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# EARLY HIV INFECTION IS ASSOCIATED WITH REDUCED PROPORTIONS OF GAMMA DELTA T SUBSETS AS WELL AS HIGH CREATININE AND UREA LEVELS

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## Abstract

Renal dysfunctions are major predictors of co-morbidities and mortality in HIV infected individuals. Unconventional T cells have been shown to regulate kidney functions. However, there is dearth of information on the effect of HIV associated nephropathies on γδ and DN T cells. It is also not clear whether γδ T cell perturbations observed during the early stages of HIV infection occur before immune activation. In this study, we investigated the relationship between creatinine and urea on the number of unconventional T cells in HIV infected individuals at the early and chronic stages of infection. Persons in the chronic stage of infection were divided into treatment naïve and exposed groups. Treatment exposed individuals were further subdivided into groups with undetectable and detectable HIV-1RNA in their in their blood.

Creatinine and urea levels were significantly higher among persons in the early HIV infection compared to the other groups. Proportions of γδ T, γδ+CD8, γδ+CD16 cells were also significantly reduced in the early stage of HIV infection ($P<0.01$). Markers of immune activation, CD4+HLA-DR and CD8+HLA-DR, were also significantly reduced during early HIV infection ($P<0.01$). Taken together, our findings suggest that high levels of renal markers as well as reduced proportions of gamma delta T cells are associated with the early stages of HIV infection. This event likely occurs before systemic immune activation reaches peak levels. This study provides evidence for the need for early HIV infection diagnosis and treatment.

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[6]Author Contributions

BO, OD and GO conceptualized and designed the study. DK contributed to analysis and interpretation of data, provided antibodies and reviewed the manuscript. OD and GO provided reagents, supervised the work and reviewed the manuscript. BO preformed experiments, analyzed and interpreted the data as well as wrote the first draft of manuscript.

[5]Ethics Statement

This study was carried out in accordance with the recommendations of the University of Ibadan/ University College Hospital (UI/UCH) Biomedical Research and Ethics Committee (UI/EC/15/0076) and the Oyo State Ministry of Health Committee on Human Research (AD13/479/951). The protocol was approved by these committees. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The results were delinked from all patient identifiers.

[b.]Conflict of Interest Statement

None declared

**Keywords**

γδ T cells; DNT cells; Renal dysfunctions; Interleukin 17; HIV-1 RNA viral load; Neutrophils

## 1. Introduction

HIV associated renal dysfunctions are major predictors of end stage renal diseases, AIDS defining events, cardiovascular diseases, metabolic diseases; all of which cause mortality in HIV infected individuals[1,2]. The incidence of HIV associated chronic kidney disease have increased in sub-Sahara Africa as well as in persons with African descent in North America[3–6]. Chronic kidney disease is a major cause of death among HIV infected individuals in sub-Sahara Africa and in persons with Africa ancestry of which the risk of death is increased six times[6]. Renal disease in HIV infection has been shown to be associated with systemic inflammation, immune activation and an impaired immune system[1,7]. Our previous study and others showed that when HIV associated nephropathies is diagnosed before acute/early HIV seroconversion is identified, it presents with uremia, high serum creatinine levels and proteinuria[3,8].

Unconventional T cells, especially gamma delta (γδ) and double negative T cells (DNT), which are unique subclasses of innate immune T cells have been shown to perform regulatory functions in humans[9,10]. DNT cells make up 1–5% of T cells in blood and lymphoid tissues and express either αβ or γδ T cell receptors, although a major percentage expresses αβ T cell receptor[9]. DN T cells expressing the αβ T cell receptor are CD3+/CD4−/CD8−, and can be found in the peripheral blood, mucosa and gut associated lymphoid tissues[11]. DNT cells have been previously described to be elevated in HIV infected individuals in order to regulate immune activation and restore immune cells to normal proportions[11]. Although, there are functional overlaps between αβ and γδ T cell receptors (TCRs), the antigen recognition format and activation for each subsets of these cells are different[10,12]. Unlike αβ T cells, γδ T cells do not process and present antigens through major histocompatibility (MHC) molecules[13]. Unconventional T cells display beneficial roles during HIV infection as they exhibit cell lytic and cytokine secretion functions[14].

γδ T cells, neutrophils and cytokines such as IL-17, IL-23, IFN-γ and TNF-α have been shown to regulate kidney functions[13,15,16]. Lower proportions of γδ T cells have been associated with kidney disorders namely diabetic kidney diseases[10], renal carcinoma[17] and end stage renal diseases[7]. Studies have also shown lower numbers and frequencies of γδ T cells in tuberculosis[18] and Hepatitis B infected patients with renal impairments[19,20]. However, there is dearth of information on the impact of HIV associated nephropathies on γδ and DN T cells proportions and subsets during early HIV infection. It is also not clear whether γδ T cell perturbations observed during the early stages of HIV infection occur before immune activation. This study was therefore designed to investigate the proportions of γδ, γδ+CD8, γδ+Cd16 as well as DNT in HIV infected individuals at different stages of infection. The timing of immune activation in relation to γδ T cells defects in these individuals was also determined.

## 2. Material and Methods

### 2.1 Patients

The study participants gave written informed consent before their blood samples were collected. Seventeen individuals at the early stage of HIV-1 infection (EHIV) were enrolled, out of which 10 were recruited from persons who presented for malaria parasite diagnosis as previously described[8]. The others were recruited from prospective volunteer blood donors whose samples were reactive for early HIV-1 infection defined by HIV-1 DNA detection along with 4th generation HIV ELISA (detection of both antigen and antibody) positive, and negative with 3rd generation HIV ELISA (detection of HIV antibody only). This definition is based on the updated CDC algorithm for laboratory testing for the diagnosis of early and chronic HIV-1 infection[21,22] Twenty-three chronically infected HIV-1 cART –naïve patients (CHIV) and 41 chronically HIV-1 infected patients on cART were randomly selected from the APIN CDC-funded PEPFAR Treatment Centre at the University College Hospital, Ibadan, Nigeria. Twenty three out of the 41 chronically HIV-1 infected patients on therapy had undetectable viral load (LVL) as defined by HIV-1 RNA copies per mL less than 20, while the remaining 18 patients in this group had detectable viral load (HVL) defined by HIV-1 RNA copies per ml greater than 150 after being on treatment for at least 2 years. HIV infected individuals in Nigeria as well as in most countries in Sub-Saharan Africa are identified and recruited for treatment at the chronic stages of infection[8]. The approximate time of HIV exposure for CHIV, HVL and LVL groups are not less than 2, 6 and 7 years respectively. HIV-1 DNA was also detected in all the samples collected from persons in EHIV group while only HIV-1 RNA was detected from other groups. A control population was established by random selection among HIV negative individuals invited to participate in the study ($n = 27$).

### 2.2 Study Population

The study participants were enrolled from July 2015 to December 2017 at the HIV diagnostic unit of the Department of Virology, College of Medicine, Ibadan, Nigeria. The analysis reported here is a sub study of a prospective cohort of early HIV-1 infected adults.

### 2.3 HIV diagnosis and clinical follow up

The study participants were over 18 years of age and residents of Ibadan, Oyo state, Nigeria. Patients' were identified as early HIV infection if HIV-1 DNA from their blood samples was detected as positive along with a 4th generation HIV ELISA that detects both antigen and antibody and negative with a 3rd generation HIV ELISA that detects only HIV antibody. Those patients with positive HIV-1 DNA with both 4th and 3rd generation ELISA were classified as chronic HIV infections as previously described[8]. Information and procedures regarding HIV diagnosis and monitoring have been previously described[8].

Determination of absolute CD4 cell counts, haematological and clinical chemistry parameters were performed on freshly collected blood samples using a Sysmex Partec ®Cyflow Counter II flow cytometer (Sysmex Partec GmbH, Gorlitz), BC-5800 ®Auto Hematology Analyzer (Mindray Bio Electronics, Shenzhen) and Roche cobas® c111 Blood Chemistry Analyzer (Roche Diagnostics, Indianapolis) respectively according to

manufacturers' instructions. Each sample was analyzed to determine absolute counts of CD4 T cells, neutrophils, monocytes, lymphocytes, basophils and eosinophils. Levels of creatinine and urea as markers of renal function were also determined. Peripheral blood mononuclear cells (PBMCs) were isolated from the cellular fraction of 5ml of blood by Ficoll density gradients and stored in liquid nitrogen until analyzed.

### 2.4 Serological assays

HIV-1 infection status of each patient was determined by testing aliquots of their plasma sample with a 4$^{th}$ generation Genscreen™ ULTRA HIV-Ag-Ab ELISA kit (Biorad, Hercules, California) followed by testing with a 3$^{rd}$ generation AiD™ anti-HIV 1+2 ELISA kit (Wantai, Beijing, China). All the assays were performed under strict biosafety conditions according to the manufacturer's recommendation. Quantitation of serum Human Interleukin 17 (IL-17) and Human Tumour necrosis factor alpha (TNF-α) was determined by using a commercial ELISA kit (Elabscience, USA) according to the manufacturer's instruction.

### 2.5 Viral load testing

Plasma HIV-1 viral load (copies/ml) was determined using the COBAS® Ampliprep/ COBAS TaqMan96®HIV-1 Test, v2:0 (Roche Molecular Diagnostics, Branchburg, NJ, USA) according to manufacturer's instruction or by an in house real-time PCR protocol

### 2.6 Immunophenotyping

Plasma and cellular fractions of each blood sample were separated by centrifugation, and peripheral blood mononuclear cells (PBMCs) were isolated from the cellular portion by gradient centrifugation and stored in liquid nitrogen at 5million PBMCs/ml in new bovine serum containing 10% DMSO. The PBMCs were thawed and washed in RPMI medium (Invitrogen, Carlsbad, CA, USA) containing 10% new born bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 2mM L-glutamine and 10mM Hepes buffer on days of assay. Frozen PBMCs were thawed and left on bench for 2 h before the phenotypic analysis. The following panels of antibodies were used: anti-CD3 FITC, anti-CD4 PE and anti-CD19 PE from Sysmex Partec; anti-CD3 PerCP and anti- CD27 PE/Cy5 from Beckman Coulter; anti-γδ PE, anti-CD16 PE/Cy5, anti-CD8 APC, anti-αβ FITC, anti-HLA-DR and DRAQ 5™ viability dye from Biolegend. Previously isolated and stored PBMCs were washed twice in phosphate buffered saline (PBS) with 2% serum and then 100μl of cocktail antibodies was added to $2 \times 10^6$ cells and incubated for 15mins. Thereafter the cells were stained with diluted DRAQ 5™ and allowed to incubate for maximum of 15 minutes before acquisition. At least 100,000 live lymphocytes were acquired on a four colour flow cytometer (Partec Cube 6, Sysmex Partec, Germany).

Absolute QC Count Check Beads green (Partec, GMBH, Munster, Germany) particles with standard settings were used for quality control. Phenotypes and fluorescence intensity measurements was consistent in all experiments. Live cells stained with single dye were used for compensation while fluorescence minus one panels (FMO) were part of the gating strategy. As shown in supplementary figure 1, Unconventional T cells such as gamma delta were gated as CD3+ γδ, gdCD8 as CD3+ γδ +CD8, gdCD16 as CD3+ γδ +CD16; double negative T cells (DNT cells) as αβ-CD8/CD4. While activated CD4 and CD8 T cells were

gated as CD3+CD4+HLA-DR and CD3+CD8+HLA-DR respectively, B cells, naïve and memory were gated as CD19+CD27- and CD19+CD27+ respectively. Forward angle and side scatter light gating were used to exclude cell debris from the analysis. The final analysis was performed using the Flowjo software (version 10; FlowJo, LLC, Ashland OR, USA).

### 2.7 Statistical analysis

Within and across groups' analysis of quantitative variables were done using ANOVA test and Tukey's multiple comparison test. Chi-square tests were used for non-parametric analysis. Pearson's r correlation was used to find linear relationships between variables. P-values were considered significant at 95% confidence limit ($p<0.05$) unless otherwise stated. Data were analyzed and compared using Graph Pad Prism version 7.00 for Windows, Graph Pad Software, La Jolla California USA.

## 3 Results

### 3.1 Baseline characteristics of the study participants

Twenty seven HIV-1-negative (HCC), 17 Early HIV infected (EHIV), 23 chronically HIV infected ART naïve (CHIV) and 41 Chronic HIV infected cART exposed individuals were enrolled for this study (Table 1). Information on participants' age, gender, absolute CD4 counts, plasma HIV-1 RNA levels, CD4/CD8 ratio, neutrophil counts as well as monocyte/lymphocyte ratio are presented in Table 1. CHIV had the lowest absolute CD4 counts ($p<0.0001$) while LVL had lower absolute neutrophil counts ($P<0.01$) and monocyte/lymphocyte ratio ($P<0.01$) compared to CHIV. There were no significant differences in parameters measured between EHIV and HCC (Table 2).

### 3.2 Early HIV infection and renal dysfunctions

The levels of serum urea and creatinine were significantly higher among early HIV-1 infected individuals compared to ART naïve persons at the chronic stage of infection and healthy controls (Fig 1A and B). Also, ART naive persons at the chronic stage of infection had significantly lower levels of serum creatinine and urea compared to those on treatment. The hypothesis inferred from the results was that pro inflammatory cytokines – IL-17 as well as TNF-alpha associated with immune regulation of kidney functions[15,23] were impacted by high urea and creatinine levels associated with HIV infection. To investigate this, levels of these cytokines in a subset of the samples were determined. It was observed that the levels of IL-17 and TNF-alpha were significantly lower in early HIV infected groups compared to the other groups. However, TNF-alpha was higher in EHIV group compared to the health controls (Fig. 1C and D).As shown in Fig. 2 (B) and (C), the level of serum IL-17 significantly correlated inversely with absolute CD4 T cell counts and directly with HIV-1 RNA viral load. Similarly, the level of serum urea correlated directly with HIV-1 RNA viral load but inversely with absolute CD4 T cell counts (Fig. 2D and E).

### 3.3 Proportions of γδ T cells and subsets, DNT cells as well as neutrophils are reduced during early HIV infection.

γδ T cells especially those expressing CD16 and CD8 have been shown to exhibit cell lytic as well as cytokine secretion functions[13,24–26]. It was observed in this study that γδ T cells

and subsets were significantly reduced during EHIV compared to CHIV. DNT cells proportions were also reduced compared to CHIV although the difference was not significant. CHIV group had significantly higher levels of γδ, γδ+CD8 and γδ+CD16 proportions compared to the treatment groups. This was also similar to what was observed for DNT cells except that the differences did not reach significant levels among the groups for this cell type (Fig. 3).

Studies have previously described the activating roles of neutrophils towards γδ and DNT cells[27]. As shown in Fig. 3, absolute neutrophil counts were lower in early HIV infected individuals compared to CHIV group, although this difference was not significant. Neutrophil counts among EHIV group were significantly higher than healthy controls. CHIV group had significantly higher neutrophil counts compared to the two treatment groups: LVL and HVL. γδ T cells significantly correlated directly with neutrophil counts levels and inversely with creatinine and urea levels in cART naïve persons, although the relationship between γδ T cells and urea was not significant (see Fig. 4).

### 3.4 Immune activation is associated with chronic HIV-1 infection

HLA-DR markers on T cells have been previously described as a marker of primary immune activation[28]. CD8+HLA-DR proportions were higher among EHIV group compared to the groups on treatment. However, CD8+HLA-DR proportions were reduced in the EHIV group compared to the CHIV group although the difference was not significant. This was different to what was observed for the proportions of CD8T cells which was higher in EHIV compared to CHIV. HVL group had a significantly higher CD8 proportion compared to EHIV (Fig. 5). We hypothesized that the early HIV infection stage investigated in this study preceded the period in which immune activation has reached the peak. To verify this assumption, we analyzed the proportions of CD8, CD4+HLA-DR and CD8+HLA-DR in a subset of EHIV groups (n=5) after six months of infection.

As shown in Fig.6, we observed that levels of CD8 T cells did not significantly improve among these individuals while CD4+HLA-DR and CD8+HLA-DR proportions increased within the six months period. This difference reached significant level for CD4+HLA-DR. Correlation analysis of DNT and γδ T cells with markers of immune activation showed that cART impacts on immune-regulatory functions of these innate immune cells (see supplementary figure 2). Without cART, DNT and γδ T cells significantly correlated directly with CD4+HLA-DR proportions but reverse was the case among persons under treatment.

Recently, it was shown that γδ T cells influence B cells development as well as affect the production of auto and natural antibodies[29,30]. Although EHIV had the lowest proportion of total B cells, this difference was not significant. They, however, had significantly higher levels of naïve ($P<0.01$) and memory B cells ($P<0.0001$) compared to CHIV group. However, mean proportions of naïve and memory B cells of healthy controls were slightly higher than EHIV group, although the differences were not significant (Fig. 7 A–C). Individuals on ART (HVL and LVL) had significantly higher proportions of naïve and memory B cells compared to the other groups studied (Fig. 7A–C). Correlation analysis also revealed that γδ T cells significantly correlated directly with total and memory B cells (Fig.

7D and E). This data therefore suggest that inhibition of γδ T cells during early HIV infection due to renal dysfunctions may have impact on their downstream functions especially those related to innate immunity like antibody-dependent cell-mediated cytotoxicity[24] as well as generation of natural antibodies[29].

## 4 Discussion

Results of this study suggest that there is reduction of γδ T cells and their subsets during early HIV infection. This reduction is associated with high levels of creatinine and urea despite the fact that this group of infected people were not on ART. Subsets of these cells that are involved in cytotoxic functions seem to be particularly affected. It is possible that systemic immune activation peaked after a reasonable compartment of γδ and DNT cells have been depleted which may occur during seeding of HIV virions into renal reservoirs as previously described[31,32]. In most sub Saharan African countries, treatment initiation for HIV infected individuals' starts in the chronic stage of infection despite the recently introduced "test and treat" policy[33], reason being that most HIV infected individuals are detected at this period[8,33]. Early HIV immunotherapy may be useful in preventing the depletion of γδ and DNT T cells proportions very early in infection before systemic immune activation. This will in turn control renal dysfunctions and further aid immune reconstitution of CD4 bearing T cells after prolonged cART[11].

Several reports have shown HIV associated nephropathies during early HIV infection, especially before treatment initiation[34–36]. However, there is conflicting information as to whether the prevalence of this condition among early HIV infected individuals is high or low. Findings from this study have shown that renal dysfunction may be more prevalent among early HIV infected treatment naïve Africans than previously recognized. This may be due to an increased risk of "HIV associated nephropathy susceptible APOL1 variants" among Africans[37,38]. High replicative and transmitting capacities of non-subtype B HIV virions in infecting renal tubules and lymphocytes may also be a factor for this disorder[34,39].

A subset of γδ T cells have been shown to serve as reservoir for HIV infection[40]. Finding in this study that IL-17 levels were higher among CHIV persons compared to EHIV is similar to reports from previous studies[41,42]. IL-17 production has previously been largely attributed to γδ, DNT and neutrophils. This IL-17/neutrophil/ γδ T cell production axis has been shown to be critical to HIV-1 disease progression[14,41,42]. Data from this study showed that high HIV-1 RNA viral load as well as urea levels may impact negatively on the IL-17/ neutrophil/ γδ T cells axis thereby limiting their activation during the early stages of infection. Other infections with similar effect on IL-17/neutrophil/ γδ T cell axis have been previously described[18,41].

The evidence provided from this study showed that the proportions of neutrophils and γδ T cell subsets were significantly reduced during early HIV infection. This is also similar to previous reports that investigated these parameters in HIV infected individuals[11,12,14,41]. However, the results of this study suggest that this inhibition may be related to the low immune activation state impacted by high creatinine and urea levels, more so that these

immune cells significantly correlated positively with markers of immune activation in the absence of antiretroviral therapy.

Chronic immune activation, which is a hallmark of HIV infection[43], has been shown recently to be significantly over expressed at the first month of infection after which it undergoes a prompt decrease before another rapid increase towards seroconversion[12,44]. The initial over expression may coincide with the period of high viral replication and seeding into various cellular reservoirs[45]. Depleted γδ and DNT cells have been shown not to fully recover even after prolonged cART[46–48]. This depletion, early in the infection may be a likely predisposing factor for end stage renal disease and other co-morbidities commonly observed among HIV infected Africans and African-Americans despite prolonged cART use[44,49]. Higher levels of γδ T and its subsets found during chronic HIV infection has been attributed to immune activation of IL-17 producing γδ T cells fractions[14].

γδ T cells have been shown to regulate humoral responses including auto antibodies, modulate size and production of pre-immune peripheral B cells as well as control levels of circulating immunoglobulins[16,29,30,50]. It has been previously shown that memory B cells defects during HIV infection may be due the impact of the virus on T cells that provide B cells help in germinal centers[43,51–53]. As observed in previous studies, treated HVL and LVL groups had significantly higher proportions of the naïve and memory B cells compared to other groups[54,55]. Also, as observed in our study, treated LVL groups had higher proportions of naïve and memory B cells compared to HVL. This supports previous studies which suggest that high viremia impacts B cell reconstitution after ART exposure[43,54,56]. We also observed that memory B cells defined by (CD19+CD27) were increased during early HIV compared to the chronic stage before commencement of ART. Previous studies have associated this finding to the observation that HIV infected persons have preserved memory B cell functions after ART exposure[56,57]. It seems that reconstitution of memory B cells can be attributed to control of HIV through the use of ART.

Accordingly, this study has shown that the lack of γδ T cells immune reconstitution to healthy levels despite cART may play a major role in B cells defects observed during HIV disease progression. This abnormally may have started from the early stages of infection. A limitation of this study was our inability to separate the expansion of $V\delta1^+$ subsets from the concomitant depletion of $V\delta2^+$ during HIV infection. These subsets have been shown to respond differently at different stages of HIV infection[14]. It would be interesting to determine which of these subsets are particularly impacted during early HIV infection especially among Africans.

In conclusion, our data has provided insights into the reduction of γδ T cells during early stages of HIV infection. This event is associated with high levels of urea and creatinine and occurs before systemic immune activation reaches set points. The kidney may possibly serve as a reservoir of HIV as this underlines the direct impact of the virus on this organ. Our findings suggest that depletion of these T cells early in HIV infection has grave implications on disease progression even after prolonged ART use. Early HIV infection diagnosis and treatment is hereby recommended.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

| | |
|---|---|
| **γδ** | gamma-delta |
| **DN** | Double negative |
| **CD** | Cluster of differentiation |
| **ELISA** | Enzyme linked immunosorbent assay |
| **PBMCs** | Peripheral blood mononuclear cells |

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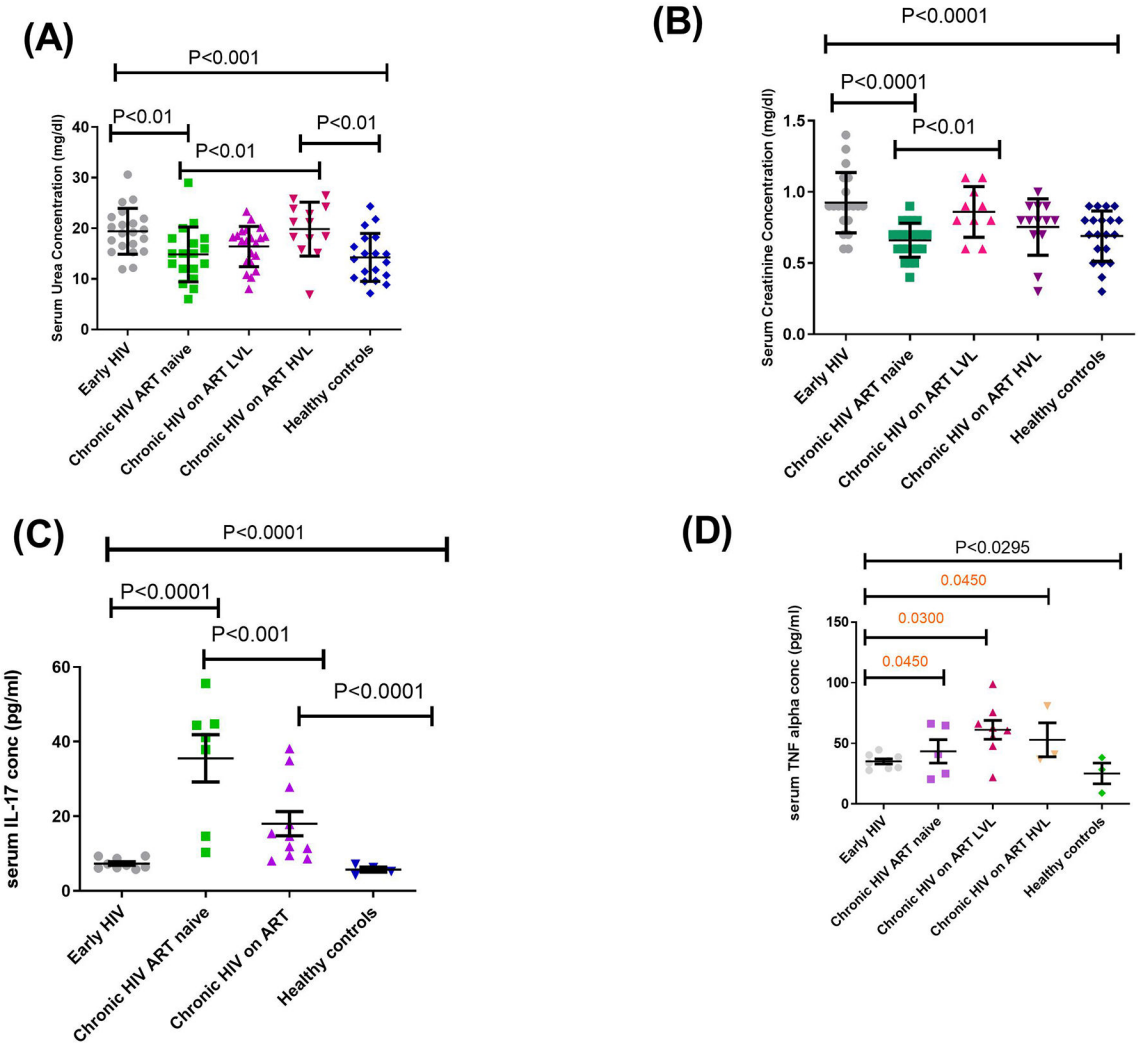


**Fig. 1. Early HIV infection is associated with renal dysfunctions.**

Levels of (A) serum urea concentration (mg/dl), (B) serum creatinine concentration (mg/dl), (C) serum IL-17 concentration (pg/ml), (D) serum TNF alpha (pg/ml). Each symbol represents an individual. Early HIV: persons at early stages of HIV infection (HIV Ab negative; HIV Ag positive, HIV DNA positive), Chronic HIV ART naïve: persons at chronic stages of HIV infection not on ART (HIV Ab positive; HIV Ag positive; HIV DNA positive), Chronic HIV on ART LVL: persons on ART for over 6months with undetectable viral load, Chronic HIV on ART HVL: persons on ART for over 6months with detectable viral load. Horizontal lines and errors bars represent standard error of the mean (SEM), 25$^{th}$ and 75$^{th}$ percentiles. Statistical test: ANOVA and Tukey's multiple comparison tests.

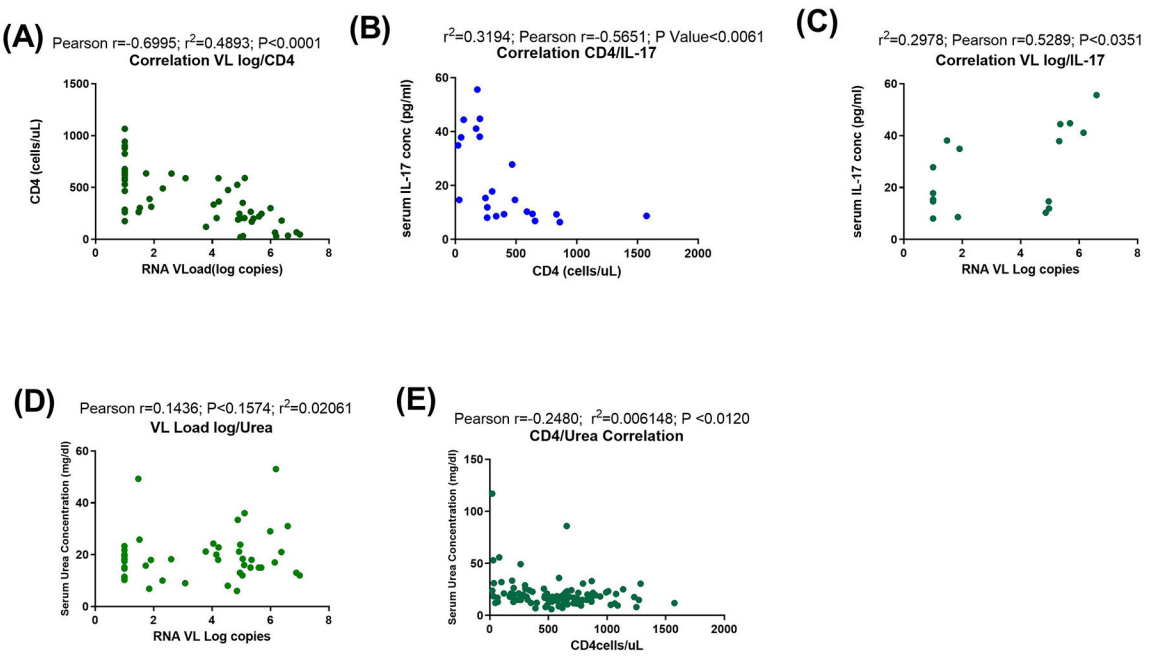


**Fig. 2. In the HIV-infected cohort, levels of serum IL-17 and urea are directly and inversely correlated with HIV-1 log RNA viral load copies and CD4 T cells proportions.**

Correlation analyses between (A) HIV-1 log RNA viral load copies and CD4 T cells, (B) CD4 T cells and serum IL-17 concentration (pg/ml), (C) HIV-1 log RNA viral load copies and serum IL-17 (pg/ml), (D) HIV-1 log RNA viral load copies and serum urea level concentration (mg/dl) and (E) CD4 T cells and serum urea level concentration (mg/dl). Blue and green symbols represent study participants. Statistical test: Pearson correlation.

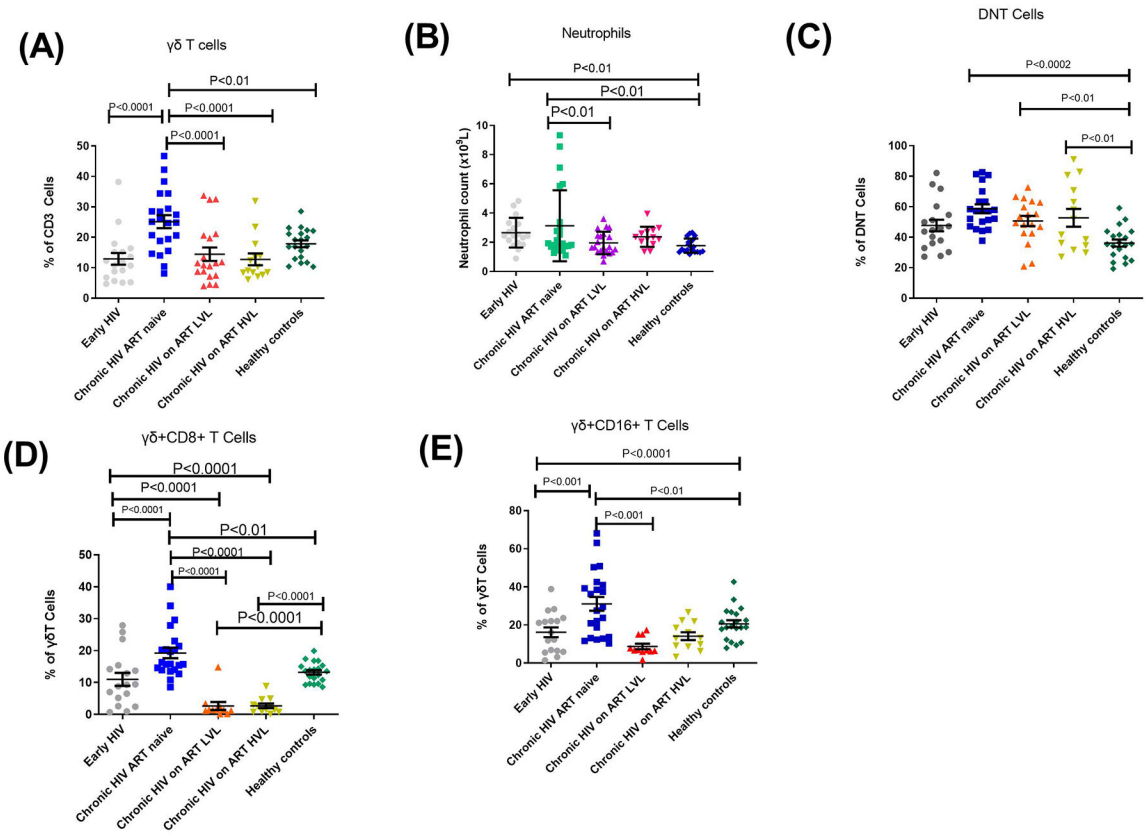


**Fig. 3. Proportions of gamma delta T cells (γδT cells) and subsets, double-negative T cells (DNT cells) as well as neutrophils are reduced during early HIV infection.**

Proportions of (A) gamma delta T cells (as % of CD3 cells, (B) Neutrophil counts ($x10^9$), (C) double negative T cells (DNT cells), (D) gamma delta + CD8 T cells, (E) gamma delta + CD16 T cells as % of gamma delta T cells . Each symbol represents an individual. Early HIV: persons at early stages of HIV infection (HIV Ab negative; HIV Ag positive, HIV DNA positive), Chronic HIV ART naïve: persons at chronic stages of HIV infection not on ART (HIV Ab positive; HIV Ag positive; HIV DNA positive), Chronic HIV on ART LVL: persons on ART for over 6months with undetectable viral load, Chronic HIV on ART HVL: persons on ART for over 6months with detectable viral load. Horizontal lines and errors bars represent standard error of the mean (SEM), $25^{th}$ and $75^{th}$ percentiles. Statistical test: ANOVA and Tukey's multiple comparison tests.

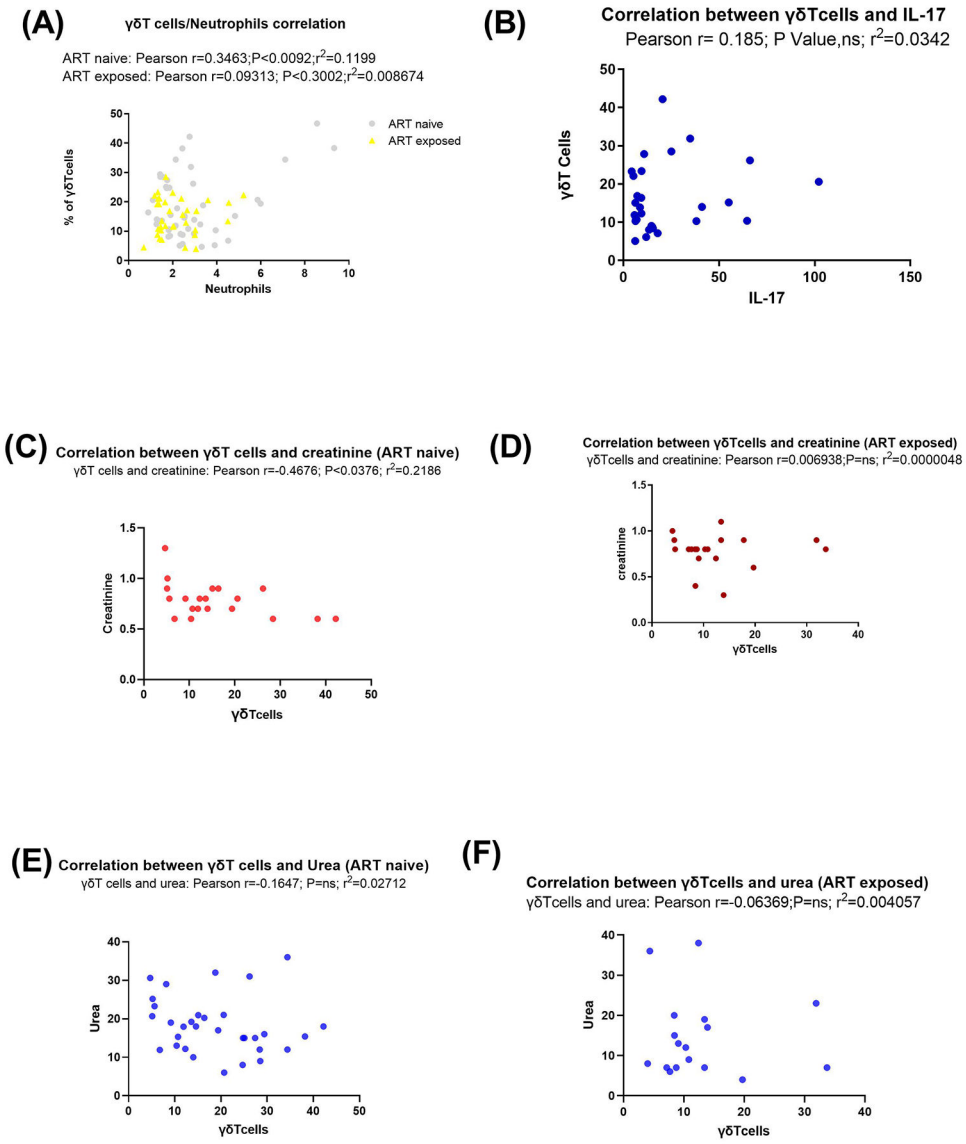


**Fig. 4. γδ T cells and subsets correlate with neutrophil counts and serum IL-17 concentration.**
Correlation analyses between (A) gamma delta T cells and neutrophil counts ($x10^9$), grey symbols represent ART naïve individuals while yellow symbols represent ART exposed individuals (B) gamma delta T cells and serum IL-17 concentration (pg/ml), (C) gamma delta T cells and creatinine among ART naïve individuals, (D) gamma delta T cells and creatinine among ART exposed, (E) gamma delta T cells and urea among ART naïve individuals and (F) gamma delta T cells and urea among ART exposed individuals. Blue symbols represent urea levels while red symbols represent creatinine levels among study participants. Statistical test: Pearson correlation.

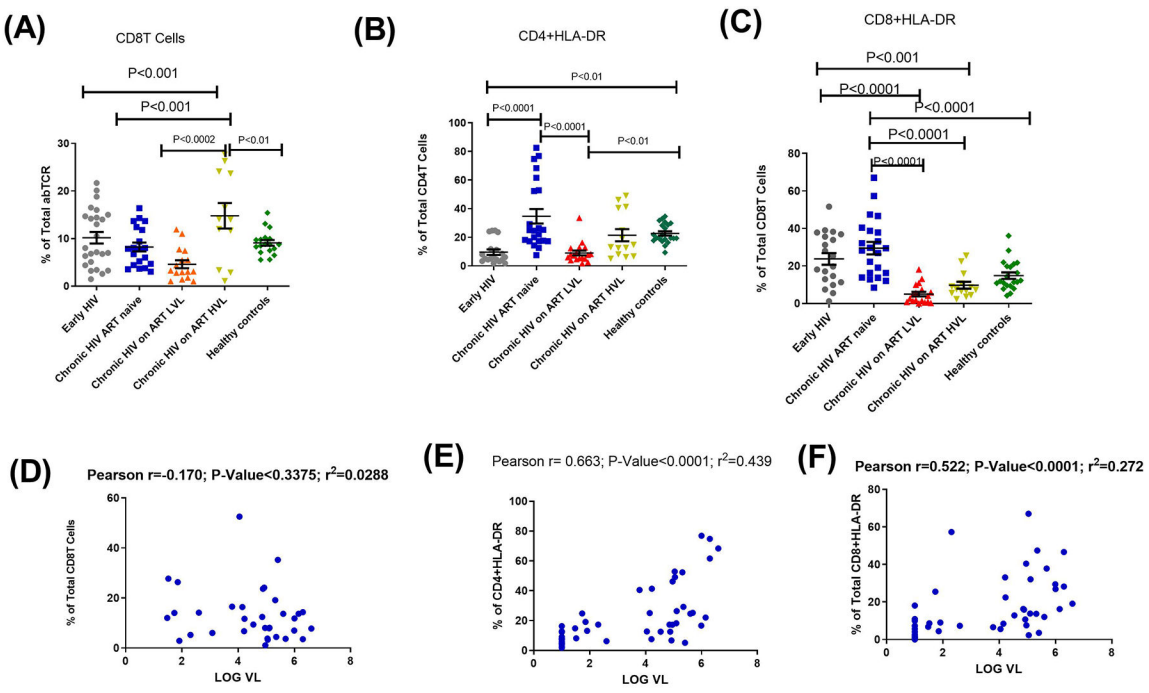


**Fig. 5. Immune activation is associated with chronic HIV-1 infection**

Proportions of (A) CD8 T cells as percentage of abTCR, (B) CD4+HLA-DR T cells as percentage of total CD4 (C) CD8 + HLA-DR T cells as percentages of total CD8 T cells. Each symbol represents an individual. Early HIV: persons at early stages of HIV infection (HIV Ab negative; HIV Ag positive, HIV Gag DNA positive), Chronic HIV ART naïve: persons at chronic stages of HIV infection not on ART (HIV Ab positive; HIV Ag positive; HIV Gag DNA positive), Chronic HIV on ART LVL: persons on ART for over 6months with undetectable viral load, Chronic HIV on ART HVL: persons on ART for over 6months with detectable viral load. Horizontal lines and errors bars represent standard error of the mean (SEM), 25$^{th}$ and 75$^{th}$ percentiles. Statistical test: ANOVA and Tukey's multiple comparison tests. Correlation analyses between (D) CD8 T cells (E) CD4+HLA-DR T cells (F) CD8 + HLA-DR T cells and Log HIV-1 RNA viral load copies. Blue symbols represent study participants. Statistical test: Pearson correlation.

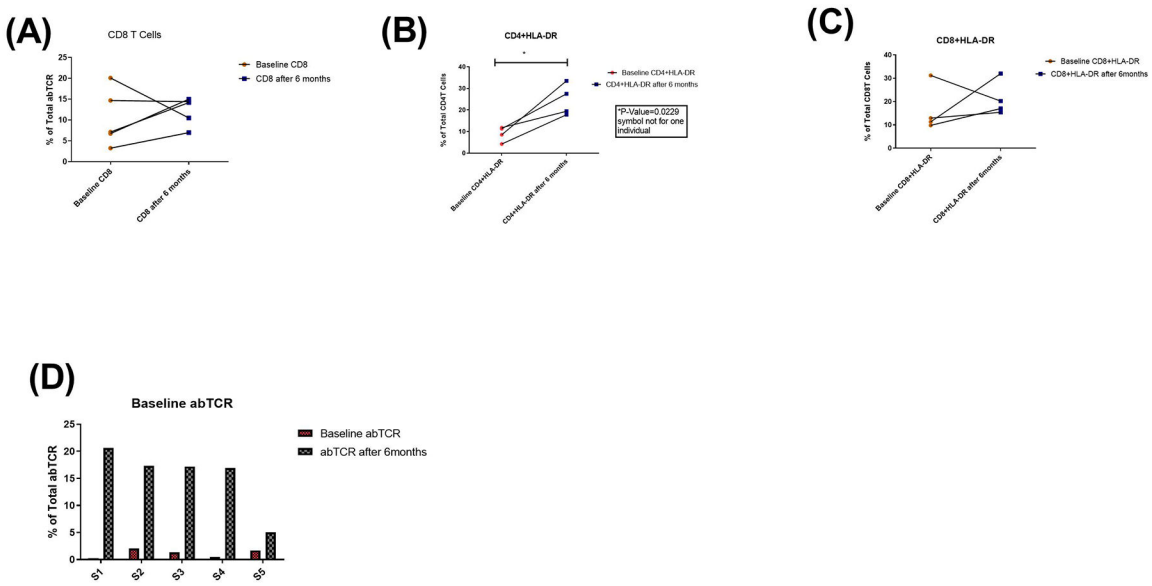


**Fig. 6. Immune activation is ongoing after six months of HIV infection among early infected individuals.**

Five Individuals longitudinal follow-up in untreated patients with early HIV infection. Immune cell activation was assessed in untreated patients by measuring the frequency of (A) CD8 T cells (B) CD4+HLA-DR (C) CD8+HLA-DR and (D) alpha beta T cell receptors at baseline and six months after.

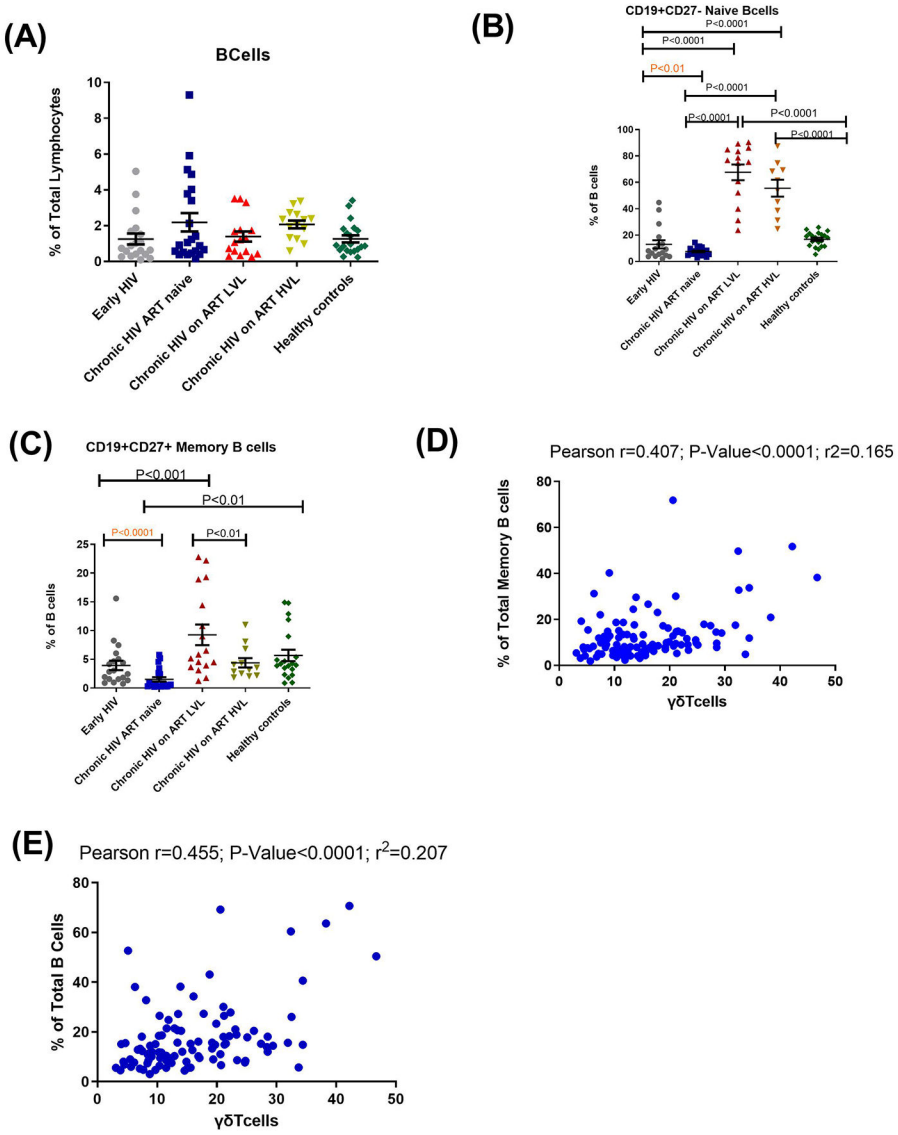


**Fig. 7. γδ T cells correlate directly with B and memory B cells**

Proportions of (A) B cells, (B) naïve B cells and (C) memory B cells. Each symbol represents an individual. Early HIV: persons at early stages of HIV infection (HIV Ab negative; HIV Ag positive, HIV Gag DNA positive), Chronic HIV ART naïve: persons at chronic stages of HIV infection not on ART (HIV Ab positive; HIV Ag positive; HIV Gag DNA positive), Chronic HIV on ART LVL: persons on ART for over 6months with undetectable viral load, Chronic HIV on ART HVL: persons on ART for over 6months with detectable viral load. Horizontal lines and errors bars represent standard error of the mean (SEM), 25$^{th}$ and 75$^{th}$ percentiles. Statistical test: ANOVA and Tukey's multiple comparison tests. Correlation analyses between (D) proportions of memory B cells and (E) Total B cells and gamma delta T cells. Blue symbols represent study participants. Statistical test: Pearson correlation.

**Table 1:**

Baseline characteristics of study participants.

| | Healthy Controls (HCC) | Early HIV (EHIV) | Chronic HIV ART naïve (CHIV) | Chronic HIV on ART | |
|---|---|---|---|---|---|
| | | | | Persons with low viral load (LVL) | Persons with high viral load (HVL) |
| | (n=27) | (n=17) | (n=23) | (n=23) | (n=18) |
| **Gender(F/M)** | 9/18 | 2/15 | 13/10 | 15/8 | 6/12 |
| **Mean Age (years)** | 27(21–41) | 35(27–48) | 43(23–69) | 47(34–69) | 47(34–69) |
| **Mean CD4(cells/l)** | 819(494–1575) | 662(401–881) | 206(68–476) | 631(175–853) | 283(172–370) |
| **Mean Plasma VL(cp/ml)** | N/A | 5,640,000(5,000,000–6,350,000) | 207,000(53,300–1,470,000) | TND | 8,620(66.8–86,300) |
| **CD4/CD8 ratio** | 5.54(4.80–10.8) | 5.7(1.92–9.91) | 3.09(1.72–6.12) | 10.7(3.63–18.0) | 0.896(0.114–3.90) |
| **Neutrophil ($x10^9$)** | 1.585(1.350–2.185) | 2.450(1.923–3.268) | 1.910(1.700–3.380) | 1.660(1.365–2.510) | 2.340(1.970–2.775) |
| **Monocyte/Lymphocyte ratio** | 0.106(0.0739–0.142) | 0.159(0.102–0.182) | 0.209(0.133–0.309) | 0.129(0.0990–0.164) | 0.168(0.135–0.218) |

Values shown for gender, age, CD4+ T cells, HIV RNA copies/ml, CD4/CD8 ratio, neutrophils, monocyte/lymphocyte ratio are medians and interquartile ranges. Statistical tests used: ANOVA and Tukey's multiple comparison tests was used for age, CD4+ T cells, HIV RNA copies/ml, CD4/CD8 ratio, neutrophils, monocyte/lymphocyte ratio. N/A—Not applicable. ns – Not significant.

**Table 2.**

P-Value of baseline characteristics of study participants.

| | EHIV vs CHIV | EHIV vs LVL | EHIV vs HVL | EHIV vs HCC |
|---|---|---|---|---|
| **Gender (F/M** | ns | ns | ns | ns |
| **Mean Age (years)** | 0.0001 | 0.0001 | 0.0001 | ns |
| **Mean CD4(cells/l)** | 0.0001 | ns | 0.0001 | ns |
| **Mean Plasma VL (cp/ml)** | 0.0001 | N/A | 0.0001 | N/A |
| **CD4/CD8 ratio** | ns | ns | ns | ns |
| **Neutrophils** | ns | ns | ns | ns |
| **Monocyte/Lymphocyte ratio** | 0.01 | ns | ns | ns |

Abbreviations: N/A, not applicable; ns, not significant.