Absolute lymphocyte count: A useful surrogate marker to initiate anti retroviral therapy in resource poor settings.

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1. Introduction

Acquired immunodeficiency syndrome (AIDS) was recognised in United States of America in 1981 and its causative agent HIV was first isolated in 1984. Since then the disease has spread virtually all over the world. HIV targets and destroys CD4 cells which predisposes the infected individual to various opportunistic infections. In areas where resources are available, decision to initiate anti retroviral therapy (ART) is routinely based on the algorithm that combine CD4, HIV load and clinical illness. But their high cost and unavailability at resource poor settings are one of its major limitations. An attempt has been made to find a correlation between CD4 count and Absolute lymphocyte count (ALC) so that timely initiation of ART could be done in peripheral areas of developing countries where automation and established technologies have not been reached.

2. Material and methods

This cross sectional study included 200 patients between 18–60 year of ages who are HIV seropositive with and without clinical evidence of oral candidiasis (100 cases; 100 controls). ALC was calculated as per the percentage of lymphocyte in total leukocyte count as seen in peripheral smear. CD4 cell count estimation was done by flow cytometry method.

Results: A good correlation was found between CD4 count and ALC in both cases (R=0.656) and controls (R=0.642). There was an increase in sensitivity (S) and decrease in specificity (Sp) of predicting CD4 count <200 cells/mm3 and < 350 cells/mm3 as the cut off value for ALC increased. ALC cut off value of 1700 cells/mm3 is likely to be the best predictor of CD4 count of < 200 cells/mm3 and ALC cut off value of 1800 cells/mm3 for CD4 count <350 cells/mm3.

Conclusions: We recommend the use of ALC as a surrogate marker for or in combination with CD4 count to determine when to start therapy and to enable routine monitoring in resource poor settings.
controls. Patients on ART, or having clinical evidence of any other opportunistic infection, diabetes mellitus, any malignancy, on corticosteroid therapy, on anti neoplastic drugs, on antibiotic therapy or on antifungal drugs for more than 2 weeks, blood dyscrasias, xerostomia and pregnant women were excluded from the study. A detailed history and thorough physical examination and relevant routine blood testing was carried out in all cases.

For isolation of candida, sample was taken with moist sterile cotton swab, in duplicate, from buccal mucosa, floor of the mouth, dorsum of the tongue or from angle of the mouth. All samples were subjected to standard mycological techniques for identification and susceptibility testing.

5 ml of peripheral venous blood was collected with anticoagulant and sera were stored in screw capped vial at -20°C. ALC was calculated as per the percentage of lymphocyte in total leukocyte count as seen in peripheral smear. CD4 cell count estimation was done by flow cytometry method.

All clinical laboratory data were entered into a relational database designed in SPSS software. Mean value of all laboratory parameters were calculated and linear regressions were applied.

### 3. Results

In our study a total of 100 patients who were HIV seropositive with oral candidiasis and 100 HIV seropositive people who were asymptomatic were included as case and control respectively. The mean CD4 counts amongst cases were significantly lower than the controls (Table 1). Majority of the patients amongst cases (77.14%) had CD4 count <200 cells/mm³. The mean ALC amongst cases were significantly lower than the controls (Table 1).

#### Table 1.

Mean count of CD4 and ALC

|                | Case  | Control | p value |
|----------------|-------|---------|---------|
| Mean CD4 cells/mm³ | 151.69 | 390.89  | <0.001  |
| Mean ALC cells/mm³ | 1456.23 | 1905.17 | 0.010   |

C.albicans was the predominant species, isolated in 54 subjects while non C.albicans species were isolated in 46

#### Table 2.

Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of varying cut off for ALC; in identifying CD4 count < 200 cells/mm³ in HIV positive patients.

| ALC (Cells/mm³) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------------|-----------------|-----------------|---------|---------|
| Case Control Case Control Case Control Case Control |
| <1000           | 48.14 28.57     | 100 89.28       | 100 40.00 | 36.36 83.33 |
| <1200           | 51.85 28.57     | 100 89.28       | 100 40.00 | 38.09 83.33 |
| <1400           | 59.25 42.85     | 87.50 75.00     | 94.11 30.00 | 38.80 84.00 |
| <1500           | 66.60 42.85     | 87.50 75.00     | 94.73 30.00 | 43.75 84.00 |
| <1600           | 64.51 57.14     | 75.00 71.42     | 95.23 33.33 | 21.42 86.95 |
| <1700           | 74.07 71.42     | 62.50 67.85     | 85.95 35.71 | 41.66 90.47 |
| <1800           | 77.77 71.42     | 50.00 67.85     | 84.00 35.71 | 40.00 90.47 |
| <1900           | 77.77 71.42     | 50.00 60.71     | 84.00 31.25 | 40.00 89.47 |
| <2000           | 81.48 71.42     | 50.00 53.57     | 84.61 27.77 | 44.44 88.23 |
| <2100           | 85.18 100       | 50.00 50.00     | 85.18 33.33 | 50.00 100   |
| <2200           | 92.59 100       | 50.00 39.28     | 86.20 29.16 | 66.66 100   |
| <2300           | 100 100         | 37.50 35.71     | 84.37 28.00 | 100 100    |
| <2400           | 100 100         | 25.00 25.00     | 81.82 25.00 | 100 100    |

#### Table 3.

Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of varying cut off for ALC; in identifying CD4 count < 350 cells/mm³ in HIV positive patients.

| ALC (Cells/mm³) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------------|-----------------|-----------------|---------|---------|
| Case Control Case Control Case Control Case Control |
| <1000           | 41.93 18.75     | 100 89.47       | 100 88  18.18 | 56   |
| <1200           | 45.16 18.75     | 100 89.47       | 100 88  19.04 | 56   |
| <1400           | 45.16 31.25     | 100 73.68       | 98 78   19.04 | 56.66 |
| <1500           | 61.29 31.25     | 100 73.68       | 98 75   25    | 56.66 |
| <1600           | 64.51 37.5      | 75 68.42       | 96.23  69   | 31.42 | 56.52 |
| <1700           | 67.74 43.75     | 69 66.15       | 95.3  60   | 36.66 | 57.14 |
| <1800           | 74.19 73.75     | 65 63.15       | 90 40   | 50    | 87.14 |
| <1900           | 74.19 75        | 55 59.89       | 90 39   50    | 87.89 |
| <2000           | 77.41 76.25     | 50 50.63       | 90.59  35   | 52.22 | 88.82 |
| <2100           | 80.64 85        | 48 50.63       | 90.3  34.14 | 55    | 91.42 |
| <2200           | 87.09 87.5      | 48 47.36       | 91.9  30.33 | 66.33 | 91.81 |
| <2300           | 96.77 93.75     | 39 47.36       | 91.75  24.69 | 66.66 | 95   |
| <2400           | 100 93.75       | 30 42.1        | 93.13  24   | 100   | 98.88 |
out of which *C. tropicalis* (19) was the most common species followed by *C. guilliermondii* (11), *C. parapsilosis* (8), *C. krusei* (4) and *C. glabrata* (4). There was a trend towards non Candida albicans species being more resistant to antifungal agents than Candida albicans, but the difference was not statistically significant (*P* = 0.914).

A good correlation was found between CD4 count and ALC in both cases (R = 0.656) and controls (R = 0.642). There was an increase in sensitivity (Sn) and decrease in specificity (Sp) of predicting CD4 count < 200 cells/mm3 and < 350 cells/mm3 as the cut off value for ALC increased (Table 2 & 3). ALC cut off value of 1700 cells/mm3 is likely to be the best predictor of CD4 count of < 200 cells/mm3 and ALC cut off value of 1800 cells/mm3 for CD4 count <350 cells/mm3.

### 4. Discussion

A free anti retroviral therapy (ART) initiative in India was launched on April 2004 by National AIDS Control Organisation (NACO) with key goals of strengthening the linkages & makes the treatment more effective by early intervention. Even though the availability of ART is gradually improving in resource poor settings, at present, the timely initiation of ART as well as opportunistic infection prophylaxis remains the most important and feasible intervention to reduce morbidity and lengthen survival time. According to WHO, the initiation of ART is based on the clinical stage and the CD4 count.8 The absolute CD4 count is a measurement of functional CD4 T-cells circulating in the blood while CD4 percentage represents the percentage of absolute lymphocytes that are CD4 cells. The CD4 counts may vary within individuals of different ethnicity; hence it is important to establish the reference ranges of CD4 count for the target population. The CD4 percentages in Indian adults were found to be lower as compared to Western countries. The mean CD4 per cent were found to be 37 per cent (range 14 to 65%). Various studies carried out have showed the range of absolute CD4 counts as 600 to 1200 cells/mm3 and < 350 cells/mm3 respectively in HIV seropositive people with CD4 count <350 cells/mm3. However various studies showed variable level of sensitivity of ALC cut off value to predict CD4 count.6 Some studies have not found ALC to be a good predictor of CD4 count and some observed low predictive values among patients with relatively early stage of HIV infection.5,11 Thus one ALC cut off value may not necessarily apply to the populations from different parts of the world but selection of appropriate ALC cut offs for prophylaxis administration should be made on a regional basis. Further studies with larger sample size and variant population would be of additional value.

To summarise, though WHO guidelines released in 2006 do not use ALC in the national ART programme for deciding on the initiation and monitoring the response of ART but in our study population we recommend the ALC cut off value of 1700 and 1800 cells/mm3 to screen CD4 count <200 cells/mm3 and <350 cells/mm3 respectively in HIV seropositive people and to be used as a surrogate marker for or in combination with CD4 count to determine when to start therapy and to enable routine monitoring.

### Conflict of interest statement

We declare that we have no conflict of interest.

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