



STUDY OF SOME BIOMARKERS THAT CAN PREDICT IMMUNOSENESCENCE IN CHILDREN BORN HIV POSITIVE AND WITH AN UNDETECTABLE VIRAL LOAD AT THE YAOUNDÉ UNIVERSITY TEACHING HOSPITAL, CAMEROON

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Abstract: In Cameroon, due to the implementation of the Test and Treat policy in 2016, antiretroviral therapy (ART) was generalized to all people affected by HIV. In people living with HIV (PLHIV) born HIV positive, although this treatment controls the plasma viral load of HIV, they are even more at risk of comorbidities compared to the general population. In addition, high levels of immune activation and inflammation persist despite control of HIV viremia, and associated to morbidities not ranking AIDS and the reactivation of other chronic viral infections, define a decline of the immune system called immunosenescence. Immunosenescence is appeared early in PLHIV born HIV positive but their follow-up is no different from those who acquired the infection late in life, leading to their early death unrelated to HIV infection. The general objective of this work was to identify biomarkers that could be included in the monitoring of PLHIV born HIV positive in order to predict the occurrence of immunosenescence. Between 2020 and 2022, we conducted a monocentric prospective study at Yaoundé on PLHIV born HIV positive from whom the socio-demographic profile was established. Biological parameters were measured in blood from two sampling periods (at recruitment and 12 months later), for the purpose of measure immune activation, inflammatory profile and markers of organ aging in order to bring out the combination of markers found relevant for the study of the immunosenescence of participants. It appears that, of the 20 biomarkers LTCD4⁺, LTCD8⁺, CD4/CD8 ratio, Platelets, Leukocytes, Monocytes, Neutrophils, Total leukocytes, Hemoglobin, TNF- α , IFN- γ , IL-2, IL-6, Creatinine, urea, AST, ALT, ALT/AST ratio, comorbidities, coinfections) described in the literature as being able to monitor immunosenescence, only 5 (LTCD4⁺, total leukocytes, platelets, IL-2 and IFN- γ) were found relevant to describe this state in our participants, parameters that can be measured with routine examinations (NFS, ELISA).

Keywords: HIV, ART, inflammation, immune response, immunosenescence.

INTRODUCTION

The human acquired immunodeficiency virus (HIV) was discovered in 1983, and belongs to Group VI of the Baltimore Classification (Huraux, 2007). In 2021, 30 million people were infected with HIV, and more than 88 million deaths since the start of the pandemic. Africa, a mainly underdeveloped area, has its sub-Saharan part which bears the heaviest burden of this pandemic, with more than 27 million PLHIV. Women and children make up two-thirds of these people (UNAIDS, 2022). Various therapeutic measures have emerged including triple antiretroviral therapy (ART) which acts at different stages of virus replication. This combination antiretroviral treatment controls plasma HIV viral load in most patients who have access to it (Suhail, 2014). In Cameroon, due to the implementation of the Test and Treat policy in 2016, ART was generalized to all people affected by HIV, regardless of the stage of infection. This has not only reduced the

number of patients diagnosed each year leaving for example from 3.4% in 2020, to 2.9% in 2023 (CNLS, 2021), but also greatly improved the prognosis of HIV infection, with a reduction in the rate of deaths due to HIV. As a result, the age and life expectancy of HIV patients continues to increase, gradually reaching that of unaffected populations to this day. As a result, we no longer speak of patients but of people living with HIV (PLHIV) (Aureau, 2019). ART has made it possible to extend the lifespan of patients by allowing them to have an undetectable viral load (viremia control), and a satisfactory level of CD4⁺ LT, thus turning this fatal infection into a chronic disease in a few months, while exacerbating the inflammatory response produced against this infection (Guy, 2015). Chronic inflammation and underlying chronic immune activation associated to behavioral factors (drug, alcohol, and tobacco use), ART, microbial transmutation, residual HIV replication, and viral coinfections (to CMV, EBV or HCV), define a physiological state of exhaustion of the immune system

known as immunosenescence (Ozanne, 2017). With patients born HIV positive, immunosenescence occurs early. Indeed, due to earlier subjection to ART in life, these people present immunological abnormalities usually described in elderly persons, which may reflect the premature development of numerous metabolic and neurological pathologies (cardiovascular diseases, diabetes and cancers, the main complications which have become the major causes of deaths in these patients on ART), demonstrating the aging of the immune system at a young age (Carron, 2017). In addition to HIV infection and daily use of ARVs, early immunosenescence is a heavy burden for PLHIV born HIV positive, and the major cause of their death. However, their follow-up is barely different from those who contracted the infection during life, and they die from non HIV-related illnesses (Assane, 2018). In the clinic, there are diagnostic tools to predict the occurrence of such events known as scores. We can list the VACS, IRP, Framingham scores and the Z-score. The latter one is regularly applied in different medical disciplines to evaluate the occurrence of health phenomena (Guessous and Durieux-Paillard, 2010). With the evolution of medicine, modern medical biology already plays a vital role in helping with decision-making, both in terms of diagnosis and in participating in the development of a treatment plan, meanwhile previous studies have already highlighted certain biomarkers of chronic inflammation and immune activation, significantly linked to an evolution of patients born HIV positive towards an early immunosenescence (Ozanne, 2017). The study of numerous immune parameters has made it possible to identify, as predictive factors of aging of the immune system, a greater frequency of CD8+CD28- T lymphocytes, a weak proliferative response, a low CD4/CD8 ratio (<1), a number decreased B2 lymphocyte count and CMV seropositivity (Poli et al., 2016). Since our research team has been interested for years in the identification of non-traditional biological markers that can explain the risk of immunosenescence in PLHIV, the general objective of this work was to identify biomarkers that could be included in the monitoring of PLHIV born HIV positive in order to predict the occurrence of immunosenescence.

MATERIAL AND METHODS

Our study, which took place between 2020 and 2022, was a monocentric prospective study, conducted at the Yaoundé University and Teaching Hospital for the recruitment of participants and biochemical analyses, and at the Study and Control Center of Communicable Diseases of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1. The aim of this study was to characterize some biomarkers of immunosenescence in patients born HIV+. This study was limited to patients on ART in order to control the possible effect of residual viral replication on immune activation and inflammation, and with a good virological response (undetectable viral load). We included participants born HIV+ from whom data were collected on a survey form to establish the socio-demographic profile (age, sex, consumption of alcohol, tobacco, drugs), the clinical profile (the HIV-1/2 serology, date of treatment start, initial protocol, modification of treatment regimen, other infections, date of diagnosis, treatment taken) and viral load. Patients were eligible for the following analyzes if frozen plasma samples obtained at the time of study inclusion and 12 months later were available. The selection of the biomarkers measured was based on two criteria: the reliability of measuring these markers on frozen plasma, and

their predictive value. The same biological markers were measured with the same kits, at study inclusion and 12 months later. The techniques used were chosen taking into account their sensitivity to measure marker levels (the ability to detect the marker at a low threshold).

(1) In order to **measure the immune activation of the cohort of study participants**, the whole blood collected was used to count TCD3+, CD4+ and CD8+ cells by flow cytometry using a FACScount (Becton Dickinson, San Jose, CA/USA), for the serology of some infection markers by immuno-chromatography with Rapid Screening Tests: HbsAg (HBV 5 in 1 Combtest (S/P)Nantong, Diagnos Biotechnologyco., China), Anti Ac -HCV (One Step Rapid Test HCV AB Test Cassettes (S/P) (Hightop Boitech, China), Anti RV Ac, Anti HSV-1/-2 Ac, Anti CMV Ac (One Step TORCH IgM/IgG kit, Bioneavan, Beijing/China), and for Blood Count by flow cytometry using the Mindray BC-30 machine).

(2) To **establish the cytokine profile of the inflammatory state of the participants**, the titration of pro-inflammatory (IL-2 and IL-6), antiviral (IFN- γ) and cell death (TNF- α) cytokines was done by the ELISA technique and RayBiotech ELISA Kits for Human Cytokines (Georgia, USA).

(3) In order to **have the biochemical profile of markers of organ aging**, the determination of the usual values of transaminases (AST and ALT), creatinemia, hemoglobin level and uremia was made by spectrophotometry with the Cromatest Linear kit, and the present comorbidities taken from the participant's medical file.

(4) In order to **bring out the combination of markers found relevant for the study of the immunosenescence of PLHIV born HIV positive**, it was necessary to make combinations of the different markers of immunosenescence by Principal Component Analysis (PCA) and compare the combination of these markers of immune activation, inflammation and immuno-senescence with Z-score, in order to bring out the most relevant combination of markers.

The data obtained were entered into Excel 2010 then analyzed with the Statistical Package for Social Sciences (SPSS) software version 12 for Windows and by the Epi Info software version 7.2.0.1.

The study was approved by the Regional Ethics Committee for Research in Human Health, and received authorization from the various study sites. Patients signed consent regarding the use of their plasma and the collection of their medical data.

RESULTS

1. The sociodemographic and clinical profile of the study participants

The participants were grouped according to the WHO classification used by the Elizabeth Glaser Pediatric AIDS Foundation. (Elizabeth Glaser Pediatric AIDS Foundation, 2018)

The average age in the study was 9.05 ± 5.09 years, and the majority of children were under 10 years old, i.e. 56.76% (n=42) of our population. The female gender represented the vast majority of participants with 68.92% (n=51), with a sex ratio of 0.45. Within our population, 24% (n=18) used drugs and 31% (n=31) tobacco in different forms, 17% (n=23) indulged in alcohol. HIV 1 infection was the majority in our cohort, with 94.60% of participants in stage I of the disease. The first line of treatment was the most

maintained, with the TDF+3TC+EFV protocol mainly used. Although the average duration of treatment was 6.66 ± 4.16 years, the maintenance of an undetectable viral load was mainly in the duration ranging from 1 to 3 years. Comorbidities not linked to HIV infection were also found (diabetes, prostatitis, renal failure).

2. Immune activation profile in the cohort

This part of the study which took place in two phases, from November 20, 2020 to July 20, 2022, made it possible to describe the T lymphocyte profile, that of blood cells, and the infectious profile.

The T lymphocyte profile was determined using LTCD4+ values and the LT CD4/CD8 ratio.

1st sample: median LTCD4+ level = 660 cells/mm³ with a CD4/CD8 T lymphocyte ratio < 1 for 89.02% of them.

2nd sample: median LTCD4+ level = 908 cells/mm³ with a CD4/CD8 T lymphocyte ratio < 1 for 87.8%. (Lymphocyte ratio <1: senescent immune system).

The blood components profile was drawn up taking into account the leukocyte lineage in particular.

1st sample: All participants had normal blood cell values. 2nd sample: Abnormal mean leucocyte (leukocytosis: $12.591 \pm 2.02.103/\mu\text{l}$) and platelet (thrombocytopenia: $370.57 \pm 136.45.103/\mu\text{l}$) values were observed. (Leukocytosis: Leukocyte level > 10,000/ mm³, Thrombocytosis: Platelet level > 500,000/mm³: senescent immune system).

The infectious profile of the study participants was drawn up on the basis of TORCH infections (*Toxoplasma gondii*, Rubella virus, Cytomegalovirus, herpes simplex virus 1 and 2), and the presence of EBV, HVB, and HCV infections, major HIV associated infections. HIV infection alone affected only one patient. Multi-infection (at least two infections associated with HIV) was widely found in our population. The co-infection that most affected the study participants was that linked to HSV-1/-2 at 93.24% (n=69) followed by that linked to EBV at 22.97% (n=17) and that to CMV at 12.2% (n=9). These are herpes viruses with high oncogenic potential. At the second sampling only the prevalence of CMV increased to 95.95% (n=71).

3. Profile of inflammatory state in the cohort

The aim was to determine the usual values of certain pro-inflammatory (IL-2 and IL-6), antiviral (IFN- γ) and cell death (TNF- α) cytokines (Table 1).

Type of cytokine	Measured mean value	Normal value	Senescent value
First sampling			
IL-6 ng/ml	50.66 $\pm$ 40.07	0.70	>0.70
IL-2 ng/ml	34.10 $\pm$ 57.11	0.21	> 0.21
INF- γ ng/ml	29.05 $\pm$ 39.51	1.4	> 1.4
TNF- α ng/ml	41.09 $\pm$ 47.74	38.6	>38.6

Second sampling			
IL-6 ng/ml	55.21 $\pm$ 91.40	0.70	>0.70
IL-2 ng/ml	36 $\pm$ 56.81	0.21	> 0.21
INF- γ ng/ml	45.96 $\pm$ 39.79	1.4	> 1.4
TNF- α ng/ml	53.35 $\pm$ 49.63	38.6	>38.6

Table 1: Cytokine levels during the 1st and 2nd samplings

Whatever the sampling period, the cytokines measured presented abnormally high levels. And these values were correlated with the age of the participants.

4. Profile of biochemical markers of organ aging in the cohort

The aging of the organs was revealed by their functioning, by determining the usual values of transaminases and hemoglobin levels, and by measuring creatinemia and uremia in the participants (table 2).

Table 2: Levels of biochemical markers during the 1st and 2nd samplings

First sampling for n=74		
	Measured mean value	Normal value
ALT (IU/L)	23.42 $\pm$ 6.71	17 - 59
AST (IU/L)	22.59 $\pm$ 7.9	10 - 50
Uremia (mmol/l)	4.79 $\pm$ 1.3	2.14 – 11.07
Creatinemia ($\mu\text{mol/l}$)	86.72 $\pm$ 6.65	71 - 115
Hemoglobin (g/dl)	11	12
Second sampling for n=74		
	Measured mean value	Normal value
ALT (IU/L)	31.29 $\pm$ 11.47	17 - 59
AST (IU/L)	31.53 $\pm$ 11.22	10 - 50
Uremia (mmol/l)	4.52 $\pm$ 1.4	2.14 – 11.07
Creatinemia ($\mu\text{mol/l}$)	15.72 $\pm$ 4.85	71 - 115
Haemoglobin (g/dl)	10.5	12

Only serum creatinine and hemoglobin levels showed abnormal values.

5. Combination of markers found relevant for the study of the immunosenescence in PLHIV born HIV positive

This part of the study aimed to propose a combination of markers known to be immune senescent, and compare them to scores existing in the literature, in order to be able to identify relevant biomarkers for the study of the immunosenescence in PLHIV in our cohort.

To do this, we first established correlations between the different markers, then a Principal Component Analysis (PCA) was carried out in order to combine all the markers of activation, inflammation and immunosenescence. The PCA was based on the *Spearman correlation coefficient matrix*.

For each recruited participant, the following 20 markers (for both samples) were used: LTCD4⁺, LTCD8⁺, CD4/CD8 ratio, Platelets, Leukocytes, Monocytes, Neutrophils, Total leukocytes, Hemoglobin, TNF- α , IFN- γ , IL-2, IL-6, Creatinine, urea, AST, ALT, ALT/AST ratio, comorbidities, coinfections.

6. Correlations between different markers of cellular activation, inflammation and organ aging

People with multimorbidity were older, more frequently women, more often presented with stage II infection, and had been on antiretroviral treatment for a longer time.

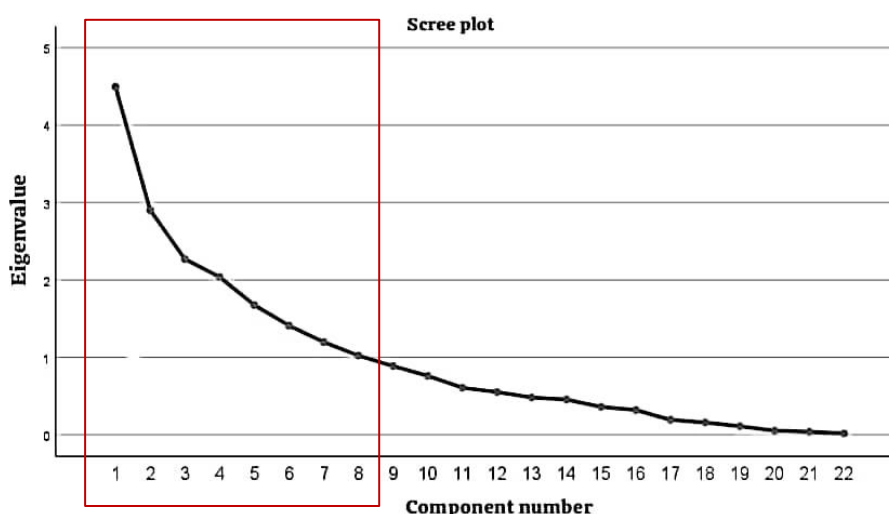
LTCD4⁺ levels were significantly correlated with positive CMV serology ($P < 0.01$).

Only variations in IL-2, INF- γ and TNF- α were correlated with biochemical parameters. Only the Urea/TNF- α association was statistically significant ($P = 0.03$).

Principal Component Analysis (PCA)

To set the number of components to be retained in the analysis, the eigenvalue scree method was used. Cattell's elbow constitutes a more severe criterion for determining the number of relevant factors, produced from the PCA's own values.

Indeed, in the resulting graph, all the points represent the eigenvalues of the components. They are connected by a line. We look for an “elbow” and we only keep the values up to this “elbow” (we only retain the factors which are located before the abrupt change in the slope). The points following this change provide little relevant information.



In the following figure which represents the evolution of the eigenvalues for the extracted components (from the 1st to the 22nd), the change in slope takes place with the 9th component, we should therefore only retain the first 8, each corresponding to one or two medical examinations.

Figure 1. Parameter scree method

It appears that the PCA allowed us to retain only the first eight main components summarizing 70% of our information, made up of 14 biomarkers: final LTCD4⁺, final LTCD8⁺, final platelets, final leukocytes, final monocytes, final neutrophils, TNF- α , IFN- γ , IL-2, IL-6, Final creatinine, final urea, final AST, final ALT.

When we look at the existing correlations between all the biomarkers constituting these 8 components, it emerges that the T lymphocyte population (component 1) is positively correlated with the cytokines TNF α , IFN- γ , IL-2 (component 4) and transaminases (component 6). The IL-6 level is negatively correlated with the AST level (component 7).

Correlation of study parameters according to Z-score

The Z score is a threshold score whose objective is to detect whether the subject is “healthy” or “pathological”, relative to a pathology.

The equation is: $Z\text{-score} = (\text{observed value} - \text{median reference value}) / \text{standard deviation of the reference population}$.

For our study,

$$Z\text{-score} = \frac{\text{Usual value of the individual's biomarker} - \text{Average value of the cohort}}{\text{Cohort standard deviation}}$$

The Z-score thus calculated allowed us to classify our participants into 2 groups:

Participants with an aged immune system (60.81%, $n=60$) with a Z-score belonging to the interval $[-2.5$ to $0.99]$ which implies chronic immune activation.

Participants with a restored immune system (14.19%, $n=14$) with a Z-score belonging to the interval $[1.01$ to $3.76]$.

When we look at the correlations existing between all 14 biomarkers, only 5 markers (LTCD4+, total leukocytes, platelets, IL-2 and IFN- γ) were statistically associated with aging or restoration of the immune system of participants.

These markers can be measured by the following examinations: lymphotyping, CBC, cytokine assay by ELISA. However, none of these five markers, taken separately, was able to tell us about the immune status of the participants.

DISCUSSION

The overall objective of this study was to identify relevant biomarkers that can be included in the follow-up of positive PLHIV, in order to predict in them the early occurrence of immunosenescence.

The Sociodemographic and Clinical Profile of the Student

Participants: Women were more represented than men, a sex ratio of 0.45, and the most affected age groups [5-10 [years for women and [0-5 [years for men. The work of Burki and Anoh have already reported a female prevalence, with 118/368 (64.7%) and 69.8% (Anoh, 2013, Burki, 2014). Half of our participants (31%, $n = 23$) took tobacco against 17 participants (22.97%) that consumed alcohol, and 24% of our participants consumed drugs. Smoking associated with some antiretrovirals is a major risk factor for metabolic complications and deaths from cardiovascular disease (MBAGA *et al.*, 2022, Vandenhende *et al.*, 2015). Alcohol is believed to both influence the incidence of infection and disease progression and is the third leading cause of mortality and morbidity in developed countries (Fontaine, 2011). There are potentially dangerous interactions between antiretrovirals with a direct action against HIV and some drugs, representing a risk of toxicity, involuntary and fatal overdose (Leclercq, 2015). Regarding the comorbidities, our patients had a type I diabetes at 4.05%, which is low compared to the results found in the study by Mbaga *et al.* (2022) which obtained a prevalence of 46.6%. However, in these patients, the prevalence of HIV-related comorbidities remains higher than that found in the general population despite an undetectable viral load for many years and a restoration of the number of LTCD4+ under ARV treatment, suggesting an accelerated aging of people infected with HIV (Sabin, 2013).

With clinical data, 70 participants were at WHO Stage I against 4 in Stage II, and the HIV-1 serotype the most found in our study with 93.24%. Indeed, HIV-1 is the most common type worldwide. As for HIV-2, it is particularly predominant in West Africa (Anoh, 2013), and Cameroon, since 2015, the implementation of Test and Treat's policy in its health system has been set up and allowed the sick to remain in early stages of the disease. The average duration of viral undetectability was 6 years, which is slightly higher than the result obtained by HAN and AL. Indeed, the rapid initiation of antiretroviral treatment very early in children just after diagnosis is recommended according to the treatment guides for HIV infection because it could promote an early decline of viral load (Han *et al.*, 2020; Thornhill *et al.*, 2016).

Immune Activation Profile: The median of the LTCD8 + was 800 cells / mm³ at first sampling and 1001 cells / mm³ at the second, both superior to the medians of the LTCD4 +, which made the ratio CD4 / CD8 less than 1 in 89.02% of patients at first sampling and 87.8% to the second, despite a ART. The persistence of a reverse CD4 / CD8 lymphocyte ratio throughout the study reflects an immune activation (Guy, 2015). Some studies on children also have shown that starting early treatment is associated with a good TCD4 / CD8 lymphocyte ratio (Han *et al.*, 2020, Seers *et al.*,

2018). Indeed, the rapid initiation of antiretroviral treatment very early in children just after diagnosis is recommended according to the treatment guides for HIV infection because it could promote standardization of the CD4 / CD8 lymphocyte ratio (Thornhill *et al.*, 2016).

A recent study has shown that in the beginning of HIV infection, there is an inflammatory environment that worsens the destruction of LTCD4 + and immune deficit *vis-à-vis* chronic viral infections frequently associated with HIV infection causing the loss of control and the reactivation of HBV or HCV, CMV or EBV. These viral infections enable, themselves, both the innate defenses and the immune responses specific to these agents, further emphasizing the chronic immune activation caused by HIV itself, creating for years a vicious circle of immunization/immune exhaustion/viral reactivation, and the increase in cancers is observed in equal age compared to the general population and relates to both viro-induced cancers (related to EBV, HBV, HCV or HPV) as well as non-induced by oncovirus (autran, 2016). In our study, it was found that participants had high concentrations of Herpes Virus antibodies.

With the blood formula counting (NFS), it is clear from our study that, in the following monthly follow-up, the number of participants with an average hemoglobin rate <12 g/dL increased from 55 (74% [64.7-86.0]) to 66 (81.08% [69.7-81.0]) 12 months later. Our work also shows that the average platelet was normal at inclusion, or 250 ± 105 cells/ μ L, then a statistically significant decline in the mean platelet count in our patients during the monitoring period. This decrease may be due to the chronicity of HIV infection in the organism (Issa *et al.*, 2017).

Profile of Inflammatory Status: There are a large number of pro and anti-inflammatory cytokines associated with aging. However, only four are usually measured (the IFN- γ being the most frequently measured cytokine, followed by the IL-6, which has already been clearly demonstrated that the rate increases in the age group of a third age (Rodriguez *et al.*, 2021, Wikby *et al.*, 2006). In this study we found that the averages of all cytokines of interest (TNF- α , IFN- γ , IL-2, IL-6) increased between the first and second sampling.

Profile of Biochemical Markers of Organ Aging in the Cohort:

The functional exploration of certain organs was performed by titration of some biochemical markers. Creatinine at an average value of 93 ± 23.19 μ mol/L in girls and 78 ± 18.24 μ mol/L in boys, ($p < 0.001$). The average uremia was 4.95 ± 1.25 mmol/L for male and 4.79 ± 1.22 mmol/L for female sex. The average ALAT was 23 ± 9.07 IU/L in men and women, from 22 ± 8.58 IU/L. The average ASAT is 22 ± 9.28 IU/L in men and women, from 21 ± 8.79 IU/L. These results were comparable to those of Coulibaly and those of Haidara, who had found an increase in the gender and the age group (Coulibaly, 2008, Haidara, 2016). In addition, 100% of our participants had normal ALAT, ASAT, Urea, creatinemia. 12 months after the average of Creatinine experienced a significant decline, it could translate from low muscle mass caused by muscle dystrophy or related to HIV infection (Deeks *et al.*, 2013).

Combination of markers found relevant for the study of the immunosenescence in PLHIV born HIV positive: With the combinations of biochemical and cytokinic biomarkers, on the 1st sampling, only the urea/TNF- α association was correlated positively, and at 2nd, only the association ASAT/TNF- α was. Our results suggest that TNF- α is a marker of the occurrence of the deterioration of the renal function and immune activation in HIV positive persons with undetectable viral load.

Correlations Between More Markers of Cellular Activation, Inflammation and Organ Aging: Studies have shown that chronic inflammation and immune activation during controlled HIV infection were found in several non-HIV-related comorbidities like. Cardiovascular diseases, diabetes, cognitive function disorder, cancer or chronic renal disease (Markowitz *et al.*, 2010).

The main component analysis has allowed us to select relevant biomarkers involved in immunosenescence and their effect on each other. It Emerges that the T Lymphocytes Population (Component 1) is positively correlated with the cytokines TNF α , IFN- γ , IL-2 (Component 4) and Transaminases (Component 6). The IL-6 level Is negatively correlated with the AST level (Component 7). They were only markers measured with the second sampling.

The LTCD4+ count is the indirect marker most often used in clinic to monitor quantitatively the evolution of the disease and the recovery of immunity in the PLHIV (Guy, 2015), with the involvement of its pro-inflammatory cytokines, in the occurrence of comorbidities characterizing the aging of the immune system (Grund *et al.*, 2016). This aging can also be determined by evaluating the liver activity, kidneys and muscles (Kone, 2018). ALAT and ASAT rates are associated with the deterioration of the renal function (proteinuria or reduced eGFR) both in PLHIV and the general population (Abraham *et al.*, 2015, Bruggeman *et al.*, 2014), the association with high values of IL-6 describes an inflammatory phenomenon during this deterioration.

By applying the Z score to the data obtained in the second sample, it appears that there are two groups of participants: the first group (n = 60) having an aging SI, with a z score belonging to the interval [-2.5 to 0.99], and the second group (n = 14) having a restored rest, with a z score belonging to the interval [1.01 to 3.76]. And when observing the markers retained by the Z score, it appears that in all HIV-related patients with an undetectable viral load and which have a low rate of IL-2 are at risk of early aging of their immune system, despite the standardization of LTCD4 + levels, leukocytes and platelets.

Our study presented many forces such as the use of the ELISA for the cytokine titration, the proposal for an immunosenescence assessment score. To our knowledge it is the first cohort study conducted in this field in Cameroon, however, some limits, such as the small size of our sample, the monocentric character of the study, the low number of chronic lymphocytic activation markers, pro-inflammatory status and the lack of evaluation of mortality in our study population.

CONCLUSION

Our study aimed to identify a set of composite markers, using easily measurable biological parameters, and capable of anticipating the early onset of immunosenescence in a person born HIV positive.

We conducted a monocentric prospective study in people born HIV positive and followed at the YUTH. Sociodemographic and clinical data were collected using a questionnaire, the information of which was completed with the medical file. Biological parameters were measured in blood from two sampling periods: at the time of recruitment, and 12 months later.

It appears from our study that, of the 20 biomarkers LTCD4+, LTCD8+, CD4/CD8 ratio, Platelets, Leukocytes, Monocytes, Neutrophils, Total leukocytes, Hemoglobin, TNF- α , IFN- γ , IL-2, IL-6, Creatinine, urea, AST, ALT, ALT/AST ratio, comorbidities, coinfections) described in the literature as being able to monitor immunosenescence, only 5 (LTCD4+, total leukocytes, platelets, IL-2 and IFN- γ) were found relevant to describe this state in our participants. Indeed, the chronicity of HIV infection is confirmed by the high levels of the antiviral cytokine IFN- γ , chronicity which induces continuous inflammation by the pro-inflammatory cytokine IL-2 and thrombocytosis, which is at the origin of the cellular activation observed: increased levels of total leukocytes, particularly LTCD4+, cells which, in turn, maintain the inflammatory phenomenon. This chronic cellular activation and the underlying sustained inflammation are the primary cause of the early immunosenescence observed in our participants.

Our study highlighted the potential of certain biomarkers to predict immunosenescence. Indeed, our results suggest that the evaluation of certain markers of immune activation (IL-2, thrombocytosis, leukocytosis) and chronic inflammation (IFN- γ), could be a useful criterion when evaluating the immunosenescence, alongside the measurement of viral load and the level of CD4+ cells.

These simple parameters to measure with routine examinations (NFS, ELISA) must be made available to medical teams to be able to complete their clinical analysis, in order to better predict the level of care that a patient will require, and therefore optimize care of PLHIV born HIV positive.

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