

Association between CD8 T-cell subsets and CD4/CD8 ratio with HS-CRP level in HIV-infected patients on antiretroviral therapy

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Abstract. Due to improved access and adherence to antiretroviral therapy (ART), most HIV-infected persons worldwide are predicted to live longer. Nowadays the cause of death for most HIV-infected persons has changed to serious non-AIDS events (SNAEs) which is due to low-grade viremia. HIV patients with ART who had undergone CD4 cell count above 500/uL and there is an increase in hs-CRP despite an undetectable viral load. Some conditions CD8 cells count do not decrease with CD4 cells repairs. We researched in Prof Kandou General Hospital with a total sample of 35 HIV patients who had received ART with the level of CD4>350/uL. CD8 levels, CD4/CD8 ratio, and hs-CRP were assessed. This research is analytic descriptive with cross-sectional study design and analysis uses Spearman correlation. The mean CD8 during the study was 1291.8 (IQR 319-2610cells/uL), the mean ratio of CD4:CD8 was 0.57 (IQR 0.16-1.24) and median hs-CRP is 2.18 (IQR 0.3-6.6mg/dL). There was a significant positive correlation between CD8 and increased hs-CRP ($r=0.369$, $p<0.05$). There was a negative correlation between CD4/CD8 ratio and hs-CRP ($r=-0.370$, $p<0.05$).

1. Introduction

Due to improved access and adherence to ART, most HIV-infected persons worldwide are predicted to live longer, healthier lives.[1] However, recent studies have identified that treated efficiently HIV-infected patients do not live as long as age-matched individuals without HIV.[2] The cause of death for most HIV-infected persons has changed from AIDS-related opportunistic infections to chronic diseases with an inflammatory pathogenesis usually associated with the elderly.[3] There is an association between premature onset and increased risk of these age-related inflammatory diseases and low points of chronic immune activation that persist during ART treatment, a process that is believed to contribute to SNAEs such as cancer, cardiovascular diseases, kidney and liver diseases.[4] This phenomenon is due to residual low-grade viremia that not eradicated by ART nowadays, other than that there is also damage to intestinal mucosal barrier so induced endotoxemia from translocation of microbe product that leads to activation immune system.[5,6] The presence of comorbid diseases like diabetes, other infection such as cytomegalovirus also contributes to inflammation.[7]

HIV patients before ART will decrease CD4 cell count and increase CD8 cell, so the ratio CD4/CD8 is low. After treatment, the majority of patients will experience CD4 cell recovery but not followed by CD8 cell decline.[8] There was an increase in inflammatory biomarkers like hs-CRP, IL-6 and others in HIV patients with ART who had undergone CD4 cell count above 500/uL and an undetectable viral load.[9]



According to the study of de Luca, et al. in 2013, hs-CRP may be a useful additional biomarker to predict cardiovascular risk in HIV-infected patients receiving ARV.[10] In previous studies by Helleberg, et al in 2015 showed an association between the persistent elevation of CD8 T-cells with an increased incidence of non-AIDS mortality.[11] Serrano-Villar et al. in 2014 explained that HIV patients receiving antiretroviral therapy had a CD4 >500 cell count but had a low CD4/CD8 ratio. This phenomenon is due to the high activation of CD8 T-cells, senescence, and higher kynurenine/tryptophan ratio, and resulted in increased risk of morbidity and mortality.[12] Another study by Shahbaz, et al. in 2015 showed that there is an association between increased CD8 T-cells and cardiovascular events.[13] Persistent elevation of CD8 T-cells may result from persistent low-level viremia as studied by Chereau et al. in 2017.[14]

Recent studies indicate that inflammatory mediators produced by monocytes predicting SNAEs incidence in HIV-infected persons treated with ART with the undetected viral load.[15] Some conditions of CD8 cells count do not decrease with CD4 cells repairs. Whether the persistent elevated of CD8 cells play a role in the inflammatory process makes it compelling to examine its correlation.

2. Methods

This study was cross-sectional to find a correlation between CD8 T cell, CD4/CD8 ratio and hs-CRP on HIV patients that used ART. The sample is outpatients HIV on ART in Internal Medicine Polyclinic of Prof. dr. R. D. Kandou General Hospital Manado starting from May to August 2017. The sampling method is on a consecutive basis. Immunoflow cytometry was used to measure CD4 T-cells, CD8 T cells use and immunometric assay methods to measure hs-CRP.

The inclusion criteria were asymptomatic HIV patients with CD4 count more than 350/uL, had been taking ARV more than six months and were willing to follow the study. Exclusion criteria were patients with comorbid rheumatoid arthritis, diabetes mellitus, hypertension and taking NSAIDs / statins. Data analysis with Spearman one-tailed correlation using SPSS statistical program version 22. Statistical significance was set $p < 0.05$.

3. Results

The total sample is 35 patients with the proportion of male sample was more than female which is 66%:34%. Only eight patients (22.8%) were ≥ 40 years. The average age group of the sample was 32.3 ± 7.7 (20-47) years. Mean length of use ARV was 43.29 ± 38.08 (6-125) months. Mean value of CD4 cells was 600.6 ± 244 /uL. Mean value of CD8 cells was 1291.83 ± 612.58 /uL. A mean number of CD4/CD8 ratio was 0.57 ± 0.31 . Mean number of hs-CRP was 2.18 ± 1.04 mg/dL. Table 1 shows the complete sample characteristics.

Table 1. It is characteristics of samples.

Characteristics	N = 35
Age (years), mean	32.3 ± 7.7
Sex, n (%)	
Male	23 (66%)
Female	12 (34%)
Body Mass Index	19.6 ± 2.3
ARV use (months), mean	43.29 ± 38.08
CD4 mean, /uL	600.6 ± 244
CD8 mean, /uL	1291.83 ± 612.58
CD4/CD8 ratio mean	0.57 ± 0.31
Hs-CRP mean, mg/dL	2.18 ± 1.04
Viral load	
Undetectable	33 (94%)
Detectable	2 (6%)

There was a favorable correlation between CD8 T-cells and hs-CRP levels ($p < 0.05$). And also there was a significant negative correlation between CD4/CD8 ratio and hs-CRP ($p < 0.05$). Table 2 shows the result of this bivariate analysis.

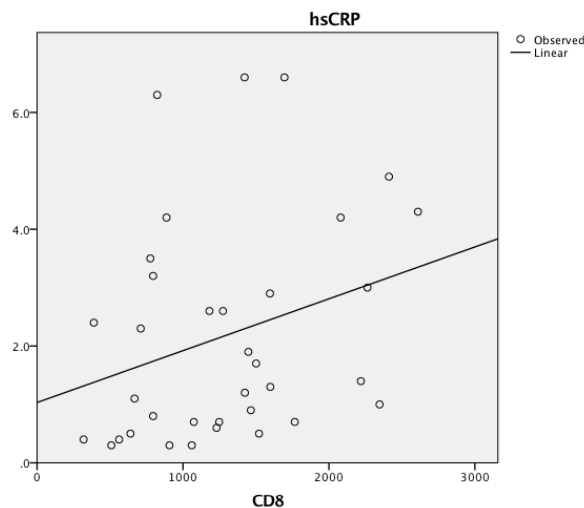


Figure 1. Association between CD8 T-Cell and hs-CRP.

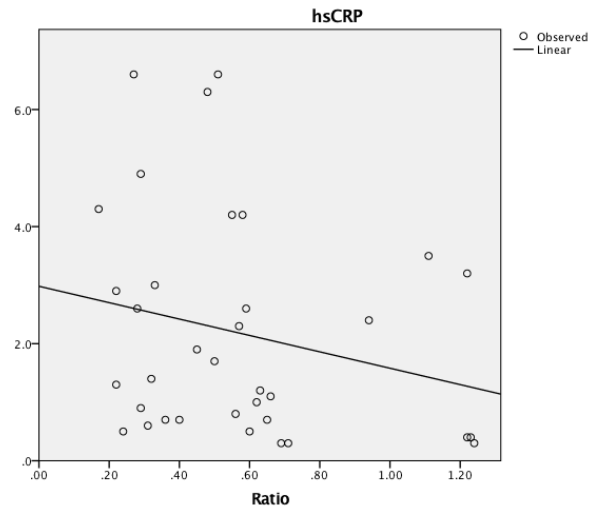


Figure 2. Association between CD4/CD8 ratio and hs-CRP.

Table 2. Analysis bivariate of CD8 T-cells, CD4/CD8 ratio, and hs-CRP.

Data	Coefficient correlation	p
CD8	0.369	0.015
CD4/CD8 ratio	-0.370	0.014

4. Discussion

In this study found that 57% of patients had high T-Cell CD8 levels and a low CD4/CD8 ratio despite having been on ARV for more than six months and 94% of patients with the undetectable viral load. This result suggests that in most HIV- infected patients treated with ART, there is still a persistent immune system dysfunction. Research conducted by Helleberg et al. focusing on the dynamics of changes in CD4 subsets before and after therapy in 3882 HIV patients reported that there was still elevation of CD8 after ten years of ARV therapy.[16] Similarly, in a cohort study by Lu Wei et al. in 109 HIV patients, the CD4/CD8 ratio remained low (mean 0.66 with range 0.19-1.84) after 4.5 years of ARVs.[17] Until now it is still unclear whether the elevated, persistent CD8 subsets were caused by persistent activation of the immune system due to residual viremia or endotoxemia due to gastrointestinal mucosal damage by gastrointestinal microbial translocation.

In this study also found the majority of patients 60% obtained an increase in hs-CRP ($>1\text{mg/dL}$). This result suggests that there is inflammation in most patients that in the future may play a role in causing SNAEs and premature aging.[18] This low-grade inflammation is thought to be due to residual viral replication, intestinal mucosal barrier damage and latent infections such as CMV, TB, and EBV.[5,6,7] Risk factors associated with increased hs-CRP are age, cigarette, body mass index (BMI), cholesterol, diabetes, and hypertension.[19] In this study, most patients had a low BMI, and only 28.6% of patients smoked. For other possible causes, the increase in hs-CRP has been excluded by exclusion criteria. The presence of co-infection of HIV patients with tuberculosis still cannot be excluded in this study. It may need further research to analyze both factors. According to a study by de Luca, there is a favorable relationship between the increase in hs-CRP and cardiovascular risk in HIV-infected patients who have received ARV.[10] Similarly, a study by Bogar et al. found an

increase in hs-CRP in HIV patients with cardiovascular risk.[20] Research by Guimarara is in 2008 showed higher levels of CRP in HIV-infected patients.[21]

The role of T-cell CD8 has been neglected in addition to CD4 because it has no part in a short-term prognosis of HIV patients.[22] There is an association between monocyte activation and increased hs-CRP. This biomarker also increases at persistently elevated of CD8 subsets.[23] There is an association between inflammation and untreated HIV infection, but ART has another effect on inflammatory markers that some may decrease but can also remain high in addition to successful ART.[18] Several studies have shown that CD4/CD8 ratio can be a predictor factor of SNAE mortality. A study in Italy showed that there was an increased risk of non-AIDS mortality in patients with suppressive VL with a CD4/CD8 ratio <0.3 compared with 0.3-0.8.[24] Research by Serrano-Villar in 2014 also showed that there was a relationship between low CD4/CD8 ratios with SNAEs incidence.[25] Another study by Serrano-Villar showed a significant association between low CD4/CD8 ratio and coronary artery disease but could not be used to determine mortality.[26]

The weakness of this study is by not excluding other confounding factors such as lipid profile, old TB infection, CMV and so on. Suggestions for future development are the study of this model in a cohort/longitudinal cohort, a study with a larger sample size and research focus on the pathomechanism of persistent elevation of CD8 levels.

5. Conclusion

There is an association between persistent increased CD8 T-cell, low CD4/CD8 ratio, and increased hs-CRP in HIV-infected patients receiving ART.

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