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**Title: T and B-cell perturbations identify distinct differences in HIV-2 compared with HIV-1 induced immunodeficiency**

Running head: T and B-cell phenotypes in HIV-2 infection

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## **Abstract**

### **Background**

For unknown reasons, HIV-2 is less pathogenic than HIV-1, and HIV-2 induced immunodeficiency may be different from that caused by HIV-1. Previous immunological studies have hinted at possible shifts in both T and B-cell subsets, which we aimed to characterize further.

### **Methods**

From an HIV clinic in Guinea-Bissau, 63 HIV-2, 83 HIV-1, and 26 HIV negative participants were included. All HIV infected participants were ART naïve. The following cell subsets were analysed by flow cytometry; T cells (maturation and activation), regulatory T cells, and B cells (maturation and activation).

### **Results**

After standardizing for sex, age, and CD4<sup>+</sup> T cell count HIV-2 had 0.938 log<sub>10</sub> copies/mL lower HIV RNA levels than the HIV-1 infected patients. Whereas T-cell maturation and regulatory T-cell profiles were similar between patients, HIV-2 infected patients had higher proportions of CD8<sup>+</sup>CD28<sup>+</sup> and lower proportions of CD8<sup>+</sup>PD-1<sup>+</sup> T cells than HIV-1 infected patients. This finding was independent of HIV RNA levels. HIV-2 was also associated with a more preserved proportion of naïve B cells.

### **Conclusion**

HIV-2 is characterized by lower viral load, and lower T-cell activation, which may account for the slower disease progression.

**Key words:** HIV, HIV-2, HIV-1, leukocyte, phenotype, activation, maturation, T cell, B cell, Guinea-Bissau

## Introduction

With an estimated 1-2 million people infected worldwide, HIV-2 is mostly found in West Africa and countries with historical relations to West Africa, such as Portugal and France [1,2]. Early surveys performed in the capital of Guinea-Bissau during the late 1980's report a prevalence of 9% among adults [3]. Since then, the HIV-2 prevalence has declined and been surpassed by HIV-1 [4-6].

Although HIV-1 and HIV-2 are structurally similar, there is only 30-60% genetic similarity on a nucleic acid basis [7]. Also, viral load is usually lower in HIV-2 compared with HIV-1 infected patients, and a large proportion of HIV-2 infected patients has undetectable viral load [8,9]. This may explain the relatively lower transmissibility of HIV-2 and that many HIV-2 infected individuals progress more slowly towards AIDS. On average, HIV-2 infection advances to cause AIDS in 25-30 years [1,3,10-13]. The clinical manifestation of HIV-2-induced AIDS closely resembles that of HIV-1 [14]. For HIV-2 infected individuals with undetectable viral loads, the mortality rate is similar to that of the background population, but viremic individuals still have an excess mortality [12,15].

HIV-2 infected patients with normal CD4<sup>+</sup> T-cell counts or low viral load have lower rates of T-cell activation compared with HIV-1 infected patients [16,17]. But during disease progression, the T-cell perturbations are very similar between HIV-1 and HIV-2 infected patients [17,18]. Although HIV-2 infected patients also experience a progressive loss of memory B-cells [19], a characteristic feature of HIV-2 infection is the development of broadly neutralizing antibodies [20].

To further understand the importance of HIV-2 RNA levels and immune perturbations, we compared immune subsets in HIV-1 and HIV-2 infected patients, and HIV negative individuals.

## **Material and methods**

### *Setting and study population*

During February 2016 – September 2017, we enrolled HIV infected patients at the HIV clinic situated at Hospital Nacional Simão Mendes, in the capital Bissau, Guinea-Bissau. All included patients were ART naïve and attended the clinic for a routine blood sampling in accordance with the national guidelines. The clinic has been described previously [21,22]. Individuals testing negative for HIV at the clinic were invited to participate as an HIV negative control group. These individuals may be regarded as high-risk seronegatives.

### *Sample collection*

Venous blood samples were collected in CPT vacutainers containing sodium citrate/Ficoll (Becton Dickinson, New Jersey, United States). Within 4 hours, samples were transported to the reference laboratory, National Public Health Laboratory, and processed. After shipment to Denmark, samples were thawed according to an optimized protocol [23]. Further description of the sample handling is available in the supplementary section.

### *Flow cytometric analysis*

PBMCs were stained in four different panels: T-cell maturation, activation and regulation, and B-cell maturation/activation (presented elsewhere). Definition of the maturation stages used for both T and B cells has been published previously [24,25]. The staining process is described in the supplementary section.

Samples were analyzed on a Sony Spectral Cell Analyzer SP6800 (Sony Corporation, Tokyo, Japan) flow cytometer. The software SP6800 version 1.6.3.7151 was used for acquisition and spectral unmixing (weighted least-squares algorithm). Cell population gating was performed on unmixed data in FlowJo v.10.4 (FlowJo LLC, Ashland, USA) as presented elsewhere.

### *HIV diagnosis and HIV type discrimination*

HIV screening was performed using Determine HIV-1/2 (Abbott Laboratories, Tokyo, Japan). HIV Confirmation and HIV type discrimination was performed using ImmunoComb HIV 1/2 BiSpot (Orgenics, Yavne, Israel) and First Response HIV 1-2.0 Card Test (Premier Medical Corporation Ltd, Kachigam, India). Patients typed with First Response, and patients typed HIV-1/2 dual reactive with ImmunoComb, had confirmatory HIV typing performed with INNO-LIA HIV I/II Score (Fujirebio, Ghent, Belgium) [26].

### *HIV RNA measurements*

HIV-1 RNA was quantified on the Abbott m2000 platform (Abbott RealTime HIV 1, version 9.00, Abbott Molecular Inc, Abbott Park, USA). As previously described [26] HIV-2 RNA was quantified using an in-house method. For both analyses, the detection limit was 50 copies/mL.

### *Statistical analyses*

Statistical analyses were performed in Stata IC 13.0 (Stata Corporation, Texas, USA). Patients with undetectable HIV RNA levels had imputed HIV RNA of 25 copies/mL equivalent to half the detection limit. Viremia was defined as HIV RNA above 100 copies/mL. Chi squared tests were used for categorical variables. After checking for normal distribution, continuous variables were compared with either T-tests or Wilcoxon Rank-sum tests. Adjustments for sex, age, and CD4<sup>+</sup> T-cell counts, and determining correlations, were performed by linear regression analysis. Maturation profiles of T and B cells were compared with the SPICE analysis. This tests whether the patient groups overall were different as a whole [27].

## *Ethics*

Ethical approval was given by the ethics committee in Guinea-Bissau (No Ref. 061/2013) after the Danish committee had given consultative approval (case no. 1305362). Patient participation was voluntary, and signed consent forms were obtained from all included patients. For illiterate patients, fingerprint signatures were obtained.

## **Results**

### *Characteristics of included patients*

We included 63 HIV-2 infected patients (47 viremic and 16 aviremic), 83 HIV-1, and 26 HIV negative controls. Sex distribution was similar between patient groups, and between patients and controls (Table 1). The HIV negative control group was younger than both HIV-2 ( $p < 0.001$ ) and HIV-1 ( $p = 0.003$ ) infected patients, and the HIV-2 infected patients were older than the HIV-1 infected patients ( $p < 0.001$ ). Despite the higher age, HIV-2 infected patients had a higher median CD4<sup>+</sup> T-cell count (351 cells/ $\mu$ L) compared with HIV-1 infected patients (217 cells/ $\mu$ L,  $p < 0.001$ ). CD4<sup>+</sup> T-cell count was not measured for the HIV negative controls. Among HIV-2 infected patients, those with aviremia tended to have higher CD4<sup>+</sup> T cell count ( $p = 0.085$ ), but there was no difference in sex ( $p = 0.165$ ) or age ( $p = 0.411$ ). HIV RNA was lower for HIV-2 than for HIV-1 infected patients ( $p < 0.001$ ) – on average 0.938 log<sub>10</sub> copies/mL (95% CI: 0.286 – 1.591) after standardizing for differences in sex, age and CD4<sup>+</sup> T-cell count. For both groups, HIV RNA correlated inversely with CD4<sup>+</sup> T-cell count (Figure 1), but there was no difference in the correlation coefficients ( $p = 0.886$ ). Despite undetectable HIV-2 RNA (aviremia), 3/16 (19%) of the HIV-2 infected patients had CD4 cell count below 200 cells/ $\mu$ L.

### *T-cell maturation*

For the overall CD4+ T-cell maturation stage distribution we found no difference between HIV negative and HIV-2 infected patients ( $p=0.307$ ), but HIV-2 infected patients presented with higher proportions of early differentiated T-cells compared with HIV-1 infected patients. Albeit, when stratified by CD4+ T-cell count (above/below 350 cells/ $\mu\text{L}$ ) there was no difference in CD4+ T-cell maturation profiles (Supplementary Figure 1, <http://links.lww.com/QAD/B456>).

For CD8+ T-cells (Figure 2), the HIV-2 infected patients differed significantly overall from both the HIV negative and the HIV-1 infected individuals (for both  $p<0.001$ ). Early differentiated CD8+ T-cells (naïve, 1<sup>st</sup> antigen experienced, and 2<sup>nd</sup> antigen experienced) were relatively scarcer in HIV-2 infected patients compared with the HIV negative individuals, whereas 3<sup>rd</sup> and 4<sup>th</sup> antigen experienced T-cells constituted a relatively larger proportion. A similar pattern was observed for the HIV-1 infected patients with even fewer early stage, and more late stage, differentiated T-cells.

When stratified by CD4+ T-cell count (Supplementary Figure 1, <http://links.lww.com/QAD/B456>) these differences diminished, and there was no overall difference between HIV-2 and HIV-1 for patients with CD4+ T cell count below 350 cells/ $\mu\text{L}$  ( $p=0.165$ ). When comparing HIV negative individuals with HIV infected patients with CD4+ T-cell count above 349 cells/ $\mu\text{L}$ , there was lower proportions of naïve CD8+ T-cells for both groups of HIV infected patients – this proportion was even lower for HIV-1 compared with HIV-2 infected patients.

### *T-cell activation*



Compared with HIV negative individuals, the HIV-2 infected patients presented with higher proportions of CD4+PD-1+ T cells compared with HIV negative individuals ( $p=0.010$ ), Supplementary Figure 2, <http://links.lww.com/QAD/B456>). HIV-1 was associated with a lower proportion of CD4+CD28+ T cells, and a higher proportion of CD4+CD38+, CD4+CTLA4+, and CD4+PD-1+ T cells. There was no differences between HIV-2 and HIV-1 for CD4+ T-cell activation markers after adjusting for sex, age and CD4+ T-cell count (Supplementary Figure 3, <http://links.lww.com/QAD/B456>).

For the CD8+ T cells, activation markers are presented in Supplementary Figure 4, <http://links.lww.com/QAD/B456>. Patient groups were stratified by CD4+ T-cell count above/below 350 cells/mL, and by viremic status for HIV-2. Both HIV-2 and HIV-1 infected patients had lower proportions of CD8+CD28+ T cells than the HIV negative individuals. This was also the case among HIV infected patients with CD4+ T-cell count above 349 cells/ $\mu$ L, and for aviremic HIV-2 infected patients. Both HIV-2 and HIV-1 infected patients also had higher proportions of CD8+PD-1+ T cells compared with HIV negative individuals.

When comparing HIV-2 and HIV-1 infected patients in Figure 3, we adjusted for sex, age and CD4+ T-cell count. Across CD4+ T-cell-levels HIV-2 infected patients had higher proportions of CD8+CD28+ and lower proportions of CD8+PD-1+ T cells. Proportions of these markers were stratified into naïve (CD45RA+) and memory (CD45RO+) CD8+ T cells (Supplementary Figure 5 and 6, <http://links.lww.com/QAD/B456>), and found that CD8+ T-cell maturation did not influence the HIV type-specific differences for these markers. Finally, we tested proportions of CD8+CD28+ and CD8+PD-1+ T cells for correlation with HIV RNA levels. Both markers tended to be associated with HIV RNA levels (Supplementary Figure 7, <http://links.lww.com/QAD/B456>), but even after adjusting for sex, age, CD4+ T

cell count and viral load, HIV-2 infected patients had higher proportions of CD8+CD28+ (p=0.008) and lower levels of CD8+PD-1+ T cells (p=0.001).

#### *Regulatory T cells (Tregs)*

The proportion of Tregs (defined as CD3+CD4+FoxP3+ cells) were higher among HIV-1 and HIV-2 infected patients than among HIV negative individuals in the crude analysis, but there were no differences after adjustment for sex and age (Figure 4). For both HIV-2 and HIV-1 infected patients a lower proportion of Tregs expressed CD25, and a higher proportion expressed CD49d. HIV-2 and HIV-1 infected patients were compared by adjusting for age, sex and CD4+ T cell count (Supplementary Figure 8, <http://links.lww.com/QAD/B456>), and there were no differences between groups.

#### *B-cell maturation and activation*

HIV-2 infected patients differed in their B-cell maturation profiles compared with the HIV negative individuals (p=0.012) and with the HIV-1 infected patients (p<0.001, Supplementary Figure 9, <http://links.lww.com/QAD/B456>). However, when stratifying by CD4+ T cell above 349 cells/ $\mu$ L most differences perished. Still, HIV-2 infected patients had higher proportions of naïve B-cells among patients with CD4+ T-cell count below 350 cells/ $\mu$ L (p=0.001).

As presented in Figure 5, proportions of the marker of functional impairment, CD21<sup>low</sup>, was greater in both HIV-1 and HIV-2 infected patients compared with the HIV negative individuals. Even aviremic HIV-2 infected patients, and those with CD4+ T-cell count above 349 cells/ $\mu$ L had increased levels compared to the HIV negative individuals. When adjusting

for sex, age and CD4+ T-cell count, there was no longer any difference between HIV-2 and HIV-1 infected patients (p=0.157) – Supplementary Figure 10, <http://links.lww.com/QAD/B456>. We found no differences in proportions of B cells with the activation marker CD80 between any groups.

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## Discussion

In this cross-sectional study we compared T and B-cell perturbations in ART-naïve HIV infected and HIV-negative individuals from Guinea-Bissau. At similar CD4<sup>+</sup> T-cell count levels, HIV-2 infected patients presented with almost 1-log<sub>10</sub> lower levels of HIV RNA. Both HIV types were associated with depletion of early stage (naïve) CD8<sup>+</sup> T cells and an increased proportion of T cells positive for activation markers. HIV-2 was associated with higher proportions of CD8<sup>+</sup>CD28<sup>+</sup> and lower proportions of CD8<sup>+</sup>PD-1<sup>+</sup> T cells than for HIV-1 even after adjusting for sex, age, CD4<sup>+</sup> T-cell count, and HIV RNA levels. Both HIV-1 and HIV-2 infected patients had perturbations in their Treg-profiles, but there were no differences between HIV types. HIV-2 infected patients had more preserved proportion of naïve B-cells than for HIV-1, but a similar high proportion of CD21<sup>low</sup> B cells.

Similar to our findings, many previous studies have described lower HIV RNA levels in HIV-2 compared with HIV-1 infected individuals [8,9,16]. A relatively large proportion of our HIV-2 infected patients had detectable viral load, which may be explained by inclusion of patients at an HIV clinic rather than enrollment of participants from a general population survey. Patients attending the HIV clinic in Bissau often present with advanced disease progression [28], and the level of viral load, also correlate inversely with CD4<sup>+</sup> T-cell count for HIV-2 infected patients [8]. We found that HIV-2 compared to HIV-1 infected patients had a viral load difference of almost 1-log<sub>10</sub> difference after standardizing for sex, age, and CD4<sup>+</sup> T-cell count. Even though aviremic HIV-2 infected individuals demonstrate a survival rate similar to the background population [15], we found 19% of the aviremic HIV-2 infected patients to have advanced disease progression with CD4<sup>+</sup> T-cell counts below 200 cells/ $\mu$ L suggesting that these patients either progress with very little viral replication or that viral load

may be fluctuating over time. CD4 cell depletion (below 200 cells/ $\mu$ L) has also been described in a proportion of aviremic HIV-2 infected Portuguese patients [16].

In the Portuguese study [16], they also investigated T-cell maturation stage distribution in HIV-1 and HIV-2 infected patients but found no differences in proportions of naïve (CD45RA+CD62L+) and memory (CD45RO+) CD4+ or CD8+ T cells at given degrees of HIV disease progression. Although late-differentiated (CD45RO+CD27-) CD4+ cells were proportionally more frequent in HIV-1 compared with HIV-2 infected individuals, Buggert et al. conclude that maturation differentiation is not a hallmark that distinguishes HIV-2 from HIV-1 infection [29]. Direct comparisons between studies are complicated by different definitions of maturation subsets and study participant selection. These other studies [16,29] investigating T-cell maturation used a less stringent definition of memory T cells than the consensus definition applied in our study [25]. Factors other than HIV may affect T-cell maturation distribution, such as age. But as age can also be a proxy for disease duration, adjustment could blur a true association. Differences in T-cell maturation profiles may result from differences in T-cell turnover rates induced by immune activation [17,30]. However, T-cell maturation profiles do not seem to differ between HIV-1 and HIV-2 at similar levels of CD4+ T-cell counts.

Immune activation is closely linked to perturbations in T-cell maturation staging. Among Guinean police officers Buggert et al. found that HIV-2 compared to HIV-1 was associated with a lower proportion of activated (CD38+HLA-DR+) CD4+ T-cells [29]. Lower levels of immune activation in HIV-2 infection correspond well with previous studies describing a prolonged disease course on average, and as for HIV-1, CD38 expression also correlates with viral load in HIV-2 infection [18]. We also found a lower proportion of CD4+CD38+ and

CD8+CD38+ T cells in the crude analyses, but after adjusting for sex, age and CD4+ T-cell count, there was no difference. Hence, the crude difference in proportions of CD38+ T cells was likely not an HIV-type-specific effect.

The importance of CD8+ T cells in HIV-1 infection has been extensively investigated, but much less attention has been given to describing and understanding this lymphocyte subset in those with HIV-2 infection. CD28 is a costimulatory receptor, and binding of CD28 with its ligand CD80 on antigen-presenting cells results in a proliferatory signal, activates telomerases, and enhance T-cell migration/homing [31]. During HIV-1 infection, the proportion of CD8+ T cells lacking CD28 increase [32], and these CD8+CD28- T-cells are considered to be at the end-stage of replicative senescence [33]. We found that proportions CD8+CD28+ T cells correlated positively with CD4+ T-cell count for both HIV-1 and HIV-2, but among HIV-2 infected patients there was a higher proportion of CD8+CD28+ T cells across CD4+ T-cell levels. This association remained even after adjusting for sex, age, CD4+ T-cell count and viral load. Khalid et al. found that Nef-mediated downregulation of CD28 correlated with suppression of T-cell activation in viremic but not in aviremic HIV-2 infected individuals [34]. Two studies investigating HIV-2-specific T cells found higher proportions of CD8+CD28+ T-cells among individuals with HIV-2. This suggest that high proportions of CD8+CD28+ T cells are associated with viral control [35,36].

The programmed death-1 receptor (PD-1) is upregulated during HIV-1 infection and limits tissue damage by suppressing T-cell-receptor-CD28 mediated activation [37]. For HIV-1, reduced PD-1 expression on virus-specific CD8+ T cells correlates with viral control, and blockage of PD-1 results in enhanced HIV-specific proliferative response of CD8+ T cells [38,39]. These cells with high level expression of PD-1 are more functionally impaired

compared to CD8<sup>+</sup> T cells with lower PD-1 expression [40], and PD-1 expression is also reduced among patients on ART [41,42]. This suggests that a high viral load drives PD-1 expression and cause functional exhaustion. Recently, it has been described that PD-1 is also involved in establishing and maintaining HIV latency [43]. In agreement with Tendeiro et al. [44], we found elevated proportions of PD-1 on CD8<sup>+</sup> T cells in both HIV-1 and HIV-2 infection compared to HIV negative individuals, and that HIV-2 infected patients had lower levels than for HIV-1. We extended this analysis by adjusting for sex, age, CD4<sup>+</sup> T-cell count, and viral load. Still, HIV-2 was associated with lower proportions of CD8<sup>+</sup>PD-1<sup>+</sup> T cells.

Our results therefore suggest, that higher CD8<sup>+</sup>CD28 and lower CD8<sup>+</sup>PD-1 proportions of T cells are true hallmarks of HIV-2 infection distinguishing it from HIV-1. This may result in more effective CD8<sup>+</sup> T-cell responses to better control viral replication and slow CD4<sup>+</sup> T-cell decline.

A number of studies have investigated HIV-specific T-cell responses in HIV-2 and HIV-1 infected individuals in attempts to understand the relatively lower pathogenicity of HIV-2 [36,45–53]. The protein Gag is most immunogenic in HIV-2 infection and the specific T-cell response to this protein relates to the degree of viremia [45,50,52]. HIV-2 infected individuals also demonstrate a more broad and polyfunctional T-cell response, which may even have an effect against HIV-1 [49,51]. This could also partly explain a potential protective effect induced by HIV-2 against disease progression during subsequent HIV-1 infection [54,55]. In contrast to HIV-1, a single HIV-2 epitope may induce a sufficient CD8<sup>+</sup> T cell Gag-specific response [53]. And whereas CD8<sup>+</sup> T cells in HIV-1 and HIV-2 infected subject are able to suppress R5 tropic viral strains equally, X4 viral replication is inhibited to

a larger extent during HIV-2 infection [48]. It is unknown why some individuals with HIV-2 infection are able to generate these favorable immune responses and others are not, but certain genetic traits are likely to be part of the explanation. We have recently shown that specific HLA-alleles (HLA-B\*58:01, HLA-DPB1\*10:01, and HLA-DRB1\*11:01) are associated with lower HIV-2 RNA levels [56].

Tregs function as immune suppressors that protect against tissue damage by promoting peripheral immune tolerance and decreasing cell activation. Chronic immune activation during HIV-1 infection induces expansion of the Treg subset, and these cells may inhibit the HIV-1-specific response, thereby favoring viral replication [57]. As HIV-2 infected individuals present with lower viral load, the relative proportion and features of Tregs could be expected to be different from patients with HIV-1. In this analysis we included the Treg surface marker CD49d. As CD49d<sup>+</sup> Tregs produce IFN- $\gamma$  and IL-17, they may be considered proinflammatory rather than immunosuppressive cells [58,59]. In Portuguese patients, Foxall et al. found similar levels of Tregs when comparing HIV-1 and HIV-2 infected patients, and as for HIV-1, frequency of Tregs increased with HIV disease progression in patients with HIV-2 [60]. We also found a similar pattern with equal proportions of Tregs for HIV-1 and HIV-2, and there was no difference in the Treg surface markers including CD49d.

Only few studies have compared B-cell perturbations in HIV-2 and HIV-1 infected individuals. After stratifying by CD4 cell count, the HIV-2 infected patients with CD4<sup>+</sup> T-cell count below 350 cells/ $\mu$ L had larger proportions of naïve B-cells. B-cell imbalances have also been described by Tendeiro et al. who found that HIV-2 infected patients have more extensive depletion of memory B-cells than the HIV-1 infected and HIV negative counterparts, despite similar numbers of total B cells [19]. Depletion of memory B-cells was



furthermore associated with viremia for HIV-2, but not HIV-1 infected patients. Our results are thus in line with this previous study showing marked HIV-type specific differences during the disease course.

To our knowledge, the CD21<sup>low</sup> B cell subset has not previously been investigated in HIV-2 infected individuals. We found that the proportion of these impaired B cells was higher for both HIV-2 and HIV-1 infected patients, but without difference between patient groups. Even HIV-2 infected patients with undetectable viral load and patients with CD4<sup>+</sup> T-cell count above 349 cells/ $\mu$ L had increased proportions of CD21<sup>low</sup> B cells compared to the HIV negative individuals. CD21<sup>low</sup> B cells have reduced responsiveness to B-cell stimuli and enhanced production of immunoglobulins, which may explain hypergammaglobulinemia during HIV infection [61]. Indeed, marked HIV-type specific differences in antibody production have been reported. HIV-2 infected individuals have high titres of neutralizing antibodies, although neither the level of heterologous nor autologous antibodies are associated with greater control of HIV-2 [62]. Nevertheless, the neutralizing antibodies from HIV-2 infected individuals have a broader effect and are more potent than antibodies derived from HIV-1 infected individuals [63,64].

## **Conclusions**

We here present a large study describing both T and B-cell phenotypes in patients from the center of the epidemic. In combination with lower viral load, HIV-2 infected patients presented with higher proportions of CD8<sup>+</sup>CD28<sup>+</sup> and lower proportions of CD8<sup>+</sup>PD-1<sup>+</sup> T cells. HIV-2 was also associated with higher proportions of naïve B-cells. These findings add to the current knowledge of immunological differences in HIV-2 and HIV-1 infection, and may partly explain why HIV-2 infected patients have slower disease progression.

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## **Authors' contributions**

BLH, MSP and CE conceived the study; BLH, MSP, BKM and CE designed the study protocol; CM and DDST were responsible for patient care; BLH, TES and TM included patients, HK performed the HIV RNA measurements; BLH and BK performed flow cytometry analyses; BLH, MSP and CE performed the statistical analyses; BLH, SJ, ALL, CW, HK, BK, and CE carried out analyses and interpretation of data; BLH, and CE drafted the manuscript; MSP, SJ, TES, TM, CW, ALL, HK, BKM and CE critically revised the manuscript for intellectual content. All authors read and approved the final version of the manuscript.

## **Conflicts of interest**

CE has held a lecture at a symposium organized by Abbott Diagnostics about an unrelated subject. Abbott Diagnostics produces HIV tests, but not contenders to the ones tested in this study. The authors have no other conflicts of interest to declare.

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## References

1. Mendoza C de, Cabezas T, Caballero E, et al. HIV type 2 epidemic in Spain: challenges and missing opportunities. *AIDS Lond Engl*. **2017**; 31(10):1353–1364.
2. Visseaux B, Damond F, Matheron S, Descamps D, Charpentier C. Hiv-2 molecular epidemiology. *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis*. **2016**; .
3. Poulsen AG, Kvinesdal B, Aaby P, et al. Prevalence of and mortality from human immunodeficiency virus type 2 in Bissau, West Africa. *Lancet Lond Engl*. **1989**; 1(8642):827–831.
4. Larsen O, Silva Z da, Sandström A, et al. Declining HIV-2 prevalence and incidence among men in a community study from Guinea-Bissau. *AIDS Lond Engl*. **1998**; 12(13):1707–1714.
5. Silva ZJ da, Oliveira I, Andersen A, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *AIDS Lond Engl*. **2008**; 22(10):1195–1202.
6. Olesen JS, Jespersen S, Da Silva ZJ, et al. HIV-2 continues to decrease, while HIV-1 is stabilizing in Guinea-Bissau. *AIDS Lond Engl*. **2018**; .
7. Hu DJ, Dondero TJ, Rayfield MA, et al. The emerging genetic diversity of HIV. The importance of global surveillance for diagnostics, research, and prevention. *JAMA*. **1996**; 275(3):210–216.
8. Ekouévi DK, Avettand-Fènoël V, Tchounga BK, et al. Plasma HIV-2 RNA According to CD4 Count Strata among HIV-2-Infected Adults in the IeDEA West Africa Collaboration. *PloS One*. **2015**; 10(6):e0129886.
9. Andersson S, Norrgren H, Silva Z da, et al. Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea-Bissau, West Africa: significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. *Arch Intern Med*. **2000**; 160(21):3286–3293.
10. Marlink R, Kanki P, Thior I, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science*. **1994**; 265(5178):1587–1590.
11. Whittle H, Morris J, Todd J, et al. HIV-2-infected patients survive longer than HIV-1-infected patients. *AIDS Lond Engl*. **1994**; 8(11):1617–1620.
12. Poulsen AG, Aaby P, Larsen O, et al. 9-year HIV-2-associated mortality in an urban community in Bissau, west Africa. *The Lancet*. **1997**; 349(9056):911–914.
13. Rowland-Jones SL, Whittle HC. Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? *Nat Immunol*. **2007**; 8(4):329–331.
14. Martinez-Steele E, Awasana AA, Corrah T, et al. Is HIV-2- induced AIDS different from HIV-1-associated AIDS? Data from a West African clinic. *AIDS Lond Engl*. **2007**; 21(3):317–324.

15. Berry N, Jaffar S, Schim van der Loeff M, et al. Low level viremia and high CD4% predict normal survival in a cohort of HIV type-2-infected villagers. *AIDS Res Hum Retroviruses*. **2002**; 18(16):1167–1173.
16. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RMM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol Baltim Md 1950*. **2002**; 169(6):3400–3406.
17. Hegedus A, Nyamweya S, Zhang Y, et al. Protection versus pathology in aviremic and high viral load HIV-2 infection-the pivotal role of immune activation and T-cell kinetics. *J Infect Dis*. **2014**; 210(5):752–761.
18. Leligdowicz A, Feldmann J, Jaye A, et al. Direct relationship between virus load and systemic immune activation in HIV-2 infection. *J Infect Dis*. **2010**; 201(1):114–122.
19. Tendeiro R, Fernandes S, Foxall RB, et al. Memory B-cell depletion is a feature of HIV-2 infection even in the absence of detectable viremia. *AIDS Lond Engl*. **2012**; 26(13):1607–1617.
20. Rodriguez SK, Sarr AD, MacNeil A, et al. Comparison of heterologous neutralizing antibody responses of human immunodeficiency virus type 1 (HIV-1)- and HIV-2-infected Senegalese patients: distinct patterns of breadth and magnitude distinguish HIV-1 and HIV-2 infections. *J Virol*. **2007**; 81(10):5331–5338.
21. Jespersen S, Hønge BL, Oliveira I, et al. Cohort Profile: The Bissau HIV Cohort-a cohort of HIV-1, HIV-2 and co-infected patients. *Int J Epidemiol*. **2015**; 44(3):756–763.
22. Jespersen S, Hønge BL, Oliveira I, et al. Challenges facing HIV treatment in Guinea-Bissau: the benefits of international research collaborations. *Bull World Health Organ*. **2014**; 92(12):909–914.
23. Hønge BL, Petersen MS, Olesen R, Møller BK, Erikstrup C. Optimizing recovery of frozen human peripheral blood mononuclear cells for flow cytometry. *PLOS ONE*. **2017**; 12(11):e0187440.
24. Johannesson TG, Søgaard OS, Tolstrup M, et al. The Impact of B-Cell Perturbations on Pneumococcal Conjugate Vaccine Response in HIV-Infected Adults. Borrow R, editor. *PLoS ONE*. **2012**; 7(7):e42307.
25. Appay V, Lier RAW van, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: Consensus and issues: Phenotype and Function of Human T Lymphocyte Subsets: Consensus and Issues. *Cytometry A*. **2008**; 73A(11):975–983.
26. Hønge B, Jespersen S, Medina C, et al. The challenge of discriminating between HIV-1, HIV-2 and HIV-1/2 dual infections. *HIV Med [Internet]*. **2018** [cited 2018 Mar 25]; . Available from: <http://doi.wiley.com/10.1111/hiv.12606>
27. Roederer M, Nozzi JL, Nason MC. SPICE: Exploration and analysis of post-cytometric complex multivariate datasets. *Cytometry A*. **2011**; 79A(2):167–174.

28. Hønge BL, Jespersen S, Aunsborg J, et al. High prevalence and excess mortality of late presenters among HIV-1, HIV-2 and HIV-1/2 dually infected patients in Guinea-Bissau - a cohort study from West Africa. *Pan Afr Med J.* **2016**; 25:40.
29. Buggert M, Frederiksen J, Lund O, et al. CD4+ T cells with an activated and exhausted phenotype distinguish immunodeficiency during aviremic HIV-2 infection. *AIDS Lond Engl.* **2016**; 30(16):2415–2426.
30. Vrisekoop N, Drylewicz J, Van Gent R, et al. Quantification of naive and memory T-cell turnover during HIV-1 infection: *AIDS.* **2015**; 29(16):2071–2080.
31. Parish ST, Wu JE, Effros RB. Sustained CD28 expression delays multiple features of replicative senescence in human CD8 T lymphocytes. *J Clin Immunol.* **2010**; 30(6):798–805.
32. Gamberg J, Pardoe I, Bowmer MI, Howley C, Grant M. Lack of CD28 expression on HIV-specific cytotoxic T lymphocytes is associated with disease progression. *Immunol Cell Biol.* **2004**; 82(1):38–46.
33. Effros RB. Replicative senescence of CD8 T cells: effect on human ageing. *Exp Gerontol.* **2004**; 39(4):517–524.
34. Khalid M, Yu H, Sauter D, et al. Efficient Nef-mediated downmodulation of TCR-CD3 and CD28 is associated with high CD4+ T cell counts in viremic HIV-2 infection. *J Virol.* **2012**; 86(9):4906–4920.
35. Angin M, Wong G, Papagno L, et al. Preservation of Lymphopoietic Potential and Virus Suppressive Capacity by CD8+ T Cells in HIV-2-Infected Controllers. *J Immunol Baltim Md 1950.* **2016**; 197(7):2787–2795.
36. Leligdowicz A, Onyango C, Yindom L-M, et al. Highly avid, oligoclonal, early-differentiated antigen-specific CD8+ T cells in chronic HIV-2 infection. *Eur J Immunol.* **2010**; 40(7):1963–1972.
37. Larsson M, Shankar EM, Che KF, et al. Molecular signatures of T-cell inhibition in HIV-1 infection. *Retrovirology.* **2013**; 10:31.
38. Brown JA, Dorfman DM, Ma F-R, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol Baltim Md 1950.* **2003**; 170(3):1257–1266.
39. Petrovas C, Casazza JP, Brenchley JM, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med.* **2006**; 203(10):2281–2292.
40. Peretz Y, He Z, Shi Y, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. *PLoS Pathog.* **2012**; 8(8):e1002840.
41. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature.* **2006**; 443(7109):350–354.

42. Trautmann L, Janbazian L, Chomont N, et al. Upregulation of PD-1 expression on HIV-specific CD8<sup>+</sup> T cells leads to reversible immune dysfunction. *Nat Med*. **2006**; 12(10):1198–1202.
43. Evans VA, Sluis RM van der, Solomon A, et al. Programmed cell death-1 contributes to the establishment and maintenance of HIV-1 latency. *AIDS Lond Engl*. **2018**; 32(11):1491–1497.
44. Tendeiro R, Foxall RB, Baptista AP, et al. PD-1 and its ligand PD-L1 are progressively up-regulated on CD4 and CD8 T-cells in HIV-2 infection irrespective of the presence of viremia. *AIDS*. **2012**; 26(9):1065–1071.
45. Zheng NN, Kiviat NB, Sow PS, et al. Comparison of Human Immunodeficiency Virus (HIV)-Specific T-Cell Responses in HIV-1- and HIV-2-Infected Individuals in Senegal. *J Virol*. **2004**; 78(24):13934–13942.
46. Andersson S, Larsen O, Da Silva Z, et al. Human immunodeficiency virus (HIV)-2-specific T lymphocyte proliferative responses in HIV-2-infected and in HIV-2-exposed but uninfected individuals in Guinea-Bissau. *Clin Exp Immunol*. **2005**; 139(3):483–489.
47. Gillespie G, Pinheiro S, Sayeid-Al-Jamee M, et al. CD8<sup>+</sup> T cell responses to human immunodeficiency viruses type 2 (HIV-2) and type 1 (HIV-1) gag proteins are distinguishable by magnitude and breadth but not cellular phenotype. *Eur J Immunol*. **2005**; 35(5):1445–1453.
48. Ahmed RKS, Norrgren H, Da Silva Z, et al. Antigen-Specific  $\beta$ -Chemokine Production and CD8<sup>+</sup> T-Cell Noncytotoxic Antiviral Activity in HIV-2-Infected Individuals. *Scand J Immunol*. **2005**; 61(1):63–71.
49. Duvall MG, Jaye A, Dong T, et al. Maintenance of HIV-Specific CD4<sup>+</sup> T Cell Help Distinguishes HIV-2 from HIV-1 Infection. *J Immunol*. **2006**; 176(11):6973–6981.
50. Leligdowicz A, Yindom L-M, Onyango C, et al. Robust Gag-specific T cell responses characterize viremia control in HIV-2 infection. *J Clin Invest*. **2007**; 117(10):3067–3074.
51. Duvall MG, Precopio ML, Ambrozak DA, et al. Polyfunctional T cell responses are a hallmark of HIV-2 infection. *Eur J Immunol*. **2008**; 38(2):350–363.
52. Foxall RB, Cortesao CS, Albuquerque AS, Soares RS, Victorino RMM, Sousa AE. Gag-Specific CD4<sup>+</sup> T-Cell Frequency Is Inversely Correlated with Proviral Load and Directly Correlated with Immune Activation in Infection with Human Immunodeficiency Virus Type 2 (HIV-2) but Not HIV-1. *J Virol*. **2008**; 82(19):9795–9799.
53. Silva TI de, Peng Y, Leligdowicz A, et al. Correlates of T-cell-mediated viral control and phenotype of CD8(+) T cells in HIV-2, a naturally contained human retroviral infection. *Blood*. **2013**; 121(21):4330–4339.
54. Esbjörnsson J, Månsson F, Kvist A, et al. Inhibition of HIV-1 disease progression by contemporaneous HIV-2 infection. *N Engl J Med*. **2012**; 367(3):224–232.

55. Esbjörnsson J, Månsson F, Kvist A, et al. Increased survival among HIV-1 and HIV-2 dual-infected individuals compared to HIV-1 single-infected individuals. *AIDS Lond Engl*. **2014**; 28(7):949–957.
56. Thomsen D, Erikstrup C, Jespersen S, et al. The influence of HLA-types on disease progression among HIV-2 infected patients in Guinea-Bissau. *AIDS Lond Engl*. **2018**; .
57. Gonzalez SM, Zapata W, Rugeles MT. Role of Regulatory T Cells and Inhibitory Molecules in the Development of Immune Exhaustion During Human Immunodeficiency Virus Type 1 Infection. *Viral Immunol*. **2016**; 29(1):2–10.
58. Kleinewietfeld M, Starke M, Di Mitri D, et al. CD49d provides access to “untouched” human Foxp3<sup>+</sup> Treg free of contaminating effector cells. *Blood*. **2009**; 113(4):827–836.
59. Akane K, Kojima S, Mak TW, Shiku H, Suzuki H. CD8<sup>+</sup> CD122<sup>+</sup> CD49d<sup>low</sup> regulatory T cells maintain T-cell homeostasis by killing activated T cells via Fas/FasL-mediated cytotoxicity. *Proc Natl Acad Sci*. **2016**; 113(9):2460–2465.
60. Foxall RB, Albuquerque AS, Soares RS, et al. Memory and naive-like regulatory CD4<sup>+</sup> T cells expand during HIV-2 infection in direct association with CD4<sup>+</sup> T-cell depletion irrespectively of viremia: *AIDS*. **2011**; 25(16):1961–1970.
61. Moir S, Malaspina A, Ogwaro KM, et al. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. *Proc Natl Acad Sci*. **2001**; 98(18):10362–10367.
62. Silva TI de, Aasa-Chapman M, Cotten M, et al. Potent autologous and heterologous neutralizing antibody responses occur in HIV-2 infection across a broad range of infection outcomes. *J Virol*. **2012**; 86(2):930–946.
63. Ozkaya Sahin G, Holmgren B, Silva Z da, et al. Potent intratype neutralizing activity distinguishes human immunodeficiency virus type 2 (HIV-2) from HIV-1. *J Virol*. **2012**; 86(2):961–971.
64. Özkaya Şahin G, Månsson F, Palm AA, et al. Frequent Intratype Neutralization by Plasma Immunoglobulin A Identified in HIV Type 2 Infection. *AIDS Res Hum Retroviruses*. **2013**; 29(3):470–478.

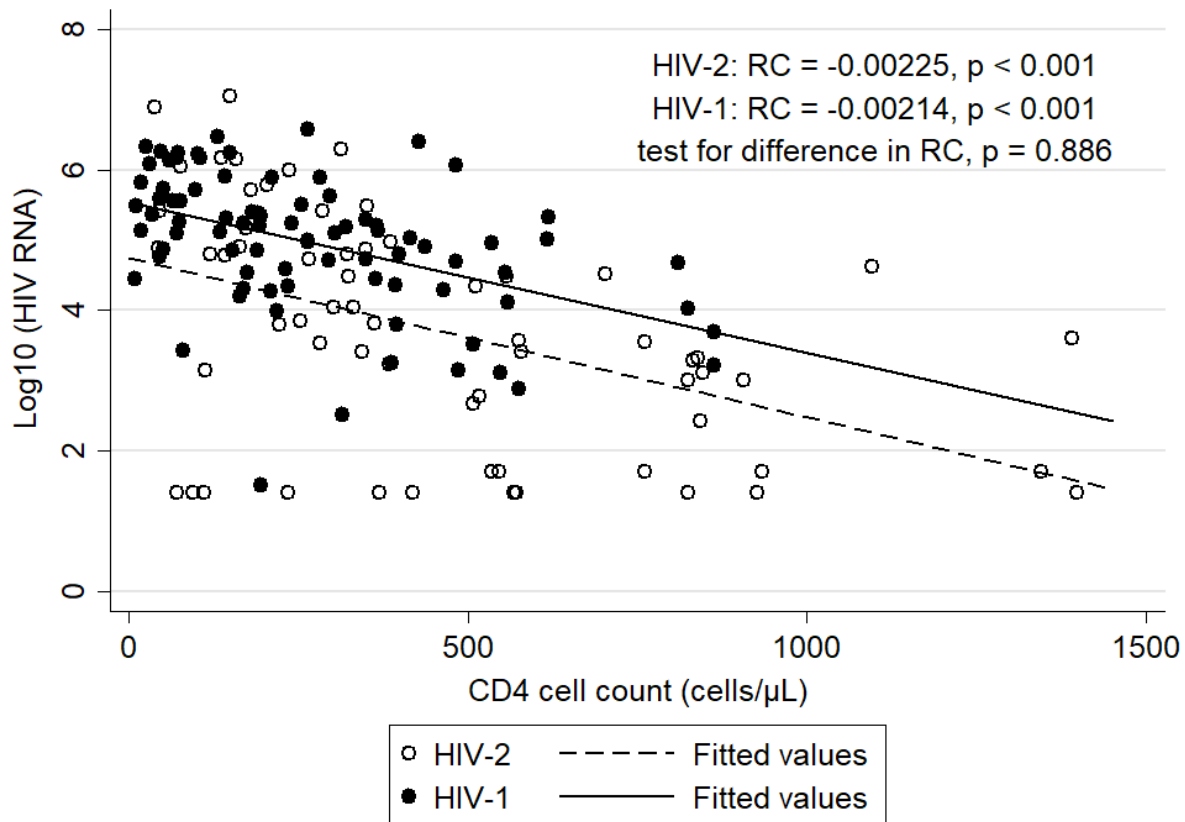


**Table 1:** Patient characteristics by HIV type and with HIV-2 infected patients stratified by viremia. Results are counts with percentages, or medians with interquartile ranges (IQR).

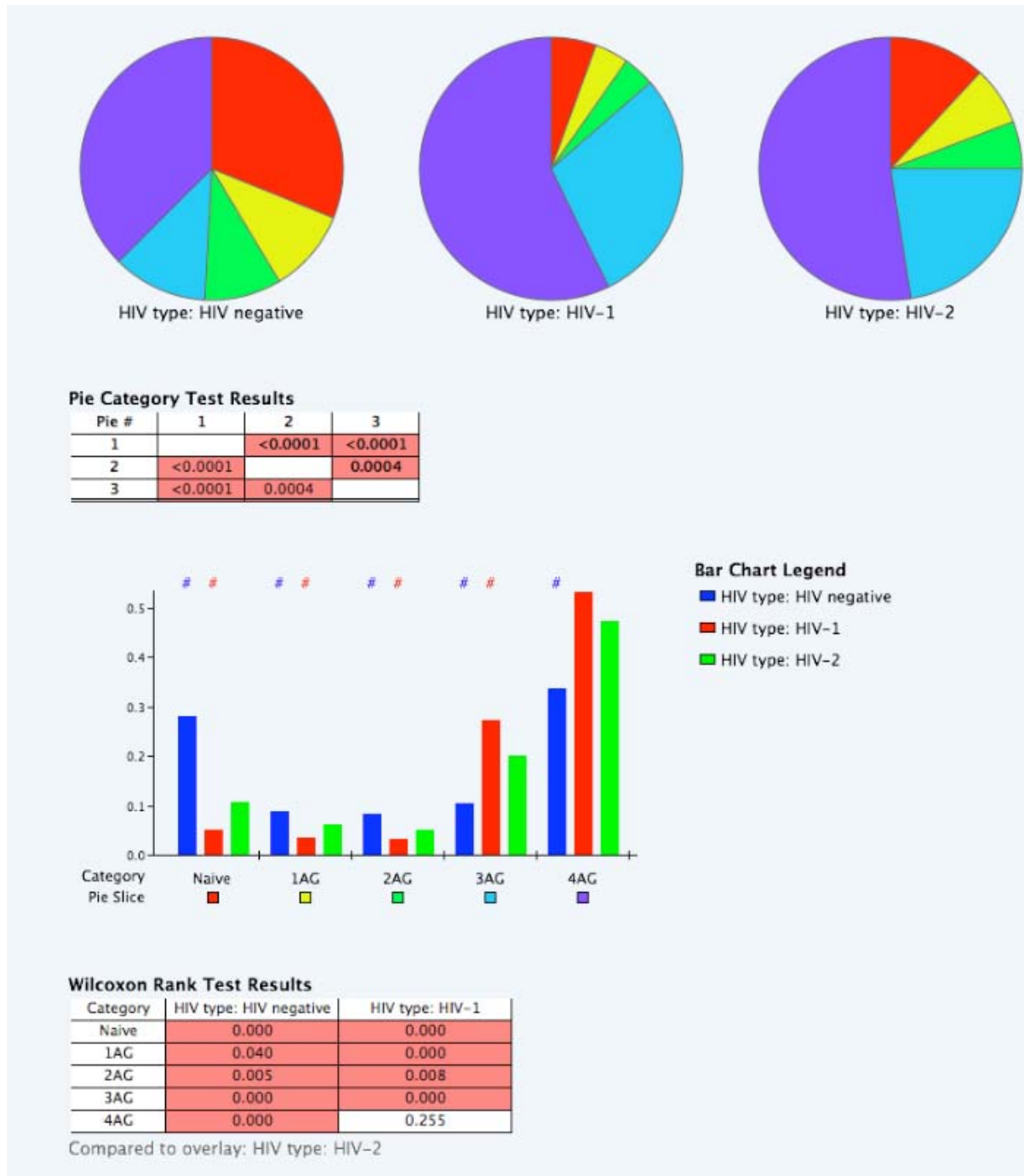
	<b>HIV neg</b>	<b>HIV-2</b>		<b>HIV-1</b>
	all	aviremic*	viremic*	all
<b>Number</b> (females/males)	26 (17 / 9)	16 (12 / 4)	47 (26 / 21)	83 (56 / 27)
<b>Age, years</b> (IQR)	30 (25 - 38)	43 (40 - 53)	49 (39 - 58)	39 (31 - 49)
<b>CD4 T-cell count, cells/<math>\mu</math>L</b> (IQR)	NA NA	557 (302 - 875)	324 (180 - 574)	217 (97 - 395)
<b>% CD4 T cells</b> (IQR)	63.4 (55.7 - 67.5)	45.0 (26.4 - 57.1)	36.0 (19.4 - 46.3)	18.2 (8.3 - 26.6)
<b>HIV-1 RNA copies/mL</b> (IQR)	NA NA	NA NA	NA NA	127.836 (27,665 - 391,516)
<b>HIV-2 RNA copies/mL</b> (IQR)	NA NA	<100 -	30,000 (2,600 - 260,000)	NA NA

Viremia defined as HIV RNA > 100 copies/mL

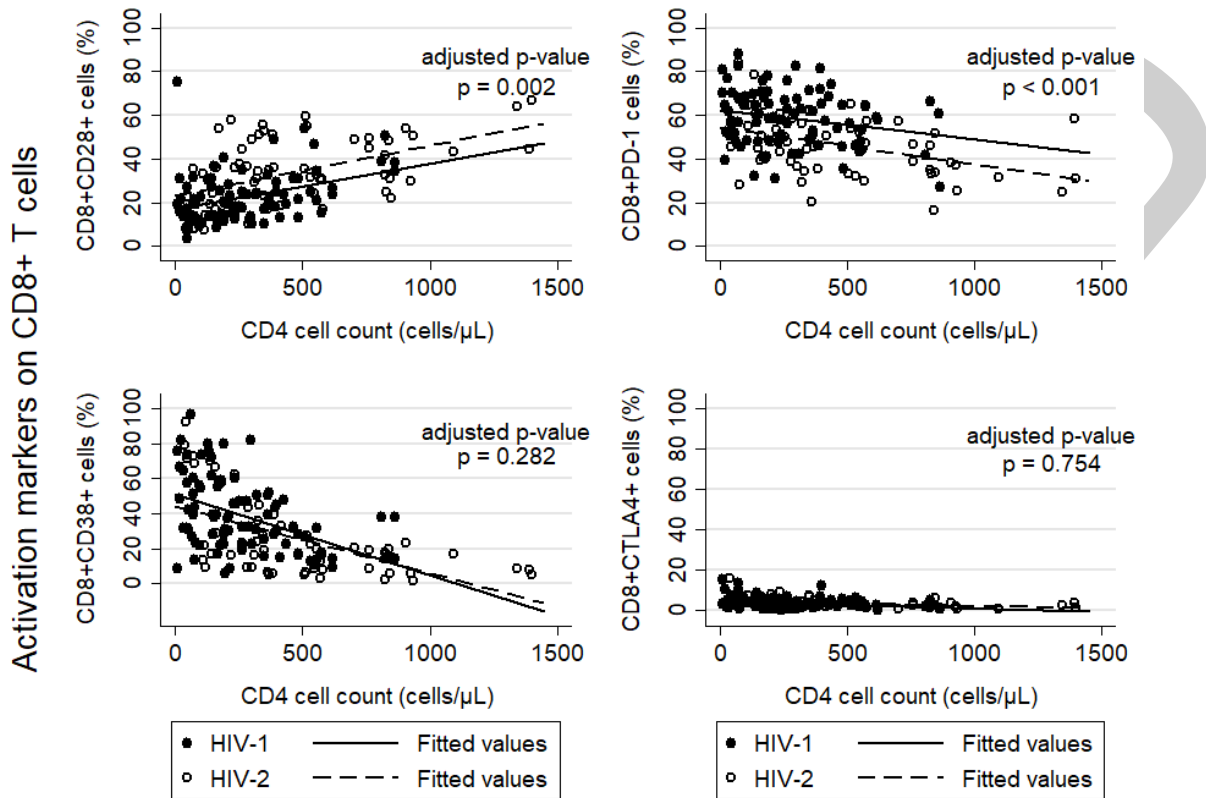
**Figure 1:** HIV RNA levels by CD4+ T cell count for HIV-2 and HIV-1 infected patients. RC = regression coefficient.



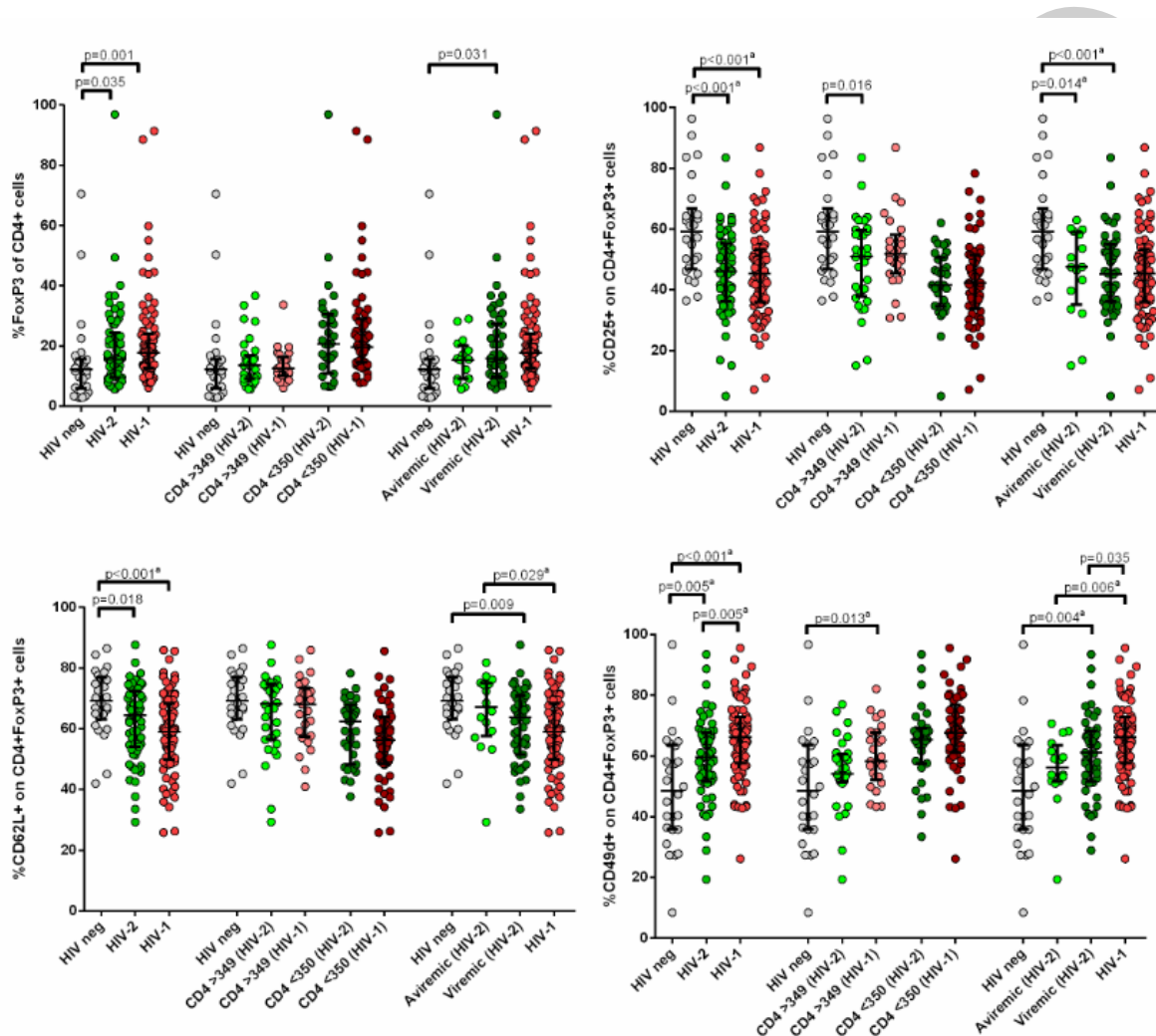
**Figure 2:** SPICE analysis of T cell maturation distribution. #1 HIV negative, #2 HIV-1, #3 HIV-2. CD8+ T-cell maturation. AG: antigen experienced. #: significant difference between groups.



**Figure 3:** T cell activation markers on CD8+ T-cells in HIV-2 compared to HIV-1 infected patients. P-values for difference in proportions of CD8+ T-cells activation markers calculated with linear regression analyses with adjustment for age, sex and CD4+ T cell count.



**Figure 4:** Regulatory (CD3+CD4+FoxP3+) T cells in HIV negative, HIV-1 and HIV-2 infected individuals. Groups were compared with Wilcoxon ranksum tests and crude p-values are presented. P-values significant after linear regression analyses adjusting for sex and age are noted with <sup>a</sup>.



**Figure 5:** Proportion of B-cells without the surface marker CD21 (CD21<sup>low</sup>) on PBMCs from HIV negative individuals, HIV-1 and HIV-2 infected patients. Data presented with medians and interquartile range bars. Wilcoxon ranksum tests were used to compare groups, and adjustment (<sup>a</sup>) for sex and age were performed using linear regression analyses.

