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# The plasma levels of soluble ST2 as a marker of gut mucosal damage in early HIV infection

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**Objective:** Following tissue barrier breaches, interleukin-33 (IL-33) is released as an 'alarmin' to induce inflammation. Soluble suppression of tumorigenicity 2 (sST2), as an IL-33 decoy receptor, contributes to limit inflammation. We assessed the relationship between the IL-33/ST2 axis and markers of gut mucosal damage in patients with early (EHI) and chronic HIV infection (CHI) and elite controllers.

**Design:** Analyses on patients with EHI and CHI were conducted to determine IL-33/sST2 changes over time.

**Methods:** IL-33 and sST2 levels were measured in plasma. Correlations between sST2 levels and plasma viral load, $CD4^+$ and $CD8^+$ T-cell counts, expression of T-cell activation/exhaustion markers, gut mucosal damage, microbial translocation and inflammation markers, as well as kynurenine/tryptophan ratio were assessed.

**Results:** Plasma sST2 levels were elevated in EHI compared with untreated CHI and uninfected controls, whereas IL-33 levels were comparable in all groups. In EHI, sST2 levels were positively correlated with the $CD8^+$ T-cell count and the percentage of T cells expressing activation and exhaustion markers, but not with viral load or $CD4^+$ T-cell count. Plasma sST2 levels also correlated with plasma levels of gut mucosal damage, microbial translocation and kynurenine/tryptophan ratio and for some markers of inflammation. Prospective analyses showed that early antiretroviral therapy had no impact on sST2 levels, whereas longer treatment duration initiated during CHI normalized sST2.

**Conclusion:** As sST2 levels were elevated in EHI and were correlated with $CD8^+$ T-cell count, immune activation, and microbial translocation, sST2 may serve as a marker of disease progression, gut damage and may directly contribute to HIV pathogenesis.



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## Introduction

Among interleukin (IL)-1 cytokine family members, IL-33 is an inflammation-induced factor with dual functions; exercising its role as an intracellular regulator of gene expression and as an extracellular alarm mediator, also considered as a damage-associated molecular pattern. This nuclear cytokine acts as an 'alarmin' in response to cellular damage induced by infection or stress at barrier sites, such as the skin, lungs, and gut [1–3]. Intracellular IL-33, which is constitutively expressed by fibroblasts, endothelial, and epithelial cells contribute to the maintenance of mechanical barriers [4,5]. Among hematopoietic cells, IL-33 is constitutively expressed by myeloid cells and contributes to initiate the differentiation of helper T cells (Th1, Th2) as the adaptive immune response is triggered [6,7]. The biologically active form of IL-33 binds to a receptor complex consisting of suppression of tumorigenicity 2 (ST2) and the IL-1 receptor accessory protein [6]. ST2 exists in two isoforms with opposing biological effects. The full-length transmembrane isoform, ST2L acts as a mediator of IL-33 activity at the site of damage whereas the soluble ST2 (sST2) isoform restricts the 'off target' systemic effects of IL-33 [8,9].

Recently, Molofsky *et al.* [10,11] further delineated the role of the IL-33/ST2 axis in the loss of epithelial integrity caused by microbial invasion of the mucosal barrier in mice. Following an acute infection, IL-33 synergizes with other epithelial cytokines and chemokines to restore damaged tissue. However, during persistent infection, a 'conversion phase' occurs during which a massive release of active IL-33 overwhelms the negative regulation induced by sST2, leading to responses by Th1 cells and by cytotoxic $CD8^+$ T cells and natural killer cells; eventually, these responses contribute to tissue fibrosis [10–12]. As the IL-33/ST2 axis is a key regulator of acute and local inflammation as well as the tissue repair process, it is therefore, considered a prognostic marker in various conditions, such as cardiac insufficiency, atherosclerosis, ulcerative colitis, Crohn's disease, and graft-versus-host disease (GVHD) [13–15].

During HIV infection, gut barrier dysfunction, and immune activation have been identified as independent predictors of morbidity and mortality [16]. We and others have reported that even when antiretroviral therapy (ART) is initiated early, plasma levels of gut damage markers remained elevated [17–19]. Given that sST2 is elevated in inflammatory conditions and that it plays a role in T-cell activation as well as in epithelial tissue repair/damage, we assessed the plasma levels of IL-33 and sST2 in relation with the early and chronic phases of infection, influence of ART initiation, and the association with the level of T-cell activation and exhaustion, gut epithelial damage, and microbial translocation.

## Methods

### Study population

In this cross-sectional study, 153 HIV-1-infected adults and 20 uninfected controls (UCs) were studied. Among these participants, 48 were in the early stage of HIV infection (EHI), defined as being within 180 days of the estimated date of infection; these patients were enrolled in the Montreal Primary HIV Infection Study Group [17]. We also assessed chronically HIV-infected (CHI) patients who were either untreated ($n = 61$) or treated ($n = 23$). Twenty-one elite controllers having undetectable plasma viral load in the absence of ART [from the Canadian Long-Term Non-Progressors (LTNP) Study Group] and 20 uninfected controls (UCs) were also included.

We prospectively assessed 41 EHI patients, of whom 17 had initiated ART within the first 6 months of infection for a median duration of 1 year, while the remaining EHI patients were untreated. Twenty CHI patients had been receiving ART for at least 2 years with a median duration of 34 months.

### Quantification of IL-33, soluble suppression of tumorigenicity 2, gut damage, and microbial translocation markers

Plasma levels of IL-33 and of sST2 were measured using the Quantikine ELISA (R&D Systems, Minneapolis, Minnesota, USA). To validate the ELISA measurements of IL-33, IL-33 mRNA expression was also measured by real time quantitative polymerase chain reaction (qPCR) in randomly selected patients in each sub-group (EHI, 9; CHI, 18; CHI-ART, 9; EC, 6 and UCs, 6). In brief, total RNA was extracted using RNeasy Mini kit (Qiagen, Hilden, Germany), and converted to cDNA using the Moloney murine leukemia virus Reverse Transcriptase (Life Technologies Inc., Burlington, Ontario, Canada). The cDNA was subjected to qPCR using LightCycler 2.0 Instrument – Roche, SYBR Green I master mix (Roche Diagnostics, Basel, Switzerland) as previously reported [20]. Intestinal-type fatty acid-binding protein (I-FABP), lipopolysaccharide (LPS), and soluble CD14 (sCD14) were measured in duplicate using commercially available ELISA kits from Hycult Biotech (Uden, the Netherlands), Cusabio (Wuhan, China), and R&D Systems, respectively. Optical densities were measured using the Biotek EL-800 plate reader (Winooski, Vermont, USA).

### Multiplex quantification of soluble inflammatory markers

Plasma IFNγ, TNFα IL-6, IL-10, and sCD40L were measured in duplicate using MAGPIX xMAP magnetic Beadlyte technology (Luminex Corp., Ontario, Canada) and the MILLIPLEX-MAP kit, according to the manufacturer's instructions (Millipore, Billerica, Massachusetts, USA). Mean fluorescence intensities for each analyte in each sample were detected using the MAGPIX instrument (Luminex); results were analyzed using the

xPONENT 4.2 software to determine the protein concentration of each soluble factor.

### Quantification of the kynurenine/tryptophan ratio as a measure of indoleamine-2,3-dioxygenase enzyme activity

Plasma levels of tryptophan (Trp) and of its immunosuppressive catabolite kynurenine (Kyn) were measured using an automated on-line solid-phase extraction-liquid chromatographic-tandem mass spectrometric method as previously reported [21]. Indoleamine-2,3-dioxygenase (IDO) enzymatic activity was determined using the plasma Kyn/Trp ratio.

### Measurement of markers of T-cell activation and exhaustion

Surface markers were analyzed using a four-laser LSRII flow cytometer (BD Bioscience, Mississauga, Ontario, Canada). The following mAbs were used: anti-CD3-Pacific blue, anti-CD4-PercpCy5.5, anti-CD8-Alexa700, anti-CD38-APC, anti-human leukocyte antigen-D related (HLA-DR)-APC.Cy7, and anti-programmed cell death 1 (PD-1)-FITC (BD Bioscience, Mississauga, Ontario, Canada). The viability marker Vivid (Invitrogen, Burlington, Ontario, Canada) was used to exclude dead cells. Data were analyzed using FlowJo software v9.6.4 (Ashland, Oregon, USA).

### Ethical considerations

Ethics approval was obtained from the Research Ethics Board of each participating institution. Participants provided written informed consent and their data were anonymized.

### Data management and statistical analyses

Patient characteristics were collected and managed using Excel software, GraphPad Prism (version 6: La Jolla, California, USA) was used to perform statistical analyses. Quantitative variables were summarized as means and SDs, or as medians and interquartile ranges. Proportions were calculated for the categorical variables. Unpaired *t*-tests or Mann–Whitney *U* tests were used, as appropriate, for comparisons of two unpaired study measures. Wilcoxon matched pairs test was used to compare paired study measures and the Spearman rank correlation test to identify association between two study measures. The Kruskal–Wallis test was used to compare multiple study groups and a 5% level of statistical significance was considered for all the analyses. The contribution of age, sex, BMI, creatinine, and total cholesterol to sST2 plasma levels were investigated to avoid bias.

## Results

### Study participant characteristics

Forty-eight EHI, 61 untreated CHI, 23 treated CHI patients, 21 elite controllers, and 20 UCs were studied. At the baseline, four of the EHI patients were classified in Fiebig stages I–IV, 10 in stage V and 34 in stage VI [22]. The majority of the study subjects were men ($n = 140$, 81%) and the mean (±SD) age was $38.1 \pm 10.1$ years (Table 1).

EHI patients had higher baseline $CD4^+$ T-cell counts compared with untreated CHI ($544 \pm 269$ vs. $331 \pm 170$ cells/μl; $P < 0.001$), although both groups had similar baseline $CD8^+$ T-cell counts ($927 \pm 505$ vs. $858 \pm 503$ cells/μl; $P = 0.485$) (Table 1). Furthermore, EHI patients had a lower baseline mean viral load compared with untreated CHI patients ($4.28 \pm 1.08$ vs. $4.75 \pm 0.93$ $\log_{10}$ copies/ml; $P = 0.030$).

**Table 1. Demographic and clinical characteristics of study participants ($n = 173$).**

| Characteristics | EHI $n = 48$ | CHI $n = 61$ | CHI-ART $n = 23$ | Elite controllers $n = 21$ | UCs $n = 20$ |
|---|---|---|---|---|---|
| Age in years | | | | | |
| (Mean ± SD) | 36.1 ± 10.3 | 38.7 ± 8.7 | 45.6 ± 8.3 | 46.4 ± 6.9 | 46.4 ± 7.2 |
| Range | 19–57 | 21–61 | 29–62 | 39–62 | 30–61 |
| Sex | | | | | |
| Men, n (%) | 47 (97.9) | 49 (80.3) | 17 (73.9) | 14 (66.7) | 13 (65.0) |
| Women, n (%) | 1 (2.1) | 12 (19.7) | 6 (26.1) | 7 (33.3) | 7 (35.0) |
| $CD4^+$ T-cell count (cells/μl) | | | | | |
| (Mean ± SD) | 544 ± 269 | 331 ± 170 | 610 ± 248 | 678 ± 246 | 913 ± 304 |
| Range | 220–1680 | 3–755 | 267–1177 | 440–1341 | 281–1559 |
| $CD8^+$ T-cell count (cells/μl) | | | | | |
| (Mean ± SD) | 927 ± 505 | 858 ± 503 | 718 ± 283 | 582 ± 339 | 450 ± 155 |
| Range | 279–2590 | 272–3496 | 245–1381 | 162–1193 | 227–843 |
| $CD4^+/CD8^+$ ratio | | | | | |
| (Mean ± SD) | 0.70 ± 0.47 | 0.46 ± 0.30 | 0.92 ± 0.34 | 1.34 ± 0.64 | 2.23 ± 0.86 |
| Range | 0.16–2.76 | 0.01–1.20 | 0.44–1.55 | 0.69–2.72 | 0.38–3.97 |
| Viral load, $\log_{10}$copies/ml | | | | | |
| (Mean ± SD) | 4.28 ± 1.08 | 4.75 ± 0.93 | <1.7 | <1.7 | NA |
| Range | 1.25–7.48 | 2.89–6.21 | NA | NA | NA |

ART, antiretroviral therapy; CHI, chronic HIV infection; EHI, early HIV infection; NA, not applicable; UCs, uninfected controls.

### Assessing IL-33 levels in plasma and IL-33 mRNA transcripts in peripheral blood mononuclear cells during the early and chronic stages of HIV infection

The plasma level of IL-33 for each participant was low and just above the level of detection as previously reported in several autoimmune disorders, including atopic dermatitis and Sjögren syndrome [23,24]. Furthermore, the mean levels did not vary between groups, including the UCs (Fig. 1a). To further ascertain that the IL-33 levels did not differ among groups, we measured the IL-33 mRNA expression levels in peripheral blood mononuclear cells (PBMC) by qPCR in 48 randomly selected study participants in each group and in line with IL-33 plasma levels; no differences were observed (Fig. 1b). Both ELISA and qPCR results showed the consistent low levels of IL-33 in all groups.

### Assessing plasma soluble suppression of tumorigenicity 2 levels during early and chronic HIV infection

sST2 levels were higher in EHI vs. CHI ($18\,538 \pm 6390$ vs. $15\,115 \pm 6174$ pg/ml, $P = 0.003$) (Fig. 1c). Plasma levels of sST2 in treated CHI and in elite controllers were similar to levels measured in UCs ($13\,394 \pm 6111$ and $14\,850 \pm 6366$ pg/ml vs. $11\,684 \pm 4507$ pg/ml, respectively; $P > 0.05$).

Elevated sST2 levels were also observed to be higher in men vs. women study participants ($16\,097 \pm 6311$ vs. $9107 \pm 4797$ pg/ml; $P < 0.001$) as reported previously [25,26]. The overall sST2 levels in untreated HIV (EHI + CHI) patients were higher than controls ($16\,758 \pm 6479$ vs. $11\,684 \pm 4507$ pg/ml; $P = 0.001$). To confirm the independent association of plasma sST2 levels with HIV infection in this cross-sectional study, we performed a multivariable analysis adjusting for age, sex, BMI, and the levels of creatinine and total cholesterol (data not shown). Prospective analysis of a subgroup of EHI patients ($n = 17$) showed that a median of 12 months of ART initiated early (i.e. during the first 6 months of infection) had no impact on plasma sST2 levels (Fig. 1d). However, after a median duration of 34 months of ART, sST2 levels decreased in CHI patients ($n = 20$; $P < 0.001$) (Fig. 1e).

### Assessing potential correlations between plasma soluble suppression of tumorigenicity 2 levels, plasma viral load, $CD4^+$ and $CD8^+$ T-cell count, and $CD4^+/CD8^+$ ratio

We did not observe any correlation between sST2 plasma levels with viral load, $CD4^+$ T-cell count or the $CD4^+/CD8^+$ ratio during EHI ($P > 0.05$) (Fig. 2a, b and d). However, sST2 levels were positively correlated with the $CD8^+$ T-cell count ($r = 0.289$; $P = 0.045$) (Fig. 2c). The correlation with $CD8^+$ T-cell count was not observed for CHI (data not shown). This suggests a link between sST2 levels and immune response involving $CD8^+$ T-cell expansion during EHI. In addition, we investigated whether using IL-33/sST2 ratio may represent a better marker of disease progression [27]; and this ratio did not improve the link with disease progression (data not shown).

### Assessing potential correlations between plasma soluble suppression of tumorigenicity 2 levels and T-cell activation and exhaustion markers

As the $CD8^+$ T-cell count correlated with plasma sST2 levels in EHI, we assessed whether there was a correlation between plasma sST2 levels and the percentage of $CD4^+$ and $CD8^+$ T cells expressing the T-cell activation markers HLA-DR and CD38. Plasma sST2 levels correlated positively with both the percentage of activated $CD4^+$ T cells ($r = 0.486$; $P = 0.001$) and with the percentage of activated $CD8^+$ T cells ($r = 0.362$, $P = 0.022$) (Fig. 3a and c). Elevated sST2 levels were also correlated positively with the percentage of $CD4^+$ T cells expressing PD-1 ($r = 0.400$; $P = 0.01$), but not with the percentage of $CD8^+$ T cells expressing PD-1 (Fig. 3b and d). Thus, the immune response following acute infection characterized by a strong T-cell activation and exhaustion was associated with elevated sST2 levels.

### Assessing potential correlations between soluble suppression of tumorigenicity 2 levels and markers of gut damage, microbial translocation and inflammation, and indoleamine-2,3-dioxygenase enzyme activity

During EHI, plasma sST2 levels correlated with plasma levels of the epithelial gut damage marker I-FABP ($r = 0.2998$; $P = 0.043$) (Fig. 4a), the monocyte activation marker sCD14 ($r = 0.3688$; $P = 0.013$) (Fig. 4b), the immune activation marker sCD40L (0.3553; $P = 0.016$) (Fig. 4c), IFNγ ($r = 0.3454$; $P = 0.036$) (Fig. 4d), and plasma IDO activity as measured by the Kyn/Trp ratio ($r = 0.3239$; $P = 0.026$) (Fig. 4e). Conversely, plasma sST2 levels were not correlated with plasma levels of LPS, TNFα, IL-6, and IL-10 levels (data not shown). Such findings evoke that the IL-33/ST2 axis 'had rung the alarm' in response to the gut damage.

## Discussion

IL-33, a member of the IL-1 family of cytokines is involved in a variety of biological functions, including its role as a guardian of skin and mucosal barriers. However, IL-33 has recently been shown to contribute to the fibrotic reaction in lung and intestinal mucosa over the course of chronic insult/infection [4]. IL-33 function is exerted via the ST2L receptor, which is highly expressed on innate lymphoid cells 2 (ILC2), regulatory T cells (Tregs), and Th2 cells present in skin and mucosal tissues [10,28]. The function of this alarmin is regulated by its decoy receptor, sST2, which inhibits and localizes IL-33

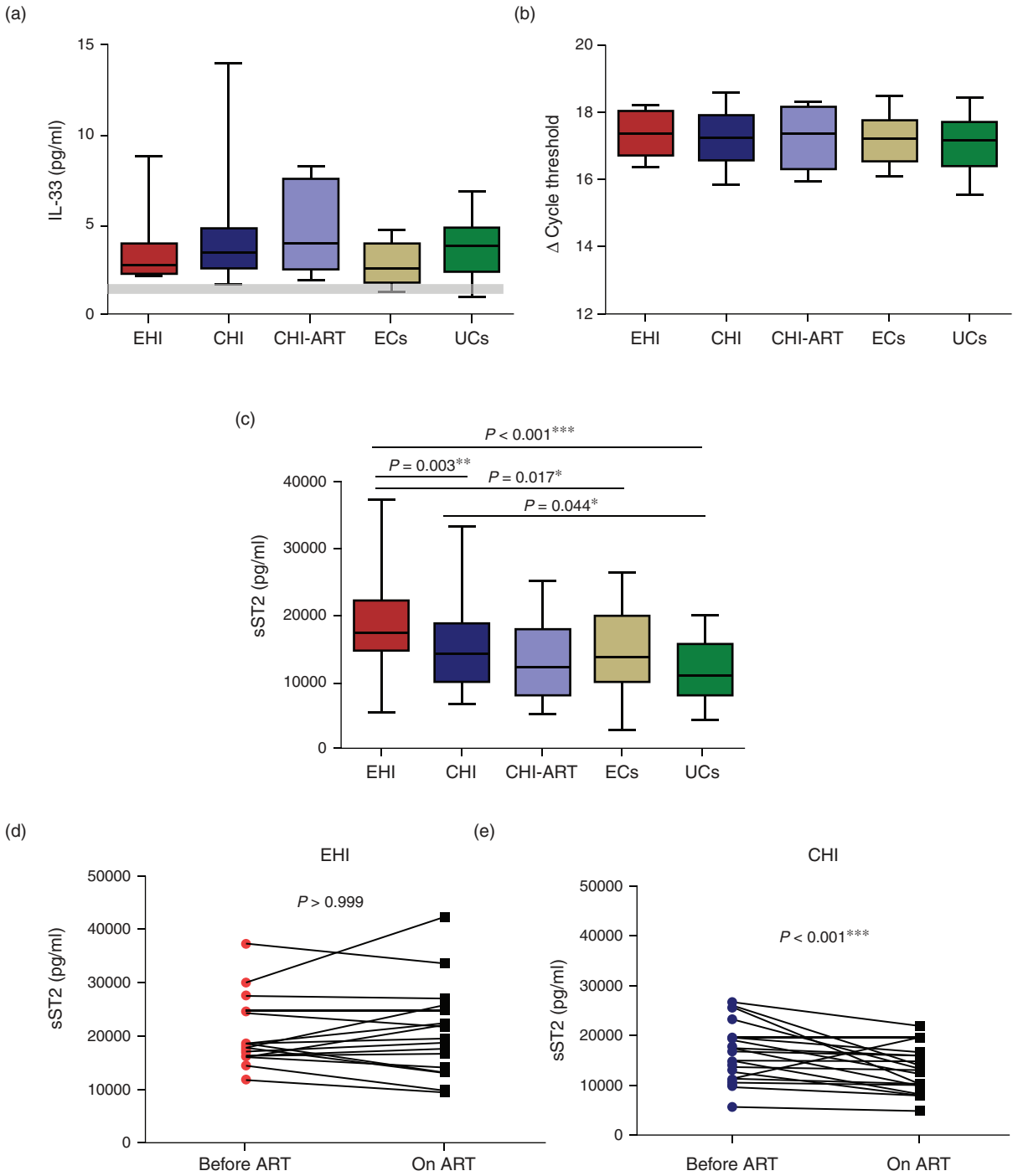


**Fig. 1. IL-33 and sST2 levels during early and chronic HIV infection.** (a) IL-33 levels in plasma. The grey horizontal line represents the minimal detectable dose of IL-33 (b) IL-33 mRNA expression in peripheral blood mononuclear cells (c) sST2 levels in plasma. Box represents the first quartile, median and the third quartile values, whereas the whiskers represent the lowest and the highest values. (d) sST2 levels before and after ART initiation in EHI patients ($n = 17$) (e) sST2 levels before and after ART initiation in CHI patients ($n = 20$). sST2, soluble suppression of tumorigenicity. ART, antiretroviral therapy; CHI, chronic HIV infection; ECs, elite controllers; EHI, early HIV infection; UCs, uninfected controls; Δ Cycle threshold = cycle threshold of IL-33 minus cycle threshold of β-actin mRNA expression. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

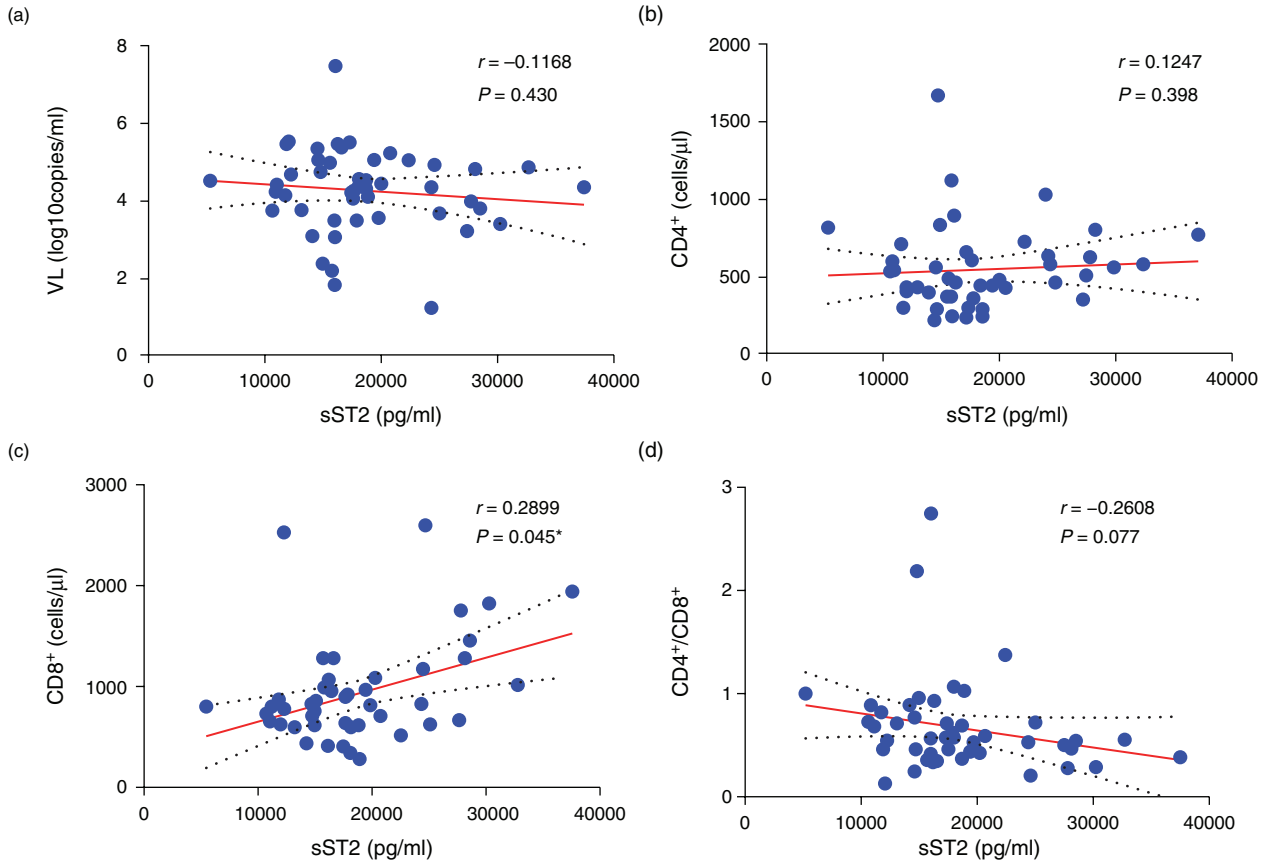


**Fig. 2. Correlation of plasma sST2 levels with the CD8$^+$ T-cell count in early HIV infection.** Correlations between plasma sST2 levels and (a) plasma viral load; (b), CD4$^+$ T-cell count; (c), CD8$^+$ T-cell count; (d), CD4$^+$/CD8$^+$ ratio. sST2, soluble suppression of tumorigenicity.

activity. The level of circulating sST2 mirrors IL-33 cellular expression and increases in response to tissue necrosis and barrier damage/repair; as such, plasma sST2 has been validated as a prognostic marker in cardiac insufficiency, colitis, and GVHD [8,14,15].

As HIV infection is associated with early onset of gut mucosal damage [29], we assessed the changes in the plasma levels of IL-33 and of sST2 during early and chronic infection and investigated whether plasma sST2 levels correlated with markers of microbial translocation and disease progression. The plasma IL-33 levels measured by ELISA were just above the level of detection in all the study subjects; these levels were validated by assessing IL-33 mRNA expression in PBMCs. Similarly, low IL-33 levels have also been reported in several autoimmune disorders [23,24] and in hepatitis B virus infection in which increased sST2 production predicted mortality [30].

We reported increased plasma sST2 levels during EHI and to a lesser extent in untreated CHI as compared with UCs. Conversely, sST2 levels were not elevated in patients receiving ART during CHI. These findings confirm and expand upon the first report of elevated sST2 in 26 HIV-infected individuals with advanced disease; these individuals also had reduced levels of IL-33 [31]. Our results are also consistent with those reported by Secemsky *et al.* [32] whose study revealed that treated CHI patients had similar sST2 levels compared with controls. Despite nonelevated ST2 levels, this marker remained a predictor of cardiac insufficiency and all-cause mortality in ART-treated patients [32]. We further showed that elite controllers had normal sST2 levels that may reflect low levels of immune activation, preservation of Th17 cells in the blood, and gut tissue with an absence of increased microbial translocation in such distinct population [33,34].

Our cross-sectional study revealed that plasma levels of sST2 increased during the EHI and were associated with certain soluble markers of immune activation, including IFNγ, sCD40L, I-FABP, sCD14, and Kyn/Trp ratio. Interestingly, when ART was initiated during the first 6 months of infection, no change was observed for plasma sST2 levels in patients followed since EHI; these participants had a median of 12-months of treatment. The persistently elevated plasma sST2 levels that are

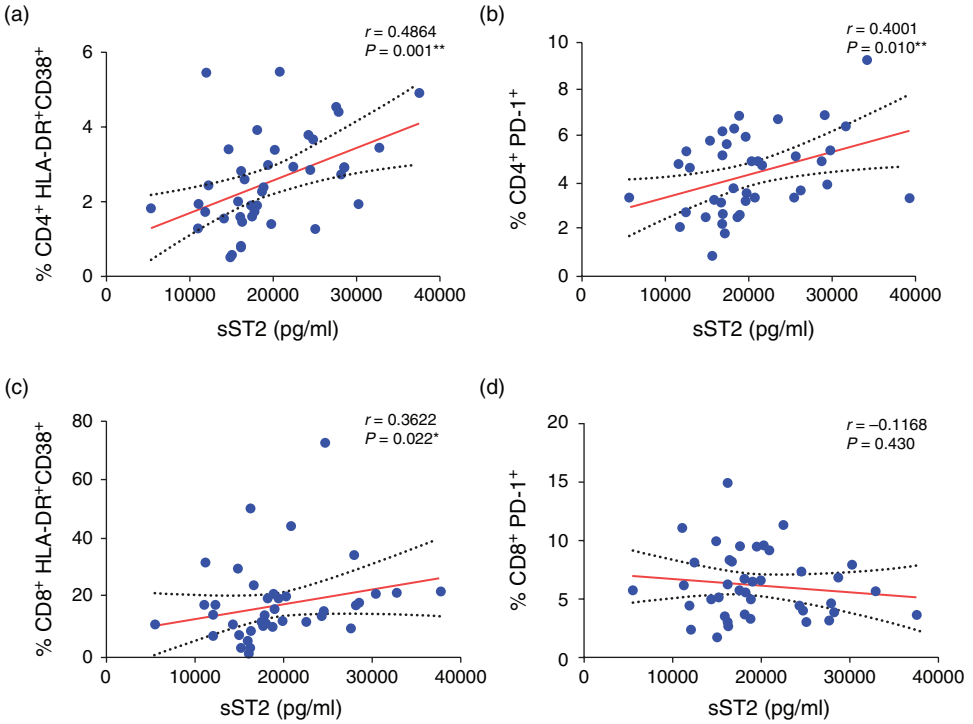


**Fig. 3. Correlation of plasma sST2 levels with expression of T-cell activation and exhaustion markers in early HIV infection.** Correlations between plasma sST2 levels and the percentage of (a) $CD4^+$ T cells expressing HLA-DR and CD38; (b) $CD4^+$ T cells expressing PD-1; (c) $CD8^+$ T cells expressing HLA-DR and CD38; (d) $CD8^+$ T cells expressing PD-1. sST2, soluble suppression of tumorigenicity. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

observed in early HIV treatment parallels the increased levels observed for other markers of gut mucosal damage in contrast to the normalization of T-cell activation markers that occurs following early ART initiation, as we previously reported [17]. Conversely, when ART initiated during CHI continued for a median of 34 months, plasma sST2 levels normalized. These chronically infected patients had no AIDS defining events and had a mean $CD4^+$ T-cell count of $333 \pm 170$ cells/μl. These observations suggest that during the chronic phase of infection, ART contributes to partially repair the damaged gut and/or to the control of viral replication. During this phase, it is also possible that IL-33 function may have switched to a tissue-repairing role (amplification phase) from a tissue-damaging role (conversion phase) leading to fibrosis [11].

Although inflammatory cytokines are elevated during the acute phase of HIV infection as viremia peaks and $CD4^+$ T-cell counts drop, we did not find any correlations between sST2 levels and either viral load or $CD4^+$ T-cell count. However, we identified a positive correlation between sST2 levels and $CD8^+$ T-cell count. Interestingly, as a tissue-derived signal, IL-33 is an inducer of $CD8^+$ T cells contributing to clonal expansion, differentiation, and cytotoxic function [35]. Bonilla *et al.* [36] showed in the lymphocytic choriomeningitis virus infection mouse model that IL-33, independently of pathogen-associated molecular patterns, primes memory $CD8^+$ T cells for cytotoxicity leading to viral control. In line with the early tissue damage, IL-33/ST2 axis was shown to be elevated at the early stage of acute pancreatitis, correlated with disease severity, and normalized over time [37]. Globally, these findings suggest that IL-33 during the 'conversion phase' induces cytotoxic function at tissue barrier and contributes to the differentiation of noncirculating tissue-resident memory $CD8^+$ T cells [18,35].

We observed correlations between sST2 levels and the percentage of $CD4^+$ and $CD8^+$ T cells expressing the immune activation markers (HLA-DR and CD38). Surprisingly, the correlation of sST2 was stronger with $CD4^+$ than $CD8^+$ T cells. This observation can be explained by the substantial $CD8^+$ T-cell death occurring during the contraction phase that follows peak of viremia during acute infection [38,39]. Similarly, we found a significant correlation between plasma sST2 levels and the percentage of $CD4^+$ $PD\text{-}1^+$ T cells in contrast to the absence of a correlation with percentage of $CD8^+$ $PD\text{-}1^+$ T cells. PD-1 expression by $CD8^+$ T cells during acute infection leads to an effective 'programed cell death' during the contraction phase [40–42]. Such study findings linking sST2 levels to $CD8^+$ T-cell elevation in absence of its association with $CD8^+$ $PD\text{-}1^+$ T cells during EHI can be explained by the global expansion of bystander $CD8^+$ T cells (non-HIV specific) that represent

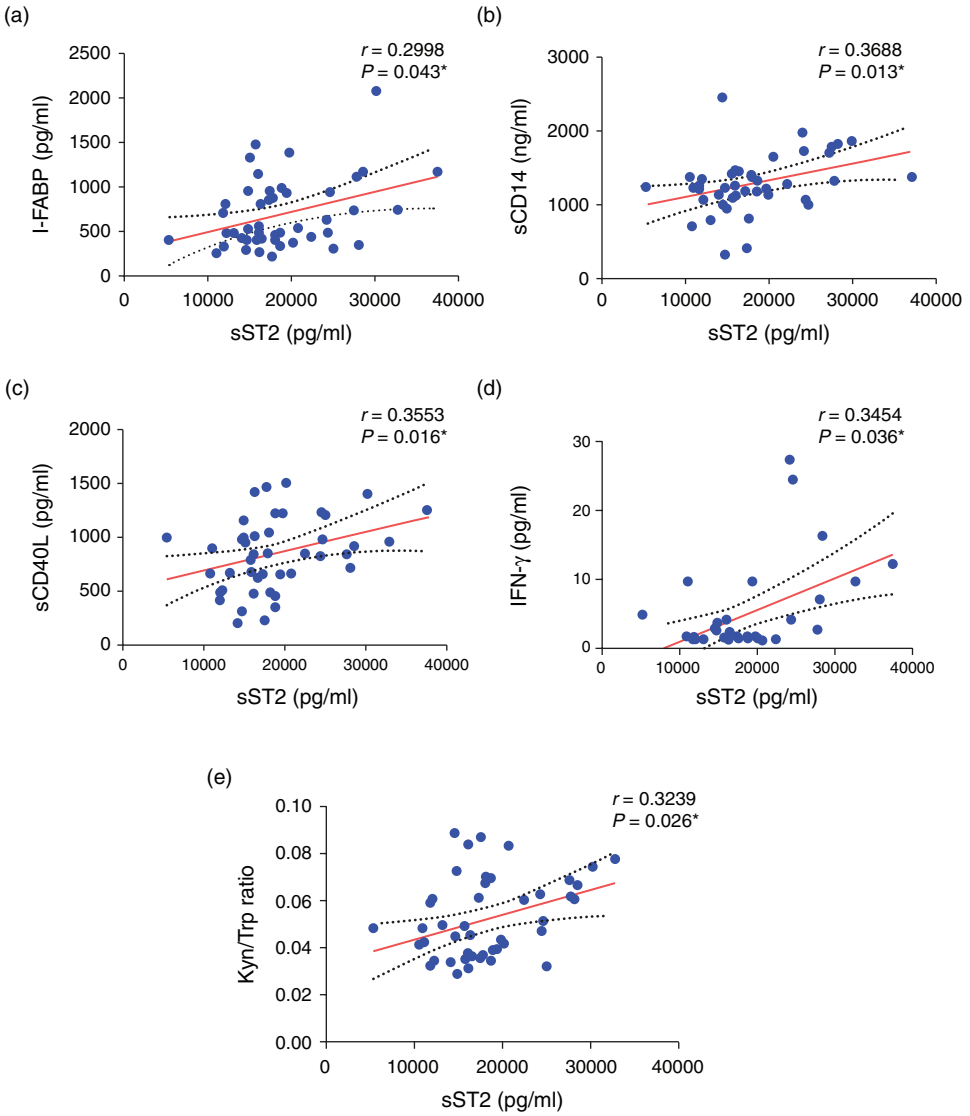


**Fig. 4. Correlation of plasma sST2 levels with markers of gut damage, microbial translocation, inflammation, and IDO enzyme activity in early HIV infection.** Correlations between plasma sST2 levels and the plasma levels of (a) I-FABP, (b) sCD14, (c) sCD40L, (d) IFNγ, (e) the Kyn/Trp ratio. sST2, soluble suppression of tumorigenicity; Kyn, kynurenine; Trp, tryptophan; $^{*}P < 0.05$.

80% of the total $CD8^{+}$ T-cell pool. Such expansion is induced by IL-15 contributing to $CD8^{+}$ T-cell survival via the induction of B-cell lymphoma 2 (Bcl-2) [43]. In contrast, $CD8^{+}$ $PD\text{-}1^{+}$ T cells that correspond to the vast majority of HIV-specific $CD8^{+}$ T cells undergo a contraction phase following acute HIV infection [18,39,41,42]. Importantly, all these relationships between plasma sST2 levels and proportion of the activated or exhausted T cells were lost in CHI (data not shown). Globally, these findings highlight the distinctive contribution of the IL-33/ST2 axis on T-cell population during the EHI, which does not persist during the CHI.

EHI leads to a massive depletion of $CD4^{+}$ T cells in the gut and altered mucosal immunity which result in epithelial damage, microbial translocation, and persistent immune activation [44]. As the IL-33/ST2 is considered to be the guardian of the mucosal barrier and given that HIV infection is associated with persistent gut damage despite early ART [17,45], we investigated the link between sST2 and gut mucosal damage. We observed a correlation between plasma sST2 and plasma levels of I-FABP, a marker of intestinal epithelium damage. Such association with plasma sST2 levels was not observed during the chronic phase for two possible reasons: the damage following early infection may be reduced because of a lower viral load and less vigorous cytotoxic T-cell response; the alarmin response may wane over time because of tissue fibrosis.

We did not observe any correlation between plasma sST2 levels and plasma LPS levels in EHI or CHI patients

regardless of whether they were receiving ART. However, it is recognized that LPS is not a predictor of HIV disease progression since LPS levels may vary with fasting or with the technique used to measure it [46,47] but its coreceptor, sCD14, which is released from activated monocytes has been linked to morbidity and mortality in HIV infection [48]. Interestingly, we observed a positive correlation between plasma sST2 levels and plasma sCD14 levels in EHI, which may be because of microbial translocation following epithelial gut damage and/or because of immune activation resulting from other causes [49].

HIV immune dysfunction has also been linked to IDO-1, the Trp catabolic enzyme. This enzyme is an immune checkpoint that is involved in induction of Tregs and inhibition of the effector T-cell functions [21,50,51]. We assessed the ratio of the plasma levels of Kyn/Trp ratio as a measure of IDO enzyme activity. We found a positive correlation between plasma sST2 levels and the Kyn/Trp ratio, which suggests that immune activation may be induced by metabolites generated either by gut microbes and/or by IFNγ following systemic viral infection [52,53]. We have previously shown that IDO activity is mediated by sCD40L [54], another marker of immune activation expressed by activated T cells [55] and a strong inducer of B cells and antigen presenting cells [56]. Among different markers of inflammation assessed herein, we observed a strong correlation of sST2 levels with sCD40L in EHI. This observation is clinically relevant as both sST2 and sCD40L plasma levels predicted cardiovascular diseases [32,57], an important cause of morbidity and mortality in HIV patients.

We subsequently assessed whether a correlation existed between sST2 and inflammatory markers IFNγ, TNFα, IL-6, and IL-10 measured in plasma. A positive correlation was found for IFNγ only. Such association is further supported by the inhibition of ST2 leading to a reduced production of IFNγ in ST2 deficient mice [58] and after administration of anti ST2 monoclonal antibodies in GVHD with an increased production of IL-33 [59]. In addition, IFNγ and IL-33 are known to counter regulate activation of ILC2s to control Tregs and type 2 responses, thus contributing to a shift toward Th1 response [11]. Further investigation is warranted to determine whether a causal link exists between elevated plasma sST2 and IFNγ expression.

Globally, our study showed that levels of sST2 were increased in EHI and correlated with the levels of certain soluble markers of inflammation (IFNγ, sCD40L, and Kyn/Trp ratio), gut damage (I-FABP) and microbial translocation (sCD14), with the percentages of $CD4^+$ and $CD8^+$ T cells expressing immune activation/exhaustion markers, and with $CD8^+$ T-cell count. Our prospective study showed that like other markers of gut damage (I-FABP), sST2 levels were not normalized following early ART initiation, in contrast with the normalized sST2 levels in the chronically infected patients who were treated for a longer duration of time.

We report that sST2 levels decrease over time and with ART in contrast to persistent elevation of gut marker I-FABP and microbial translocation marker sCD14. We speculate based on a model where the antigenicity (T-cell receptor ligation and HIV-specific response) is combined with 'adjuvanticity', the degree to which the immune system is called upon to take action by damage-associated molecular patterns that include IL-33/ST2 axis [60]. During the steady-state phase between the virus and the host and during ART, we hypothesize that the gut damage persists to a lesser extent than in early EHI, decreasing the need to 'sound the alarm'.

An elevation of sST2 may reflect its buffering activity with respect to its ability to inhibit function of IL-33, a cytokine that is released into the circulation during the acute phase of infection where gut damage occurs. This study supports a role for IL-33/ST2 axis during amplification (acute) and conversion (chronic) phases of an infection, as previously described by Molofsky *et al.* [11] in an animal model. During an acute infection, IL-33, an alarmin links inflammation, immune function, and tissue repair, whereas in the case of persistent infection, IL-33 may contribute to gut fibrosis [29,61].

As sST2 levels were elevated in EHI and were correlated with $CD8^+$ T-cell count, immune activation, and microbial translocation, sST2 may be a marker of gut damage and disease progression. Furthermore, sST2 may directly be involved in HIV pathogenesis through its role on T-cell activation, as blockade of ST2 improved the outcome of gut GVHD in a humanized mouse model [59]. Whether and how HIV itself or virally induced cell damage directly regulates IL-33 and ST2 expression in epithelial, myeloid, and lymphoid cells during the different phases of infection need to be further studied. As the role of IL-33 and ST2 is phase dependent, therapeutic strategies need to be rationally designed according to each phase. Therefore, blockade of sST2 should be of therapeutic value during acute phase, whereas blockade of IL-33 should be considered for chronic phase when fibrosis develops in lymphoid tissues [62]. Such therapeutic interventions can benefit from further studies on intracellular and cytoplasmic IL-33 expression during tissue damage in the gut with a focus on epithelial cells, ILC2, and other cells expressing ST2 receptor. Based on our study findings, the IL-33/ST2 axis represents a promising immunotherapeutic target for HIV infection.

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V.M. performed the experiments, analyzed the data, wrote the first draft, and made revisions to the final draft of the manuscript. J.P.R. designed the study, contributed to the data analysis and critically revised the first and the final draft of the manuscript. M.A.J. contributed to the experiments, analyzed the data, and critically reviewed the final draft. R.P., B.L., C.C., R.T., J.G.B., R.L., J.C., C.T., and J.P.R. contributed to patient study participation and critically reviewed the manuscript. All the authors have read and approved the contents of the manuscript.

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## Conflicts of interest

There are no conflicts of interest.

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