EVIDENCE FOR LOWER CD4⁺ T CELL AND HIGHER VIRAL LOAD IN ASYMPTOMATIC HIV-1 INFECTED INDIVIDUALS OF INDIA: IMPLICATIONS FOR THERAPY INITIATION

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Abstract

Purpose: We have earlier documented that the south Indian population had lower CD4 counts. The aim of this study was to investigate a previous suggestion on a new CD4⁺ T cell cut off and association with HIV-1 RNA levels for decision on anti retroviral therapy in India (south). Methods: We evaluated a new methodology i.e., artus real-time PCR and CD4⁺ T cell count by Guava EasyCD4™ system. From 146 HIV infected individuals seen at a tertiary care centre, blood was collected for CD4⁺ T cell and HIV-1 RNA estimation. Results: The receiver operating characteristic curve cut off value for the CD4 counts to distinguish between CDC clinical categories A and B was 243 cells/μL, and to distinguish B and C was 153 cells/μL. The RNA level that differentiated CDC A and B was 327473 RNA copies/mL, while for CDC B and C was 68543 copies/mL. There was a significant negative correlation (r = -0.55, P < 0.01) between the RNA estimated and CD4⁺ T cell counts in HIV infected individuals. Conclusions: A majority with CD4 counts of 201-350 cells/μL in our population had higher viral load than the treatment threshold suggested by the International AIDS society and the above two methodologies are useful in monitoring HIV infections.

Key words: Artus HIV-1 real-time PCR, Guava easyCD4 assay, HIV, India

A report from this centre had earlier shown that the south Indian population had lower CD4 T cells counts and hence requires a different cut off for clinical classification for HIV infected individuals. Anti retroviral therapy (ART) for human immunodeficiency virus disease is currently available even in countries with resource poor settings.[1] At present most of the attention is also focused at low cost methods for the estimation of CD4⁺ T cell counts and viral load for the monitoring of infection.[2-3] There are different CD4⁺ T cell estimation assays that have been evaluated with the standard technique of flow cytometry.[2-4] Recently we have evaluated the Guava EasyCD4™ system (Guava Technologies, Hayward, CA, USA) for the T cell estimation with flow cytometry.[5] The aim of this study was to investigate further the previous suggestion on new CD4⁺ T cell and HIV-1 viral loads for initiating ART in India (south). In this study we applied the presently available commercial assays for viral load estimation (Qiagen artus real-time PCR assay,GmbH, Germany) and the CD4 cell count (Guava EasyCD4™ system, CA, USA) in HIV-1 infected individuals to find evidence for proposed cut offs.

Materials and Methods

Blood samples was collected from 146 serologically identified HIV infected treatment naïve individuals who came to the clinical virology department of a tertiary care centre in India (south) for monitoring tests. The samples were collected always between 8:00 am and 10:00 am during the period of March 2005 through September 2006 after an informed consent. The individuals were classified into CDC clinical categories: asymptomatic (A), symptomatic (B) and AIDS (C) by the appropriately trained examining physician. Category C differed from B in having AIDS-defining illnesses like disseminated or extra pulmonary tuberculosis, cryptococcal meningitis, oesophageal candidiasis, chronic diarrhoea with wasting syndrome. In addition, stratification based on CD4⁺ T cell counts into three categories as per CDC guidelines was carried out for analysis. The categories were individuals with CD4⁺ T cell count ≥ 500 (category 1), 200 - 499 (category 2) and <200 (category 3).

Absolute CD4⁺ T cell counts were estimated by the Guava EasyCD4™ System (Guava Technologies, Hayward, CA, USA) as reported earlier.[5] The CD4 evaluation in our laboratory by this instrument is a part of a regular quality assurance program carried out by WHO and a laboratory in Thailand under the auspices of NACO, India. The HIV-1 viral load was estimated using real-time PCR, RotorGene 3000 (Corbet Research Scientific, Australia) with artus HIV-1 RG RT-PCR assay (Qiagen GmbH, Germany). The manufacturer’s instructions were followed for the extraction of RNA and for the reverse transcriptase PCR.
RNA was isolated using the QIAamp® Viral RNA assay (Qiagen, GmBH, Germany) from 140 μL of plasma and eluted in 50 μL of elution buffer. Twenty microlitre of the extracted RNA was used in a 50 μL RT-PCR reaction which contains the internal control as well in each tube. Each run of this assay had the four quantitation standards provided by the manufacturer. The cycling condition used were 50ºC for 10 minutes (RT) followed by 95ºC for 10 minutes (for the activation of Hot start Taq polymerase enzyme) subsequently 45 cycles at 95º C for 20 seconds, 50ºC for 30 seconds and 72ºC for 30 seconds. The analytical sensitivity reported by the manufacturer for the artus HIV-1 RG RT-PCR assay is 53 Copies/mL.

Statistical analysis

The viral load and the CD4+ T cell count was analyzed by Pearson’s correlation test (r). A Receiver Operating Characteristic (ROC) curve analysis was carried out using SPSS 11.0 for windows statistical software to determine the best CD4+ T cell count cut-off values that classified the CDC clinical categories A and B and CDC categories B and C. A ROC curve was also generated to find the best cut-off for viral load level that classified the CDC clinical categories A and B and CDC categories B and C of HIV infected individuals and CDC categories based on CD4+ T cell counts. The area under the curve (AUC) was used as measure of the overall accuracy of CD4+ T cell counts and viral load levels in defining CDC clinical categories. The values of AUC can range from 0.5 to 1. An AUC of 1.0 or close to 1.0 indicates perfect accuracy. The comparisons of means were done by the Kruskal-Wallis one-way analysis of variance test using the EPI Info statistical software program ver. 6.04c.

Results

Among the 146 individuals, 62 were asymptomatic (CDC A), 33 were symptomatic without AIDS (CDC B) and the remaining 51 were symptomatic individuals with AIDS (CDC C). There were 19 individuals in CD4 category 1, 60 individuals in the category 2 and 67 individuals in the category 3. The table shows the mean, median and the standard deviation of the CD4+ T cells and HIV-1 viral load obtained for the three CDC clinical categories of infected individuals. The difference in the mean CD4+ T cells and the viral load among the three categories were significant ($P < 0.0001$). There was a significant negative correlation (Fig. 1) between the CD4+ T cell estimated and the viral load with a r of -0.55 ($P < 0.01$).

![Figure 1: Scatter diagram showing the correlation between CD4+ T cell counts (Guava® EasyCD4™ system (Guava Technologies, Hayward, CA, USA) and the viral load (artus HIV-1 RG RT-PCR assay Qiagen GmbH, Germany).](image)

**Table:** The mean, median and the standard deviation (SD) of the CD4+ T cells (Guava® EasyCD4™ system, Guava Technologies, Hayward, CA, USA) and HIV-1 viral load (artus HIV-1 RG RT-PCR assay Qiagen GmbH, Germany) obtained for the three CDC clinical categories of infected individuals

<table>
<thead>
<tr>
<th>CDC clinical and CD4 categories</th>
<th>CD4+ T cells (cells/μL)</th>
<th>Viral load (copies in log/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>A1 ($n = 18$)</td>
<td>765</td>
<td>676</td>
</tr>
<tr>
<td>A2 ($n = 34$)</td>
<td>346</td>
<td>336</td>
</tr>
<tr>
<td>A3 ($n = 10$)</td>
<td>158</td>
<td>168</td>
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<tr>
<td>A ($n = 62$)</td>
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<td>378</td>
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<tr>
<td>B1 ($n = 1$)</td>
<td>594</td>
<td>-</td>
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<tr>
<td>B2 ($n = 18$)</td>
<td>276</td>
<td>236</td>
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<tr>
<td>B3 ($n = 14$)</td>
<td>112</td>
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<td>C1 ($n = 0$)</td>
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<td>C2 ($n = 9$)</td>
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<td>C3 ($n = 42$)</td>
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<td>96</td>
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<tr>
<td>C ($n = 51$)</td>
<td>129</td>
<td>105</td>
</tr>
<tr>
<td>All ($n = 146$)</td>
<td>279</td>
<td>215</td>
</tr>
</tbody>
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The ROC curve predicted the best CD4\(^+\) T cell count that classified clinical staging A and B as 243 cells/μL (sensitivity: 76\%, specificity: 73\%). The predicted CD4\(^+\) T cell count to classify the CDC categories B and C was 153 cells/μL (sensitivity: 73\%, specificity: 72\%). The CD4\(^+\) T cell counts had a good accuracy in defining CDC clinical categories (A and B) and (B and C), with an AUC of 0.79 and 0.73 respectively. This is shown in Fig. 2. The ROC curve was also generated for the viral loads based on the three CDC clinical categories and CD4\(^+\) T cell categories. The best viral load that predicted an individual to be symptomatic (CDC B) was RNA ≥ 327473 copies/mL (sensitivity: 73\%, specificity: 71\%). The accuracy was similar to the accuracy of CD4\(^+\) T cell counts with an AUC of 0.75. A RNA of ≥ 688543 copies/mL predicted clinical AIDS (CDC C) (sensitivity: 65\%, specificity: 52\%) (Fig. 3). The viral load level that best correlated with CDC CD4 categories were 76990 copies/mL for CDC CD4 categories 1 and 2 and 487772 RNA copies/mL between CDC 2 and CDC 3 (Fig. 4).

**Discussion**

It is reported that the mean CD4\(^+\) T cell counts among normal south Indians is significantly lower than that in the western population.\cite{6-9} We earlier proposed a modified classification based on the CD4\(^+\) T cell counts for HIV infected individuals in southern India. The categories of CD4 counts proposed were cell count > 300, 81-300 and ≤ 80 cells/μL, instead of the > 500, 201-500 and ≤ 200 recommended by CDC.\cite{6} In this study reported by ROC curve analysis it was found that a cell count of 243 cells/μL has a sensitivity of 76\% and a specificity of 72\% to differentiate CDC A and B while a count of 153 cells/μL best correlated for categorization of CDC B and C clinical categories. We reiterate the findings of our earlier study that the south Indian population had a lower CD4 counts and hence requires a different cut-off for clinical classification.\cite{6}
A viral load of $3.27 \times 10^5$ RNA copies/mL was best correlated with clinical staging categorized as asymptomatic (CDC A) or symptomatic (CDC B) while a viral load of $6.88543 \times 10^5$ copies/mL correlated with clinical staging of symptomatic (CDC B) and symptomatic with AIDS (CDC C). Among the three different CDC CD4 categories the viral load level that best correlated with CDC category 1 and 2 was $7.6990 \times 10^5$ RNA copies/mL, while for CDC 2 and 3 it was $4.87772 \times 10^5$ RNA copies/mL. This high viral load seen in our population may be due to the constant immune activation that is driven environmentally. Increased HIV replication in activated CD4+ T cells takes place with higher expression of CCR5 co-receptors. In this context, it was found that the AUC for the viral loads were low when compared to CD4+ T cells indicating lower accuracy of viral load levels in classifying different CDC categories.

The optimum time to initiate ART is before the patients become symptomatic. As per the WHO criteria and British HIV association (BHIVA) guidelines ART should be recommended for asymptomatic HIV infected individuals having less than $200 \times 10^6$ CD4+ T cell counts. International AIDS society recommends the initiation of ART be considered in asymptomatic individuals with CD4+ T cell count $>200$ to $350 \times 10^6$ cells/μL and viral load $50000$-$100000$ copies/mL. Interestingly, 34 (56%) of the 61 individuals who had a CD4+ T cell count of 201-500 and 10 (15%) of the 66 individuals in CDC category 3 (CD4 < 200) were asymptomatic. In our study population, 50% of the individuals with CD4 counts of 201-350, were found to be asymptomatic. It was also observed that the T cell count ($243 \times 10^6$ cells/μL) associated with symptomatic status is almost the same as the cell counts prescribed by WHO and BHIVA for the initiation of treatment. These guidelines also suggest that if the individual’s CD4+ T cell count is 201-350, other factors like rapidity of CD4 decline, viral load and symptoms should be considered before the initiation of therapy. The mean HIV RNA copies observed in 18 (50%) asymptomatic (CDC A) individuals with CD4+ T cell count of 200 to 350 cells/μL in our population was $5.3 \times 10^5$ copies RNA/ml (Median $1.3 \times 10^5$ copies/mL). While the mean HIV RNA copies observed in 18 (50%) symptomatic (CDC B and C) individuals with CD4+ T cell count of 200-350 cells/μL in our population was $1.4 \times 10^5$ copies RNA/ml (Median $6.1 \times 10^5$ copies/mL). Only 9 (25%) of these 36 individuals had a viral load <100,000 copies of RNA/mL.

It could be speculated that the lack of classical clinical symptoms with higher levels of viral load in our population of HIV infected individuals may be a reflection of the differential virus-host relationship. This phenomenon may be due to the genetic make up (ethnicity) of individuals. The populations in other parts of country also have a lower CD4+ T cell count than the Western countries. The best way to suppress the virus replication and to achieve the desirable clinical outcomes for patients is treating them early. Therefore, even though the current cut-off appears to be not quite right for southern India, it might serve as “a blessing in disguise”. Treating HIV-1 infected individuals earlier may help to slow down the spread of the virus in India by reducing the chance of infected individuals from harbouring transmissible virus. Our data shows that majority (75%) of the individuals with CD4 counts of 201-350 cells/μL in our populations should be considered for treatment, as the viral loads are high irrespective of the clinical condition.

The GuavaEasy CD4 system is a newly introduced methodology in the market. Hence, in developing countries like India, it is important to evaluate the system with the other new commercial methods of HIV-1 viral load estimation. We had earlier analysed the correlation of the CD4+ T cells estimated by the GuavaEasy CD4 system and the flow cytometry and had shown that both systems are usable interchangeably as there was a very significant
The CD4+ T cells and CD3+ cell values of all the quality control samples were within the expected acceptable range. In the present study, we have also analysed the association of CD4+ T cell estimated by GuavaEasyCD4 system and the viral load level estimated by artus HIV-1 RG RT-PCR assay and found to be significant ($r = -0.55$).

In conclusion, our study showed a significant negative correlation between the CD4+ T cell estimated by the GuavaCD4 system and the artus real-time viral load assay. This study showed that a cut off CD4+ T cell count of 243 cells/μL distinguished individuals who were asymptomatic (CDC A) from symptomatic (CDC B). Our study also showed that majority of the individuals with CD4 counts of 200-350 cells/μL in our populations had higher viral load than that suggested by the International AIDS society to be considered for treatment and this need to be taken in to account in the recommendation of the national guidelines.

References


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