PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Combining CD4 recovery and CD4/CD8 ratio restoration as an indicator for evaluating the outcome of continued antiretroviral therapy: an observational cohort study
AUTHORS	Lee, Shui Shan; Wong, Ngai Sze; Wong, Bonnie; Wong, Ka Hing; Chan, Kenny

VERSION 1 - REVIEW

REVIEWER	Santiago Moreno University Hospital Ramón y Cajal. Alcalá University. Madrid. Spain.
REVIEW RETURNED	17-Apr-2017

GENERAL COMMENTS	The article deals with an important aspect of immune reconstitution associated with antiretroviral treatment, that is, the fact that not only CD4 recovery is important but also the normalization of CD4/CD8 ratio. The authors evaluate the factors associated with a composite endpoint, termed "optimal immune outcome", which includes a CD4 count >500 and a CD4/CD8 ratio >0.8. The results show that pre-treatment CD4 and CD8 count, as well as gender, age and the duration of treatment are associated with an optimal immune outcome. The article is well-written and is easily followed. The analyses are well done, and the results are clearly shown in tables and figures. Comments for the authors 1. It is not clear why the authors have chosen a combination of CD4 and CD4/CD8 ratio restoration. They do not state if the combination of these two measurements add any value to the measurement of simply the CD4/CD8 ratio. Previous papers, cited by the authors, have shown that the ratio is a better predictor of overall mortality and development of non AIDS defining events than the CD4 count. If the authors are aware of any report showing that taken together the post-treatment CD4 count and CD4/CD8 ratio predict better the clinical outcomes should include this in the Introduction and Discussion. It would make more meaningful the research. 2. Similarly, the authors have chosen a convenience cut-off value for the ratio, i.e. 0.8. Previous reports have shown that a CD4/CD8 ratio=0.4 better predicts the risk of an adverse outcome. It would be important to see if the results obtained with a cut-off of 0.8 are the same with the validated cut-off of 0.4. 3. In several parts of the manuscript (conclusion of the abstract, introduction, discussion), the authors state that "our results suggest that a combination of CD4 count and CD4/CD8 ratio provides a better reflection of immune outcome, compared to the reliance on CD4 alone". However, the study does not allow to conclude nothing similar to this statement. It should be rephrased accordingly,

	especially the conclusion of the abstract.

REVIEWER	Bruno Giuseppe Clinic of Infectious Diseases, University of Bari (Italy)
REVIEW RETURNED	10-May-2017

GENERAL COMMENTS	Reviewer BMJ open The authors evaluated the effects of combining CD4 count and CD4/CD8 ratio as indicator of immune recovery in a large cohort on ART for ≥ 4 years. Despite, as reported by the authors in the limitations of the study, no clinical data were presented in terms of non-AIDS events and morbidity and mortality, the study provide interesting information for clinical practice in monitoring immunological changes in ART treated patients. Main impression: As reported in other works (PMID:26908792, PMID:28182620), immunological recovery and a normalized CD4/CD8 ratio in HIV-infected patients might not be evident, despite effective ART. In addition, CD4 count could not be the only marker of immunological improvement following an effective ART. The article is well written and purposes are elucidated. However, few issues need to be clarified, as below specified. Thus, after a minor revision the manuscript should be considered for publication. Major comments: In Table 2 differences between HIV-infected individuals with high and low pre-HAART CD8 count are shown. Heterosexual mode of transmission were more likely to have a pre-HAART CD8 count < 800; this feature deserves a comment in the discussion. Moreover, cumulative HIV-RNA viremia has been associated (PMID: 25715104) with a lower likelihood of achieving a normalized CD4/CD8 ratio. A clear evaluation of the impact of cumulative HIV viremia on the achievement of a normalized ratio (>0.8) should be added in table 4 comparing those with optimal immune outcome or conventional outcome. Minor comments: If data are available, the impact of HCV and/or CMV co-infections (anti-CMV IgG) should be assessed. In fact, as reported in a recent studies HCV (PMID HCV: 26355305, PMID: 27371888), and CMV (PMID: 26400999 PMID: 27601222), particularly, may alter CD8 count because of persistent immune activation state thus modifying CD4/CD8 ratio. Otherwise, the authors should add these lacking data in the limitations of the study. Of note, all suggested references, should be added to the text.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1. It is not clear why the authors have chosen a combination of CD4 and CD4/CD8 ratio restoration. They do not state if the combination of these two measurements add any value to the measurement of simply the CD4/CD8 ratio. Previous papers, cited by the authors, have shown that the ratio is a better predictor of overall mortality and development of non AIDS defining events than the CD4 count. If the authors are aware of any report showing that taken together the post-treatment CD4 count and CD4/CD8 ratio predict better the clinical outcomes should include this in the Introduction and Discussion. It would make more meaningful the research.

R: Some studies have reported the development of comorbidity in HIV patients despite achieving satisfactory CD4 level following antiretroviral therapy, while a suboptimal CD4/CD8 was shown to be related with HIV associated non-AIDS conditions. The observation motivated us to explore the use of a combined marker to measure immune recovery, using regularly collected investigation results. To date, we could not find any published articles using both CD4 and CD4/CD8 ratio markers to predict clinical outcome.
We have added a sentence in INTRODUCTION to explain the non-concurrent rise of CD4/CD8 ratio and CD4 count to strengthen the rationale of using combined marker: “Low CD4/CD8 ratio was observed in patients despite high CD4 level (>500/μL).”[ref 12]

2. Similarly, the authors have chosen a convenience cut-off value for the ratio, i.e. 0.8. Previous reports have shown that a CD4/CD8 ratio=0.4 better predicts the risk of an adverse outcome. It would be important to see if the results obtained with a cut-off of 0.8 are the same with the validated cut-off of 0.4.
R: The ratio of 0.8 was chosen as cut-off with reference to a previous study [ref 17] (Menozzi M, Zona S, Santoro A, et al. CD4/CD8 ratio is not predictive of multi-morbidity prevalence in HIV-infected patients but identify patients with higher CVD risk. J Int AIDS Soc 2014;17(4 Suppl 3):19709. doi: 10.7448/IAS.17.4.19709), which has been cited in METGHODS and DISCUSSIONS.
As the objective of this manuscript is examining the optimal immune recovery, setting a cut-off of 0.4 for examine the adverse outcome would deviate from the original objective. We agree with the Reviewer to examine the context of a low CD4 count threshold. In response to the comment, we have further classified the distribution of CD4/CD8 ratio into <0.4, 0.4-0.79 and >=0.8 and the corresponding CD4 levels. This is now added as Supplementary Table 2 to show the distribution at baseline and at the end of Year 4. We believe that this would form useful reference for supporting the planning of followup studies.

3. In several parts of the manuscript (conclusion of the abstract, introduction, discussion), the authors state that "our results suggest that a combination of CD4 count and CD4/CD8 ratio provides a better reflection of immune outcome, compared to the reliance on CD4 alone". However, the study does not allow to conclude nothing similar to this statement. It should be rephrased accordingly, especially the conclusion of the abstract.
R: The statement has been rephrased in the (a) CONCLUSION: Our results suggested that a combination of CD4 count and CD4/CD8 ratio offers another potentially useful approach to assessing immune outcome, compared to the use of CD4 alone, and ABSTRACT: A combination of CD4 count and CD4/CD8 ratio could be a useful approach for the characterisation of treatment outcome over time, on top of monitoring CD4 count alone.

Reviewer: 2

Major comments: In Table 2 differences between HIV-infected individuals with high and low pre-HAART CD8 count are shown. Heterosexual mode of transmission were more likely to have a pre-HAART CD8 count < 800; this feature deserves a comment in the discussion. Moreover, cumulative HIV-RNA viremia has been associated (PMID: 25715104) with a lower likelihood of achieving a normalized CD4/CD8 ratio. A clear evaluation of the impact of cumulative HIV viremia on the achievement of a normalized ratio (>0.8) should be added in table 4 comparing those with optimal immune outcome or conventional outcome.
R: Pre-HAART CD8 was positively correlated (r=0.50, p<0.001) with pre-HAART CD4 (Supplementary Figure 1d). As heterosexuals had lower pre-HAART CD4 than MSM, they were more likely to have lower pre-HAART CD8 than MSM in univariate analysis, but the difference was not significant after

the adjustment by confounder of pre-HAART CD4. We have added a sentence in the DISCUSSION to this effect.
We are aware of the association of cumulative HIV-RNA viremia with a lower likelihood of achieving a normalized CD4/CD8 ratio reported in PMID: 25715104 [ref 5 in our manuscript]. The study referred to the outcome of primary HIV infection following antiretroviral therapy, which is different from that of chronic infection examined in this study. We have performed another set of GEE models with cumulative viral load as an independent variable. It was not a significant predictor of an optimal ratio, though the number of patients eligible for the analyses was just 187. The results are not shown in details in the manuscript (see APPENIDIX overleaf)

Minor comments: If data are available, the impact of HCV and/or CMV co-infections (anti-CMV IgG) should be assessed. In fact, as reported in a recent studies HCV (PMID HCV: 26355305, PMID: 27371888), and CMV (PMID: 26400999 PMID: 27601222), particularly, may alter CD8 count because of persistent immune activation state thus modifying CD4/CD8 ratio. Otherwise, the authors should add these lacking data in the limitations of the study. Of note, all suggested references, should be added to the text.
R: We agree that HCV and CMV coinfections could be important factors in shaping immune recovery. Unfortunately, such data were not available for analyses in this study, which we shall definitely consider in future studies. The limitation has been added in the DISCUSSION, alongside the suggested references:
……..our dataset did not include other inflammatory markers or infectious disease outcomes (e.g. HCV and/or CMV co-infections [ref 32-35]) and therefore these could not be analysed in perspective.

APPENDIX

Multivariable logistic regression model with cumulative viral load from seroconversion to diagnosis (n=187)
a) optimal immune outcome; b) conventional outcome
aOR 95%C.I.; aOR 95%C.I.
Male gender 2.01 (0.71-5.71); 0.84 (0.28-2.58)
Age at HAART initiation 0.97 (0.94-1.01); 0.94 (0.90-0.97)*
Pre-HAART CD4 (/μL)
<=100 ref; ref
101-200 3.60 (1.49-8.70)*; 1.31 (0.58-2.97)
201-300 5.70 (1.93-16.84)*; 1.73 (0.60-4.98)
>300 7.15 (0.72-71.12); /
Months on treatment
49-72 ref; ref
73-96 1.82 (0.72-4.60); 2.80 (1.09-7.18)*
>=97 2.55 (1.20-5.41)*; 2.33 (1.10-4.96)*
Pre-HAART CD8≤800/μL 0.998 (0.997-0.999)*; 1.001 (0.9996-1.002)
Cumulative viral load from seroconversion to diagnosis 0.98 (0.95-1.01); 1.00 (0.97-1.04)
Constant 1.72; 8.04
aOR – adjusted odds ratio;
*p<0.05

Multivariable logistic regression model with cumulative viral load from seroconversion to HAART initiation (n=187)
a) optimal immune outcome; b) conventional outcome
aOR 95%C.I.; aOR 95%C.I.
Male gender 1.90 (0.67-5.42); 0.90 (0.29-2.75)

Age at HAART initiation 0.97 (0.94-1.01); 0.94 (0.90-0.97)*
Pre-HAART CD4 (/μL)
<=100 ref; ref
101-200 3.77 (1.56-9.08)*; 1.31 (0.58-2.97)
201-300 6.18 (2.10-18.17)*; 1.72 (0.60-4.90)
>300 7.87 (0.82-75.50); /
Months on treatment
49-72 ref; ref
73-96 1.72 (0.69-4.34); 2.88 (1.11-7.43)
>=97 2.52 (1.19-5.35)*; 2.34 (1.10-4.99)
Pre-HAART CD8≤800/μL 0.998 (0.997-0.999)*; 1.001 (0.9997-1.002)
Cumulative viral load from seroconversion to HAART initiation 0.99 (0.96-1.01); 1.01 (0.99-1.04)
Constant 1.72; 6.17
aOR – adjusted odds ratio;
*p<0.05

Multivariable logistic regression model with cumulative viral load from seroconversion to data end point (n=187)
a) optimal immune outcome; b) conventional outcome
aOR 95%C.I.; aOR 95%C.I.
Male gender 1.93 (0.68-5.48); 0.89 (0.29-2.72)
Age at HAART initiation 0.97 (0.94-1.01); 0.94 (0.90-0.97)
Pre-HAART CD4 (/μL)
<=100 ref; ref
101-200 3.75 (1.56-9.02)*; 1.31 (0.58-2.97)
201-300 6.10 (2.08-17.94)*; 1.73 (0.61-4.93)
>300 7.75 (0.80-74.56); /
Months on treatment
49-72 ref; ref
73-96 1.73 (0.69-4.35); 2.87 (1.11-7.40)
>=97 2.53 (1.20-5.37)*; 2.33 (1.09-4.96)
Pre-HAART CD8≤800/μL 0.998 (0.997-0.999)*; 1.001 (0.9997-1.002)
Cumulative viral load from seroconversion to data end point 0.99 (0.96-1.01); 1.01 (0.99-1.04)
Constant 1.69; 6.16
aOR – adjusted odds ratio;
*p<0.05

VERSION 2 – REVIEW

REVIEWER	Santiago Moreno Department of Infectious Diseases University Hospital Ramón y Cajal Madrid Spain
REVIEW RETURNED	18-Jun-2017

GENERAL COMMENTS	The authors address the kinetics and factors associated with a combined immune restoration outcome in HIV-infected patients on long-term successful antiretroviral therapy in Hong-Kong. They find that an optimal immunological restoration (i.e. >500 CD4/mm3 and a CD4/CD8 ratio >0.8) is achieved in a moderate percentage of patients and identify predictive factors of such restoration. The issue of full immune recovery during ART is important, since it has been shown in multiple reports that the CD4/CD8 ratio is an

	independent predictor of overall mortality and development of comorbidities, in additional to the more traditional CD4 count. The article under review offers a clear picture of the kinetics of the recovery, and identifies associated factors which are very consistent with previous reports. Besides, it adds some good information on new predictive factors associated to, what they call, optimal immune restoration. The paper is well-written and can be easily followed. Tables and graphics are also clearly presented. References are adequate and up-dated. Comments for the authors (minor). 1. To comply with requirements for observational studies, it would useful to have a Strobe diagram/checklist. 2. While the suggestion of combining CD4 count and CD4/CD8 ratio to define a full immune restoration with high predictive value of clinical outcomes is well-accepted, it is not clear the cut-offs chosen. It would be interesting to confirm the reproducibility of the results with different cut-offs, which have been identified in previous reports as clinically predictive. For instance, a CD4/CD8 ratio >0.4 has been associated with a decreased risk of non AIDS events and comorbidities, and >1 is used as a definition of immunological normalization. 3. Same comment for the pre-HAART CD8 count which has been used as a predictor of recovery (800 CD8/mm3).

REVIEWER	Giuseppe Bruno Clinic of Infectious Diseases
REVIEW RETURNED	19-Jun-2017

GENERAL COMMENTS	The authors provided careful responses to our queries. Thus, the paper should be considered for publication.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1
Comments for the authors (minor).
1. To comply with requirements for observational studies, it would useful to have a Strobe diagram/checklist.

RESPONSE: We have provided a STROBE checklist. Thanks for your kind reminder.

2. While the suggestion of combining CD4 count and CD4/CD8 ratio to define a full immune restoration with high predictive value of clinical outcomes is well-accepted, it is not clear the cut-offs chosen. It would be interesting to confirm the reproducibility of the results with different cut-offs, which have been identified in previous reports as clinically predictive. For instance, a CD4/CD8 ratio >0.4 has been associated with a decreased risk of non AIDS events and comorbidities, and >1 is used as a definition of immunological normalization.

RESPONSE: There is stronger association between pre-HAART markers (CD8≤800/μL, and CD4/CD8 ratio >0.4) and outcome variable of CD4≥500/μL & ratio ≥1 than outcome variable of CD4≥500/μL & ratio ≥0.8. However, the proportion of patients achieving CD4≥500/μL & ratio ≥1 will become very small (6%). We have added a couple of sentences on p12, lines 14-17 to address this

observation: “The association of pre-HAART CD8 with optimal immune outcome was stronger with a cut-off ratio of ≥1 but the proportion of patients achieving the target outcome would be very low at 6% (46 out of 718).”

We confirm that our analyses did show the significant association between pre-HAART CD4/CD8 ratio >0.4 and both the optimal immune outcome (CD4≥500/μL & ratio ≥0.8), and the conventional outcome (CD4≥500/μL). However, as the dataset for this study had not included non-AIDS events and comorbidities, we are unable to examine their association with CD4/CD8 ratio >0.4.

3. Same comment for the pre-HAART CD8 count which has been used as a predictor of recovery (800 CD8/mm3).

RESPONSE: We have tried to use pre-HAART CD8 ≤600/μL and ≤1000/μL as cut-off. Both the cut-offs (Pre-HAART CD8 ≤600/μL and ≤1000/μL) were not significantly associated with outcome variables at year 4 (CD4 ≥500/μL and the optimal immune outcome of CD4≥500/μL & ratio ≥0.8).