# REACTIVE OXYGEN SPECIES (ROS) IN T-CELL SUBSETS OF HIV+CART NAIVE AND HIV+CART+ INDIVIDUALS

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**BACKGROUND:**

The cellular activation and chronic inflammation are a consequences of the increased production of reactive oxygen species (ROS). Identifying and understanding the mechanisms of oxidative stress in HIV infection is an important element of an integrated approach to antiretroviral therapy (cART) monitoring.

## MATERIALS AND METHODS:

- Peripheral blood samples (Li-heparin) were collected from cART+HIV+ with sustained viral suppression and HIVVL<40 copies/ml (A, n=28), cART-HIV+ individuals (B n=10) with HIV VL>1000 copies/ml, and HIV- volunteers (C, n=10) of similar age and sex.
- The viral load was determined in plasma by Abbott real time HIV-1 assay (LLOD 40 copies/mL).
- Direct flow cytometry was used to determine the absolute number (AC) and percentage of CD4+ and CD8+ T lymphocytes (fig.1A).
- We measured ROS levels in cells by incubating CD4 + and CD8 + stained peripheral mononuclear cells (PBMCs) at 37°C for 30 minutes with a sensor, forming fluorescent ROS complex (fig.1B).
- ROS levels were quantified according to the mean fluorescence intensity ($MFI_{ROS}$/100) by flow cytometry (FACSDiva 6.1.2).

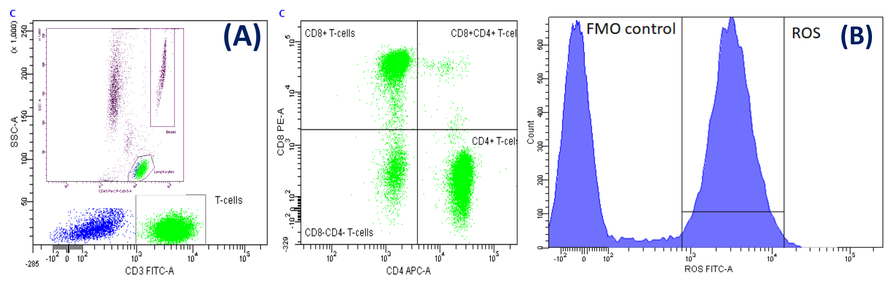


**Fig.1 (A) Flow cytometry gating strategy for CD4+ and CD8+ T-cell subsets. Lymphocytes were initially gated on side scatter properties and CD45 expression (upper left panel), T cells were then gated on the expression of CD3 (left panel), and further subdivided into CD4+ and CD8+ T cells (middle panel). (B) Flow cytometric determination of intracellular ROS in CD8+ and CD4+ T cells using a ROS sensor. MFI was measured in the FITC-channel. A Fluorescence Minus One (FMO) control was used to set markers for the ROS-positive population.**

## AIM:

**To compare intracellular ROS in CD4+ and CD8+ T-cells of cART-naive HIV+ individuals (cART-HIV+) to those on continuous cART (HIV+cART+), with suppressed HIV viral load (VL), and to HIV-negative healthy volunteers.**

## RESULTS:

No difference in CD4 AC was found between groups A and C in contrast to group B (935±261 vs. 866±434, p=0.66 vs. 422±296, p<0.01). The CD4/CD8 ratio in both patients' groups was lower as compared to group C (1.4±0.4 and 0.5±0.4, vs.2.4±0.8,p<0.001) (fig2.A). $MFI_{ROS}$ in CD4+T was significantly higher in both HIV+ groups as compared to C (28.8±12.3 and 44.3±23.6 vs. 18.3±7.9,p<0.01 for both) (fig2.B). $MFI_{ROS}$ in CD8+T was not significantly different between groups A and C (30.6±11.9 vs. 22.9±11.6 p=0.11) while in group B we observed significantly higher levels (40.8±16.5,p<0.01) fig2.C). Noteworthy, $MFI_{ROS}$ in CD4+T correlated positively with HIV VL (R=0.4,p<0.01) and inversely with CD4/CD8 ratio (R=-0.4,p<0.01) (fig2.D), unlike $MFI_{ROS}$ in CD8+T.

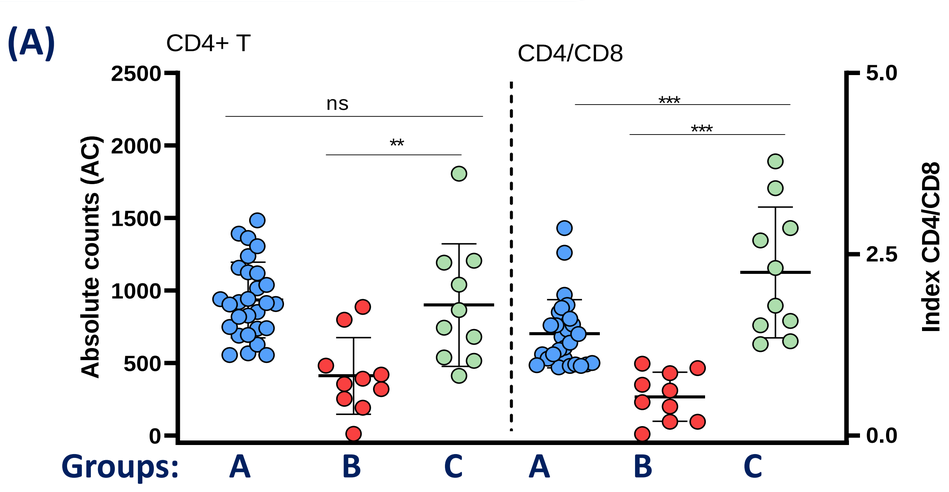


**Figure 2. (A): Absolute count of CD4+ cells and CD4/CD8 ratio in HIV+ individuals on continuous cART with suppressed viral load (group A), HIV+ cART-naive people with detectable VL (group B) and HIV-negative volunteers (group C);**

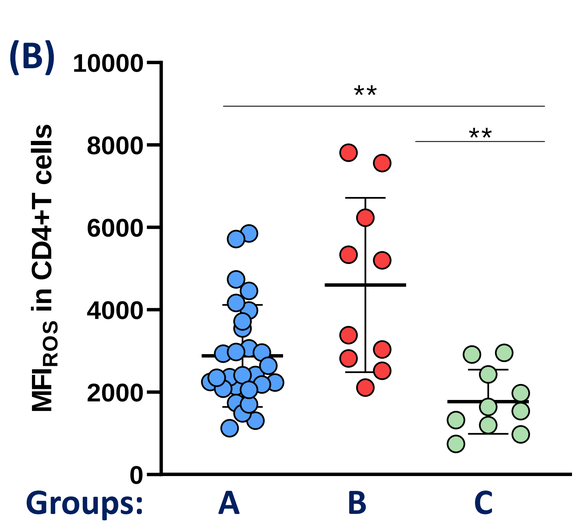


**Figure 2.(B): $MFI_{ROS}$ in CD4+T-cells in group A, group B and group C, unpaired ANOVA, p<0.01**

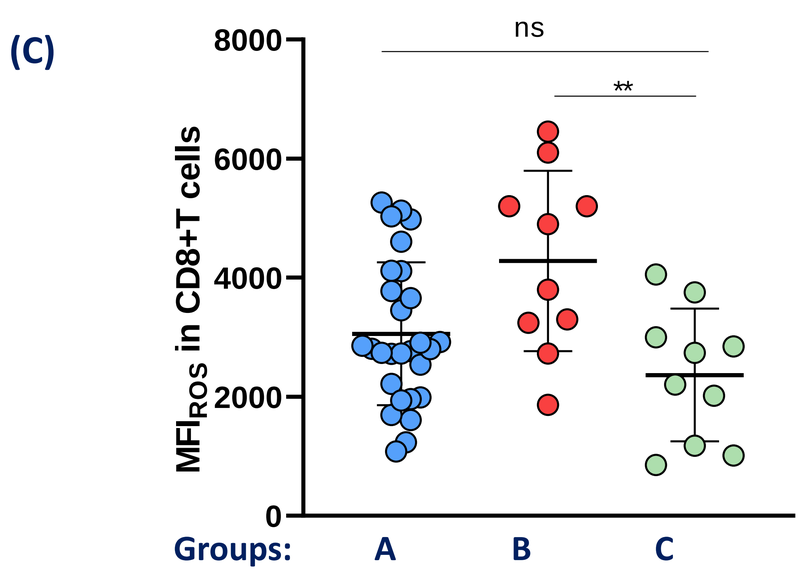


**Figure 2.(C): $MFI_{ROS}$ in CD8+T-cells in group A, group B and group C unpaired ANOVA, p<0.01**

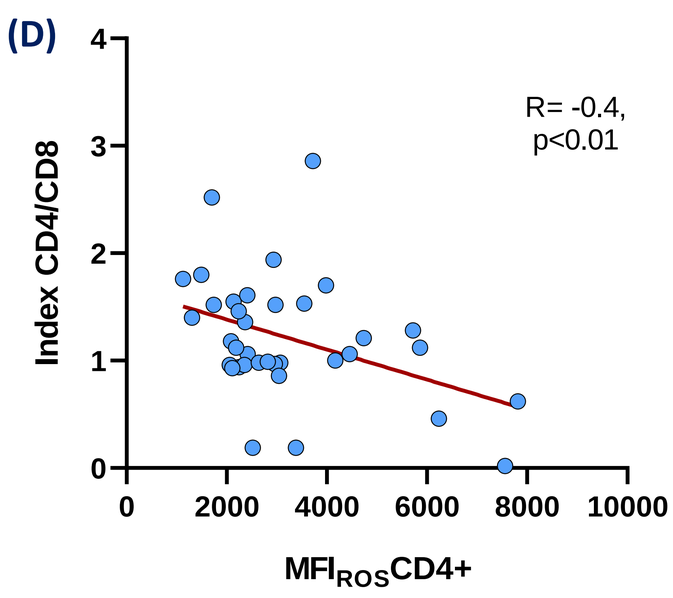


**Figure 2.(D) Correlation between $MFI_{ROS}$ in CD4+T and CD4/CD8 ratio in HIV+ individuals.**

## CONCLUSIONS:

$MFI_{ROS}$ in CD4+T production may be an indicator of residual HIV activity in the settings of undetectable HIV VL. A better understanding of the relationship between ROS in CD8 and CD4 T cells could lead to improved cART monitoring.

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