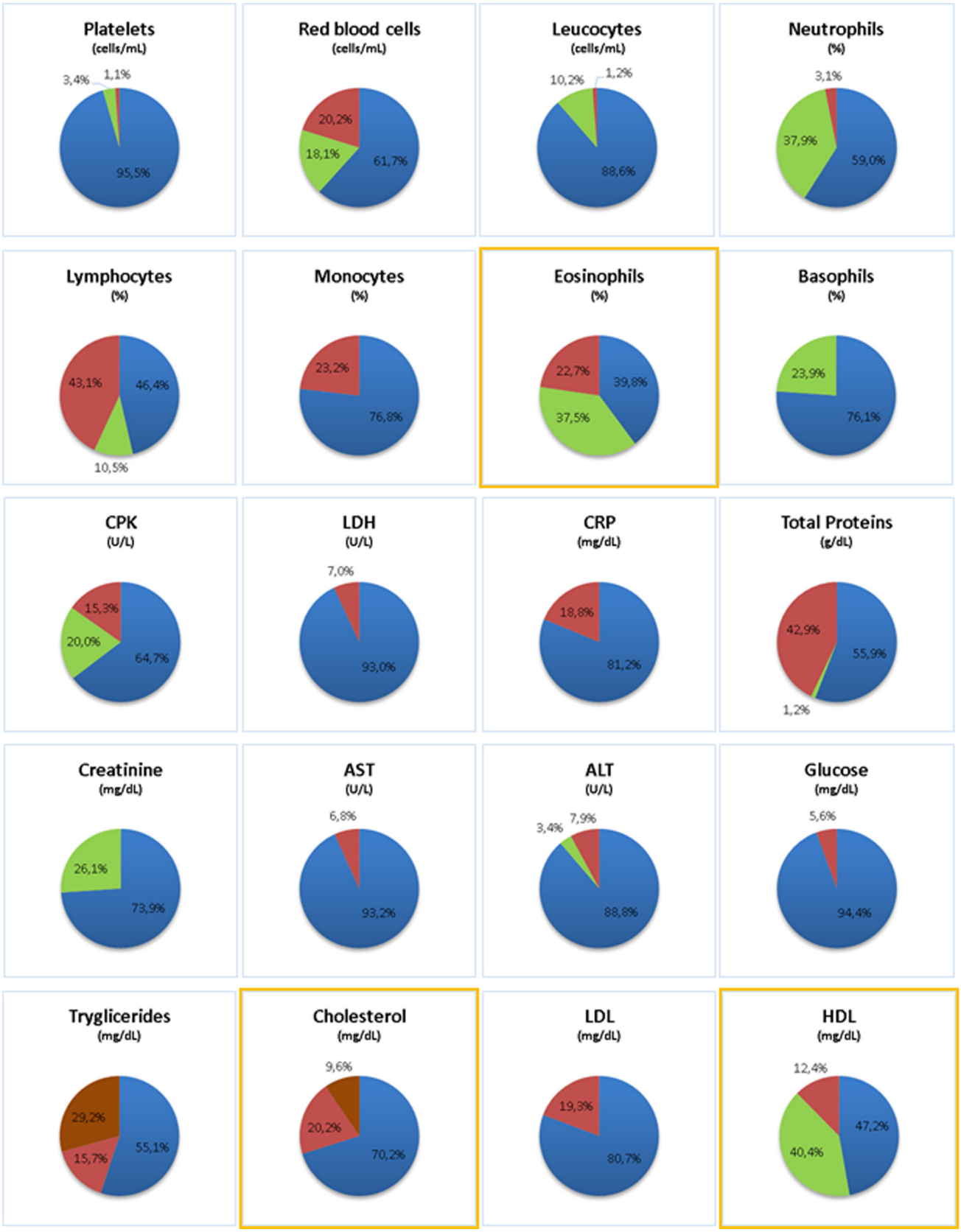

FIGURE 2 - Abnormal exams in all HIV-infected groups. Significant difference among groups ($p \leq 0,05$) to those graphs with a yellow board. Qui-square was used for all variables studied. Normal results in blue; Low values in green; High values in red; Very high values in brown.

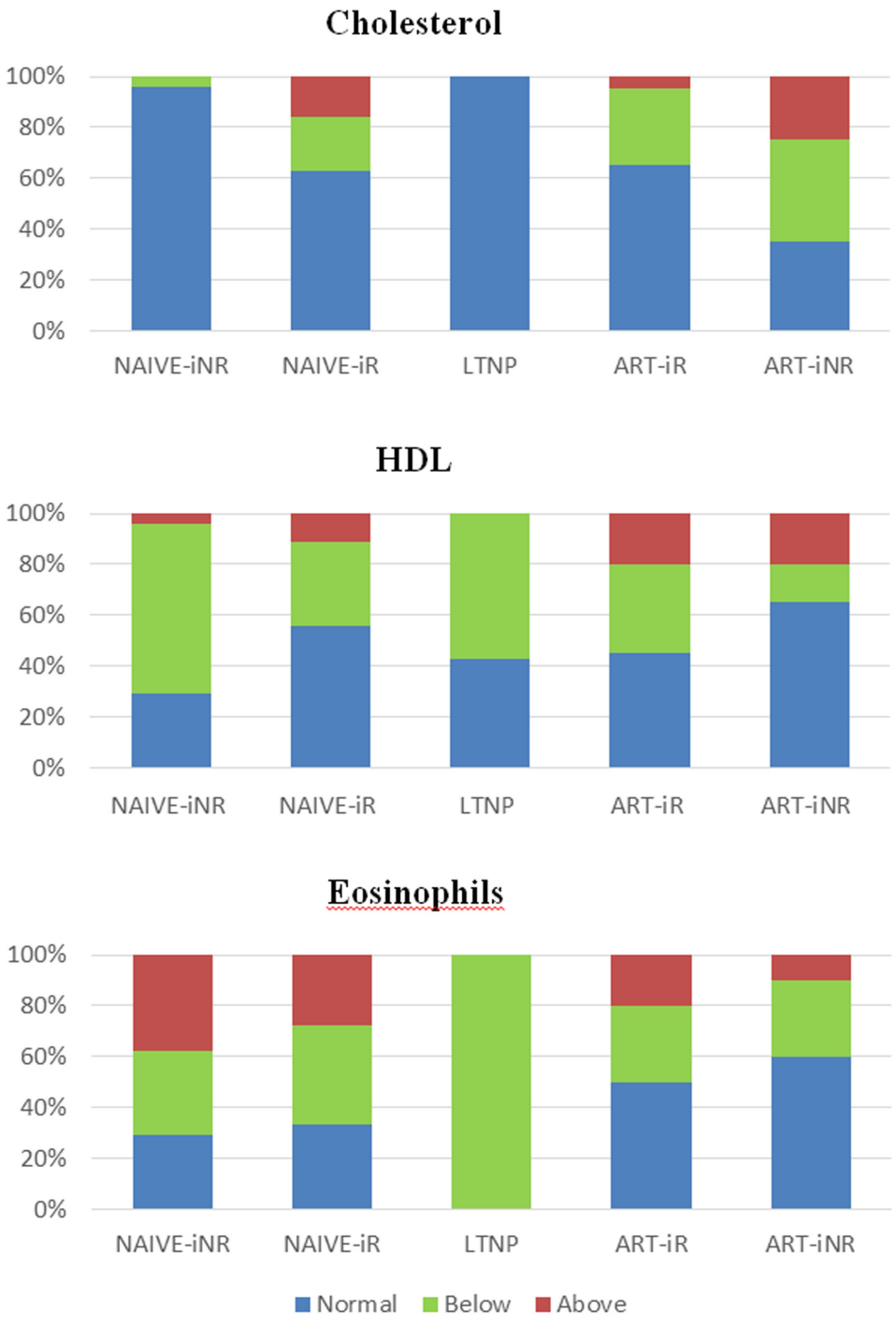

FIGURE 3 - Difference in the Proportions of Abnormal Cholesterol, HDL and Eosinophils in all HIV-infected groups. Difference in the Proportions ($p<0,05$). Normal results in blue; Low values in green; High values in red; Very high values in brown. ART: antiretroviral therapy; Naïve: ART-naïve; iR: immunological responder; iNR: immunological non-responder; LTNP: long-term nonprogressors.

For cytokines, the only difference found was for IL-10, with decreased levels presented in cART patients, a median value in LTNP following the increased levels in the other naïve-groups (Figure 4).

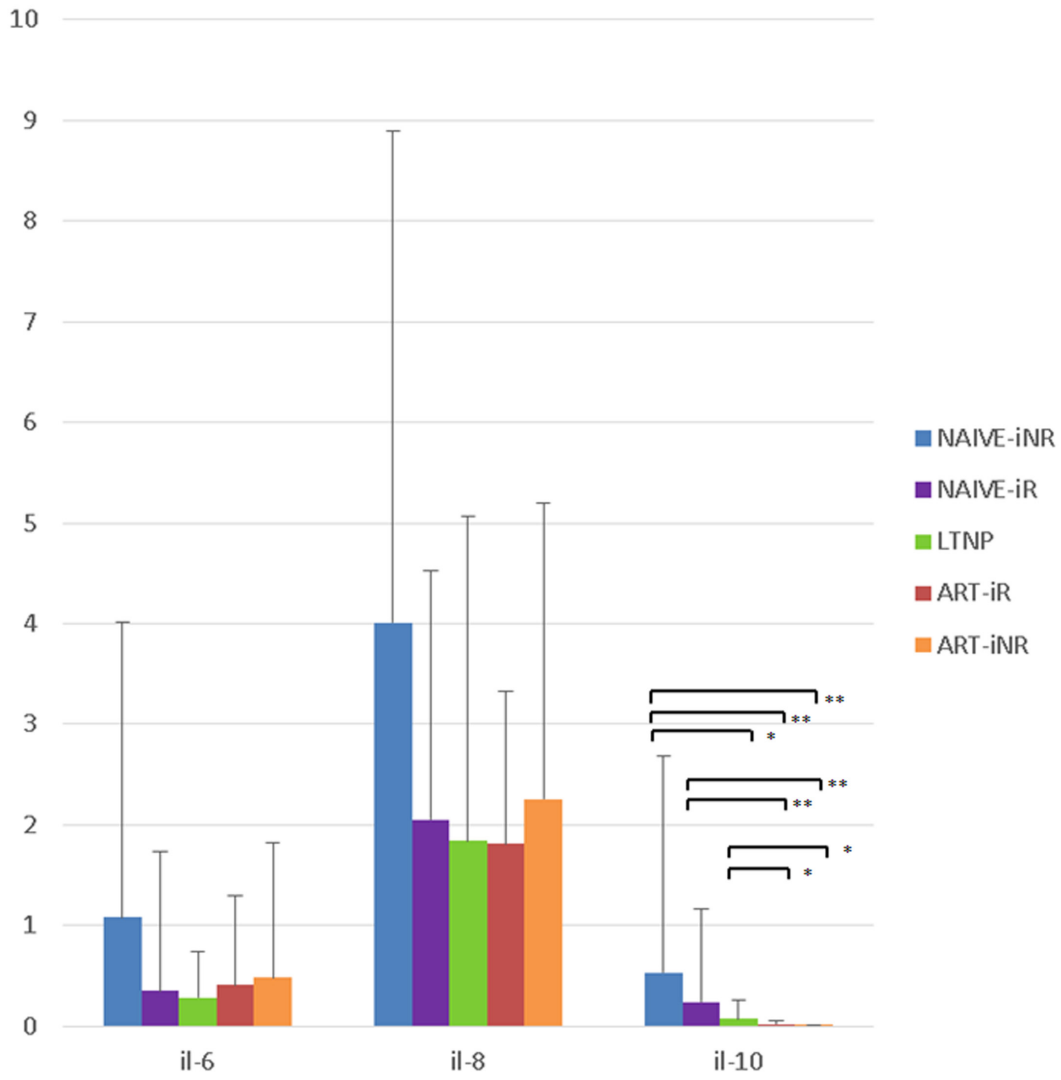

FIGURE 4 - Cytokines values in the five HIV-infected groups. Significant difference between the specified groups: * ≤0,05, ** ≤0,01. Gamma test was used for all cytokines. ART: antiretroviral therapy; Naïve: ART-naïve; iR: immunological responder; iNR: immunological non-responder; LTNP: long-term nonprogressors.

DISCUSSION

CD4+ T / CD8+ T lymphocytes count and complete blood count

It is known that the increased risk of HIV progression to AIDS and death is related to high viremia and low CD4+ T-cell counts, which represent the best markers for clinical follow-up of patient's status (Palella *et al.*, 1998; Langford, Ananworanich, Cooper, 2007). Here, LTNP showed an effective and upregulated CD4+ and CD8+ T-cell response, that could be able to target some envelope's epitopes in HIV, limiting the viral replication and showing improving the cell proliferation (Pantaleo

et al., 1995; Zaunders, Bockel, 2013). In addition, they presented similar time of infection (data not showed) and CD4+ T counts as cART-patients with a good immunovirologycal response.

A higher similarity was found between NAÏVE-iR and LTNP in relation not only to CD4+ but also to VL, CD8+ counts, lymphocytes, total leukocytes and other variables. This suggests even that some NAÏVE-iR patients could be possible LTNP people, however this assumption could only be proved through identification of genetic markers or by following these people without cART to monitor the evolution of the infection - which is no longer possible due to current recommendations for early treatment initiation. According to Streeck and Nixon (2010), an early suppression of HIV VL by the host might be critical for the generation of functional CD8+ T cell responses, an important mechanism to preserve CD4+ T cell function; both of these groups (LTNP and NAIVE-iR) seem to have this potential.

Regarding CD8+ T cells, we found difference only in the NAÏVE-iR group, which presented the highest values. Although there is evidence of CD8+ increased numbers as a consequence of high HIV-replication or even as a compensatory mechanism of CD4+ T lymphocytopenia (Streeck, Nixon, 2010), our patients from NAÏVE-iR and LTNP groups presented a low VL and high CD4+ T counts, suggesting that they could present a high number of antigen-specific CD8+T cells. Moreover, their CD4+ T cells could influence in the magnitude and quality of the CD8+ T-cell response (Krawczyk, Shen, Pearce, 2007; Streeck, Nixon, 2010; Zaunders, Bockel, 2013) improving the effector functions, including the production of cytokines or degranulation and cytolytic activity to suppress viral replication (Migueles *et al*., 2002).

Even the general average not representing leukopenia, total leucocytes were significantly lower in NAÏVE-iNR than NAÏVE-iR, maybe because of our criteria of groups division in relation to CD4+ T counts. However, this difference did not appear between ART-iR and ART-iNR. We observed a decline beyond 10% of the lower limit in total leukocytes in 10,2% of our patients, but not directly related to the group to which they belonged. In this sense, other authors (Parinitha, Kulkarni, 2012) showed a correlation between leucopenia and low values of CD4+ T cells. In a prospective study with HIV-infected men, Lau *et al.* (2003) observed this decline in more than 77% of the cases that progressed to AIDS and in only 23% of those that did not. For this reason, evaluation of this parameter can also help in understanding the patient's clinical condition, especially in health services that do not have CD4 + T count analysis.

Significantly higher lymphocyte counts were observed in LTNP when compared to all iNR groups demonstrating the stable clinical status of them. Only 10,5% of our patients were lymphopenic but no difference was found among groups. On the other hand, 37,9% of patients were neutropenic. Alike the literature report that about 35% of asymptomatic HIV-patients or patients with AIDS present at least one episode of neutropenia due to the infiltration of HIV or other pathogens into the bone marrow, myelotoxicity due antiretroviral drugs use, apoptosis of progenitor and mature hematopoietic cells, and nutritional deficiency, among others (Moses *et al*., 1996).

In this study, the LTNP presented the lowest eosinophil counts. Beyond that, cART-individuals had lower eosinophil averages than other naïve-groups. Analyzing the frequency of individuals with "abnormal values" of these cells, almost 40% of patients showed low values and 23% had values above the normal range, with a statistical difference among groups. These findings are similar to the study of Parinitha and Kulkarni (2012), who demonstrated eosinophilia in 21.6% of their patients. In contrast, Price *et al.* (2006) found no alterations in the number of these cells in patients with HIV/AIDS.

In our study, the HIV-groups did not present difference to platelets, red blood cells, hematocrit and hemoglobin (data not showed), and the most patients had normal ranges to these variables. Nonetheless, anemia is a frequent complication that occurs in 20 to 80% of HIV-infected subjects and this condition has been associated with progression to AIDS and lower survival (Parinitha, Kulkarni, 2012; Venkataramana, 2013) thereby, it is important to evaluate all these markers in HIV-patients.

Triglycerides, Lipid profile and Glucose

The metabolic parameters demonstrated many significant differences among our groups, highlighting

the increased levels of cholesterol and triglycerides in ART-iNR, comparing to the other groups. To triglycerides, ART-iR showed elevated values compared to NAÏVE-iR and a very high frequency of HIV-patients (45% of all) presented increased triglycerides levels, with no differences among the groups. We noticed that almost 30% of them had an altered value of cholesterol and the groups showed statistical difference among them. The highest frequency of these changes was presented by cART-patients, compared to naïve-patients; no subject of LTNP group showed changes in total cholesterol levels, results corroborated with others (Venkataramana, 2013; Souza *et al*., 2013; Neto *et al*., 2013). However, naïve patients usually have some lipid disorders associated with the HIV infection itself, such a low total cholesterol, LDL and HDL levels, and increased triglycerides (Souza *et al*., 2013).

Dyslipidemia is known to be more frequent among HIV-infected patients than in the general population and, after the beginning of cART, they start to present different profiles of changes (Souza *et al*., 2013; Neto *et al*., 2013). In general, approximately 40 to 80% of patients develop hypercholesterolemia and 40 to 65% develop hypertriglyceridemia (Neto *et al*., 2013) and some of the major nutritional problems are overweight, obesity and lipodystrophy conditions (Geraix, Carvalhaes, Pereira, 2008), which contribute significantly to the development of cardiovascular diseases (Venkataramana, 2013). As expected, in our study these changes also occurred on a large scale.

Regarding LDL, in the present study the iNR-groups showed a significant increase of this variable in relation to LTNP group, but the frequency of abnormal values did not differ among them. The HDL was greater in both cART-groups than the NAÏVE-iNR. This fact could have occurred due to the negative correlation found between HDL and VL (-0.262, p=0.013, data not showed). According some authors (Fontas *et al*., 2004), there is an association between the seroconversion itself with a reduction in lipid levels as a result of weight loss and protein depletion, and a subsequent increase of HDL after cART initiation. For altered HDL levels, we reported statistical difference among the groups.

In a review by Souza *et al.* (2013), the alterations in metabolic parameters seem to be characterized differently according the cART-schemes. More than 50% of our cART-patients received regimens containing NNRTI, a fact that may explain the cases of hypercholesterolemia and hypertriglyceridemia (Neto *et al*., 2013). About 25% of these NNRTI-patients were in use of Nevirapine (not showed), a drug associated with an elevation in HDL concentration (Neto *et al*., 2013), which is consistent with our findings. However, in our study no correlation was found among the parameters analyzed and the patient's cART-scheme. Some protease inhibitors, particularly Indinavir and Lopinavir, were commonly associated with hypercholesterolemia, hypertriglyceridemia, increased LDL and a reduced HDL range (Souza *et al*., 2013; Neto *et al*., 2013), which could also explain the decreased HDL value in 40% of our patients.

At the moment of patient inclusion, the diabetic ones were excluded; even so, a small portion of patients (5,6%) presented an elevation in glucose values, without difference among groups. Despite that, the diabetes is a frequent comorbidity in HIV-infected patients (Hadigan *et al*., 2001; Neto *et al*., 2013) and deserves to be screened in all HIV-services.

Liver and renal functions and other biochemical exams

Although many liver diseases initially cause only mild symptoms, their early detection is important (Mgogwe *et al*., 2012) and liver transaminases are useful markers to be monitored. Studies have shown that minimal to moderate increases in liver enzymes (specially AST) are associated with a higher risk of mortality in patients with AIDS (Viana *et al*., 2011). The present study showed a normal range of both variables in most of the patients, with a difference only in ALT averages, which was higher in ART-iR than in ART-iNR group. This result somehow indicates that adherence to cART could be a potential responsible to the higher liver enzyme levels, which deserves to be periodically analyzed in HIV-patients, once that, in the study by Viana *et al.* (2011), 55% of HIV-infected patients with AST levels above the normal range died. Furthermore, we did not observe any correlation between the cART-scheme and these liver alterations (not showed), but Mgogwe *et al.* (2012) even found greater increases in these enzymes

as a result of toxicity related to the use of cART drugs, such as Nevirapine. The mechanism of cART-induced liver injury is driven by diverse mechanisms, such as immune reconstitution, mitochondrial toxicity, metabolic host-mediated injury and hypersensitivity reactions (Pillaye *et al.*, 2020).

In regard to kidney function, we analyzed only serum creatinine levels, which were within the normal range in 74% of our patients, with no difference among the groups. This finding agrees with those found by others researchers (Neuhaus *et al.*, 2010; Mgogwe *et al.*, 2012; Venkataramana, 2013) who also did not find changes in creatinine levels during the use of cART. Indeed, the prevalence of HIV-related nephropathy has remained stable since the 1990s, after the success of modern cART (Atta, 2010). Since these renal dysfunctions are present in some HIV-subjects, regardless of the cART use, periodic assessment of renal function in these patients is, therefore, essential for an early diagnosis of kidney disease.

In the present study, in addition to lipid profile, we investigated some parameters reported as predictors of non-AIDS illnesses, including increased cardiovascular risk. There were differences in the LDH (NAÏVE-iNR > ART-iNR), CRP (increased in iNR groups) and CPK levels (elevated specially in ART-patients). To these last three variables, we observed the following frequency of altered results, respectively: 7%, 19% and 35%, no difference among the groups. Other studies (Venkataramana, 2013; Tenorio *et al.*, 2014) previously showed an increase of these markers in HIV-patients, and a negative correlation between CRP values and CD4+ T cells (Venkataramana, 2013). Despite that, we believe in the need of attention to these markers in the clinic, especially in patients on cART at greater risk of non-AIDS events. Our data also suggest that the caution in the cART early indication is needed for the LTNP and NAÏVE-iR, since they did not show high levels of these inflammatory markers.

Cytokine profile

In the present study, extremely low plasma cytokines levels were observed in all participants. Similar findings have been reported by Fahey (1998), although serum cytokine levels are frequently related to their *in vivo* activity, there are many explanations for the difficulty in detecting these molecules, e.g., circulating cytokines reflect the imbalance between their production and absorption by cells that express the respective receptors and/or migration to tissues and soluble receptors that are present for many cytokines, which may block their detection.

We showed negative correlation between IL-6 and CD4+ T cells and *nadir* and a positive correlation between IL-6 and VL and CRP (data not showed); the first correlation could explain the IL-6 average found. Levels of IL-6 were not significantly different among our groups. Similarly, the introduction of cART had not been affected circulating IL-6 levels in the study conducted by Brazille *et al.* (2003), despite that other authors have observed a noticeable decline in circulating levels of this and other inflammatory cytokines after cART initiation (Stacey *et al.*, 2009). To IL-8, we found no difference among the groups, but this inflammatory cytokine showed negative correlation with *nadir* and CD4+ T counts, and positive correlation with IL-6 and IL-10 (not showed). Wada *et al.* (2015) also did not find any difference in the IL-8 levels between naïve and cART patients. Nevertheless, since these cytokines are also associated with a poor prognosis (Tenorio *et al.*, 2014), further studies are needed to minimize their levels in treated and untreated HIV/AIDS patients.

Very low levels of IL-10 were found in the present study, mainly in cART-patients. Other studies have described the same observation between suppressive-cART patients and those naïve (Wada *et al.*, 2015). In this line of reasoning, some studies (Stylianou *et al.*, 1999; Aukrust *et al.*, 1999) have shown that the cART initiation induces a gradual decline in IL-10 levels. However, the role of IL-10 in HIV infection continues to be not fully understood. We, and others (Haissman *et al.*, 2009), found negative correlation between IL-10 and CD4+ T cells, differently of the others cytokines that we have analyzed (not showed). Similarly, Streeck and Nixon (2010) observed higher levels of IL-10 in patients who are at a more advanced phase of the disease and/or debilitated immunovirological parameters.

Finally, despite the limitations of the present study including its small sample size and cross-sectional design,

we carefully have excluded several individuals who could provide confounding variables of the chosen markers. So, our findings provide a rational basis for studying the effect of different CD4+ T counts and cART regimens in combination with more effective and long-term anti-inflammatory interventions, which could modify not only the prognosis of the AIDS progression, but also the risk of the early development non-AIDS comorbidities.

Briefly, this study revealed the presence of various metabolic, hematological, biochemical and immunological disturbances in asymptomatic HIV-infected patients. Our original results are related to the multiple comparisons among the groups, which are dichotomized in the presence / absence of cART and type of immune response presented by the patients.

We observed that in almost half of the patients, altered levels of triglycerides and cholesterol, especially in those under cART, which is worrying, considering the potential risk of developing non-AIDS comorbidities. In addition, the treated and immunological responder's individuals (ART-iR) presented more increased levels of CPK and ALT, which suggests the influence of the medication on metabolic parameters since the treated groups were homogenous in terms of time since HIV diagnosis, age, gender and drug classes used.

In relation to naïve-groups, we demonstrated more changes in nonresponders (NAÏVE-iNR) individuals. They presented increased levels of inflammatory cytokines, LDH, CRP and LDL, and the high viral load found in this group may have some role in changing these variables, fact that deserves our attention because they may progress faster to AIDS. Not surprisingly, the LTNP group showed better immunological, biochemical and metabolic status, especially due to high lymphocytes count and low values of cholesterol, LDL, CPK, LDH, CRP.

Lastly, the present results highlight the importance of regular patient monitoring regarding the progression of HIV infection, adherence to therapy, balanced diets, regular physical activity and maintenance of expected biochemical and metabolic values, in addition to controlling inflammatory parameters. Larger prospective studies are needed for the long-term assessment of these parameters in treated and untreated HIV-patients, as well as interventional studies and new therapies studies. Besides that, for future therapies using cytokines or other immunomodulants properties, we have to considerate individualized treatment for HIV-patients, analyzing each predominant cytokine profile together with other clinical parameters, including socio-behavioral and, perhaps, genetic factors.

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