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Original Article

Utility of absolute lymphocyte count as a surrogate marker of CD4 cell counts: Is it useful?

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ABSTRACT

Background: Human immunodeficiency virus (HIV) management has witnessed paradigm changes over the past decade. In the early era, Absolute lymphocyte counts (ALC) were used as an inexpensive, indirect marker of immunity status. With time, CD4 lymphocyte counts and HIV RNA levels have become a standard of care for follow up of people living with HIV/AIDS (PLHA). Wide disparities between resource rich and poor countries, rekindles the need for an inexpensive surrogate marker for CD4 lymphocyte counts. Multiple studies in the past including one by Sen S et al, in 2011 did not validate ALC as a surrogate marker of CD4 lymphocyte counts and had recommended a similar study at another centre to validate the same.¹ Recently few publications have suggested that ALC may be used as a proxy marker to CD4 lymphocyte counts in resource poor areas.² With this backdrop we decided to evaluate the association if any, of ALC as a surrogate marker to CD4 lymphocyte counts.

Methods: ALC and CD4 lymphocyte counts measurements of 241 patients at our HIV/AIDS referral centre were assessed over a period of 13 months.

Results: Pearson correlation coefficient, coefficient of determination and standard statistical methods revealed modest linear correlation between ALC and CD4 lymphocyte counts which was statistically significant but did not have clinical significance.

Conclusion: We recommend that time has come to call curtains down on ALC as a surrogate marker for CD4 lymphocyte count.

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Introduction

Majority of Human immunodeficiency virus (HIV) positive people now live in developing countries like Sub-Saharan Africa and South East Asia contributing to nearly 80% of the global share.¹ This also happens to be the region where there is a limitation of resources for CD4 estimation, availability of ART and thereafter follow up. Mindful of this problem, the World Health Organization (WHO) in 2005 acknowledged that ALC may be used to make treatment decision in resource poor settings when CD4 lymphocyte counts are either not available or not affordable but in the subcategory of mildly symptomatic patients only.³ The rationale for the WHO's recommendation was that most studies at that time had concluded that a decline in ALC was strongly correlated with a decline in CD4 count, though there were some discrepancies.^{4–8} Over the years our guidelines have been in line with standard recommendations.

By 2007–09, data evaluating the utility of ALC as an inexpensive surrogate marker of CD4 lymphocyte counts to guide therapeutic decisions was found lacking.^{9,10} It was shown that ALC <1200/cumm was not optimal for identifying patients requiring ART since it showed low sensitivity and specificity to predict CD4 lymphocyte counts below 200 cells/mm.¹⁰ This difference was further highlighted in the study conducted in 2008 by Derrese Daka and Eskinder Loha. In their study they concluded that ALC as a surrogate marker for CD4 lymphocyte counts had a low sensitivity and specificity.¹¹ In Nov 2009, WHO in its revised guidelines of ART in adults and adolescents outlined an expanded role of laboratory monitoring including CD4 lymphocyte counts and strategic introduction of viral load monitoring in HIV care.¹² They also recommended that in areas where CD4 testing/viral load levels were not available, clinical parameters were to be used to guide treatment decisions.

This reflected a paradigm shift in the management of PLHA from unreliable ALC to more accurate measurements CD4 lymphocyte counts/HIV viral load estimation. Access to ART has now improved. Over the years there has been better penetration of CD4 and viral load testing facilities. The testing itself is now more accurate and at a lower cost.²

However in the recent past there has again been a resurgence of studies published which advocate the utility of ALC as a proxy marker for CD4 estimation in management of HIV/AIDS.² Multiple studies in the past including one by Sen S et al, in 2011 did not validate ALC as an effective surrogate marker of CD4 lymphocyte counts.¹ They suggested more work at a different centre, on the subject, to validate their outcome. This necessitated a revisit on the relationship between ALC and CD4 count.

Materials and methods

This cross sectional study was conducted at a regional HIV/AIDS referral centre. The hospital provides HIV care such as counselling, treatment of opportunistic infections, out patient as well as in patient care for all HIV related complications and laboratory support (including CD4 lymphocyte

counts and outsourced viral load testing in select cases), all free of cost. The study was conducted from May 2009 to June 2010.

To evaluate the relationship of ALC and CD4 lymphocyte counts in PLHA.

Sample size for correlation coefficient was calculated using software available at <http://www.cct.cuhk.edu.hk/stat/other/correlation.htm>. For a power of 80% and alpha error of 0.5, the sample size required for a correlation 'r' of 0.35, 0.6 and 0.9 were 62, 19 and 7 respectively. We enrolled 241 patients for the study.

All consecutive HIV positive patients reporting to the hospital (Inpatients and outpatients) were included in the study. The patient spectrum in the study included asymptomatic HIV infection to advanced AIDS.

There were only two female patients who reported during the study duration. They were excluded to prevent any selection bias (if any).

ALC and CD4 lymphocyte counts were carried out on the same blood sample, the same day. These study subjects were assessed clinically with other relevant laboratory investigations as required. A standardized data collection form was completed for each patient which included demographics, age, mode of infection, year of detection, clinical and laboratory data. Complete past medical records of all patients were either in their possession or in our data bank, our centre being the regional referral centre for HIV/AIDS. Two study physicians determined the WHO clinical stage.

All patients underwent analysis for CD4 lymphocyte counts at first detection, six monthly for first one and half years and yearly thereafter. A basic haemogram, urine analysis, liver function tests and serum creatinine, screening for syphilis, hepatitis B and C was also done. Yearly Chest X ray, ultrasound of the abdomen, baseline and thereafter two yearly Mantoux test was also done. Computerised tomography (CT) scan of abdomen, head and chest was carried out in relevant cases. Serial weight records and any other relevant tests required for treatment of any specific cases were also undertaken. CD4 lymphocyte counts were estimated using Fluorescence Activated Cell Sorting (FACS Counter) and complete blood counts including white blood cell count (WBC) and Absolute lymphocyte count (ALC) was done by FACS Count Becton Dickinson, USA. All blood indices were done on the same sample for each patient. Quality Control was monitored by internal controls and external proficiency programmes. The data set was de-identified and no addresses, unique identifiers or patient visit dates were included. Informed consent was obtained.

Pearson correlation between CD4 lymphocyte counts and ALC was calculated. Coefficient of determination was also calculated. Statistical analysis was done using EpiInfo 2011 and Microsoft excel sheet 2007 by qualified statistics expert.

Results

A total of 241 subjects were included in this study, all of which were males. The mean age was 32.4 years (standard deviation: 8.3, range: 23–51 years). A little over one third of the study subjects had CD4 lymphocyte counts less than 200 cells/mm³,

and 75% had a count of less than 350 cells/mm³. The mean of CD4 lymphocyte counts was 280.79 (standard deviation: 168.89) and the mean ALC counts were 1950.28 cells/mm³ (standard deviation: 989.18) for all subjects.

The lowest and highest CD4 lymphocyte counts were 5 and 939 cells/mm³ (Tables 1 and 2). There was a normal distribution of the patients' ALC counts with respect to stratified CD4 lymphocyte counts. The Pearson correlation coefficient was calculated to determine any linear correlation between ALC and CD4 levels. The Pearson correlation coefficient for the entire data set was 0.35 and after adjusting for the extreme values (outliers) it was 0.38. Similarly the coefficient of determination was 0.12 for the entire data set and 0.14 after adjusting for the outliers (Table 1).

It was found that there was a modest linear correlation between ALC and CD4 lymphocyte counts as evidenced by a Pearson coefficient of 0.35 (entire data set) and 0.38 (after adjusting the outliers). Graphical representation by scattered diagram of the correlation between the CD4 lymphocyte counts and ALC is depicted in Fig. 1.

Discussion

During early part of the decade 2000–10, CD4 testing was unavailable in most parts of developing worlds (where it was needed the most). Even where it was available the cost of testing was prohibitive for mass usage except when funding was available through research projects or insurance.

Studies done prior to 2005, showed a relationship between the declines in ALC and CD4 lymphocyte counts as a strong predictor for short term disease progression. Thus during the era of scarce availability of testing laboratories as well as prohibitive cost of CD4 testing, ALC became an acceptable surrogate marker of immune function. This was acceptable as the standard of care at that time.^{4–8} With progression of time, better ART drugs and regime combinations became available leading to an improvement in life expectancy and quality of life of PLHAs, in both the developing and developed countries. Over the years, with more data accumulation, new evidence emerged in 2007–08 that ALC has a low sensitivity and specificity as a proxy marker for CD4 levels.^{9–12} By 2009, WHO also came up with a position paper in which ALC was no longer a part of management strategy. They now included CD4 and preferably viral RNA levels for guiding management decisions.¹²

Thus the standard of care shifted from ALC to HIV RNA/CD4 levels. Over the years CD4 testing has become widely available and its cost has also decreased markedly (due to scales of economy). Government funded programmes have also penetrated deeper into remote areas with better

Table 2 – ALC distribution within grouped CD4 counts.

CD4 cell counts grouped data	N	Minimum	Maximum
<50	ALC 10	350	3450
50–100	ALC 21	600	2200
100–150	ALC 30	1150	3750
150–200	ALC 47	1100	2650
200–250	ALC 101	950	2800
250–300	ALC 7	1900	1900
300–350	ALC 4	2100	2100
350–400	ALC 3	1850	1850
400–450	ALC 2	4000	4400
450–500	ALC 1	2240	2240
500–550	ALC 1	2100	2100
550–600	ALC 2	2250	2294
600–650	ALC 1	1600	1600
650–700	ALC 3	2500	2500
700–750	ALC 1	14400	14400
750–800	ALC 1	1600	1600
>800	ALC 5	2400	2450

availability of CD4 testing. The CD4 testing equipment and kits have themselves been improved upon, over the years. Even HIV RNA levels are now available in many areas where earlier they were unheard of, enabling HIV RNA testing in select cases a possibility.¹³

With better tolerable HIV drugs and availability of generic formulations in the developing world these patients have now started showing better responses to therapy with resultant increased longevity. Today with modern drugs, the life expectancy of a PLHA is near normal compared to a non HIV infected person.¹⁴ Thus the focus of HIV pandemic has shifted from early mortality to longer life spans. Premature ageing manifesting as early osteoporosis, cataract and coronary artery disease, are the new burning issues in PLHAs today.

Two recent studies one by Nyawira Githinji et al and other by Sreenivasan et al have again highlighted the existence of a relationship between ALC and CD4 lymphocyte counts.^{2,15} Even these two studies reveal a weak-modest relationship between these two markers and definitely no capability of detecting immunological and virological failures likely to contribute of ART resistance. A similar study as ours by Sen S et al, in 2011 did not validate ALC as an effective surrogate marker of CD 4 lymphocyte counts.

Pearson correlation coefficient values < 0.35 are weak/low correlation, 0.36–0.67 modest/moderate correlation and >0.68–1.0 are considered strong/high correlations.¹⁶ Correlation coefficient is more of an abstract measure and does not give a precise interpretation. Coefficient of determination is more meaningful (determined by squaring the correlation coefficient).

Table 1 – Correlation of CD4 with ALC.

	N	Minimum	Maximum	Mean	Standard deviation
CD4	241	5	939	281	169
ALC	241	200	14400	1950	989
Pearson correlation coefficient 'r'	For entire data set: 0.35		After adjusting the outliers: 0.38		
Coefficient of determination 'r ² '	For entire data set: 0.12		After adjusting outliers: 0.14

Fig. 1 – Correlation graph between CD4 and ALC counts.

In our study, the Pearson correlation coefficient was 0.35 for the entire data set and 0.38 after adjusting the outliers, which at best can be called modest. The coefficient of determination was 0.12 and 0.14 for the complete data set and after adjusting the outliers respectively. Even though the Pearson correlation coefficient of 0.35–0.38 was statistically significant (at an alpha error of 0.05 and required sample size of 62 for correlation coefficient 0.35, actual sample size 241) the coefficient of determination was 0.12–0.14. This means that only 12–14% of the variation of ALC can be explained or accounted for by variation in CD4 levels. This translates to the fact that ALC levels cannot be relied upon as a surrogate marker of CD 4 levels and are not dependable for clinical decision making. Now, in such a scenario it is more important that management of PLHAs to be carried out using validated scientific tools like HIV Viral RNA levels or CD4 lymphocyte counts which will tell us about virological and immunological failure in addition to being more accurate in their own measurements. Use of ALC count will thus be a retrograde step in practise of current evidence based medicine.

Conclusion

Our study done at a different HIV/AIDS referral centre (in reference to the study by S Sen et al has also objectively revealed a statistical correlation between ALC and CD4 counts but not amounting to clinical significance and we agree with the findings of the latter).

The resurgence of publications reflecting a weak to modest relationship between CD4 lymphocyte counts and ALC should not change our practise of HIV care. To conclude, it's time we call curtains down on ALC as a surrogate marker for CD4 count.

Intellectual contribution of authors

Study concept: Lt Col Amitabh Sagar, Lt Col Biju Vasudevan
Drafting and manuscript revision: Lt Col Amitabh Sagar, Maj Vikas Ambiya, Brig Nardeep Naithani, Lt Col Biju Vasudevan

Statistical analysis: Lt Col Amitabh Sagar, Lt Col Sunil Agrawal
Study supervision: Lt Col Amitabh Sagar, Brig Nardeep Naithani, Maj Abhishek Pathak, Lt Col Biju Vasudevan

Conflicts of interest

All authors have none to declare.

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