Total Lymphocyte Count and Haemoglobin Concentration Combined as a Surrogate Marker for Initiating Highly Active Antiretroviral Therapy in a Resource-limited Setting as against CD_4 Cell Count

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ABSTRACT

Aim: *To find a sensitive and low-cost surrogate marker for CD_4 count for initiating highly active antiretroviral therapy (HAART) [CD_4 < 200 /mm^3], in the form of total lymphocyte count (TLC) < 1200 /mm^3 combined with haemoglobin (Hb) with multiple Hb cut-offs.*

Method: *Two hundred and three consecutive treatment-naïve adult HIV positive outpatients attending the virology clinic in World Health Organization (WHO) clinical stage 1, 2 or 3 were enrolled in the study. Their complete blood counts and CD_4 counts were done. Descriptive statistics was done by two methods correlating TLC alone with CD_4 and the other using combined marker of TLC and Hb with CD_4 count.*

Result: *Total lymphocyte count alone did not correlate well with CD_4 counts (r = 0.13; p = 0.065). Sensitivity of TLC < 1200 /mm^3 to predict CD_4 < 200 /mm^3 was low (23.27%) and the sensitivity of the combined marker (TLC + Hb) increased with higher Hb cut-offs.*

Conclusion: *Adding Hb to TLC markedly improved the sensitivity of the marker to predict CD_4 count < 200/mm^3. We also recommend a trade-off Hb cut-off of 10.5 g/dL for optimum sensitivity and specificity in this population subset.*

Keywords: Haemoglobin, HIV, surrogate marker, total lymphocyte count

Conteo Total de Linfocitos y Concentración de Hemoglobina Combinados como Marcador Sustituto para Iniciar la Terapia Antirretroviral Altamente Activa en un Contexto de Recursos Limitados Frente al Cconteo de Células CD_4

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RESUMEN

Objetivo: *Encontrar un marcador sustituto sensible y de bajo costo para el conteo de CD_4 para iniciar la terapia antirretroviral altamente activa (HAART) [CD_4 < 200 /mm^3], en forma de conteo total de linfocitos (CTL) < 1200 /mm^3 combinado con la hemoglobina (Hb) con múltiples valores de corte de Hb.*

Método: *Doscientos y tres pacientes adultos ambulatorios consecutivos, con SIDA pero sin tratamiento previo, que asistían a la clínica de virología en las etapas clínicas 1, 2, ó 3 de la Organización Mundial de la salud (OMS) 1, 2 ó 3, fueron captados para este estudio. Se les realizaron conteos sanguíneos y conteos de CD_4 completos. Se hizo una estadística descriptiva mediante dos métodos: uno que correlacionaba solo el CTL con el CD_4, y el otro que combinaba el marcador de CTL con el conteo de CD_4.*

Resultado: *El conteo total de linfocitos solo no tuvo una buena correlación con los conteos de CD_4 (r = 0.13;* p = *0. 065). La sensibilidad del CTL < 1200 /mm^3 para predecir CD_4 < 200/mm^3 fue baja (23.27%) y la sensibilidad del marcador combinado (CTL + Hb) aumentó en la medida que los valores de corte de Hb fueron mayores.*

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Conclusión: *La adición de Hb al CTL mejoró notablemente la sensibilidad del marcador para predecir el conteo de CD_4 < 200/mm³. También recomendamos un valor de corte de Hb negociable de 10.5 g/dL, para una óptima sensibilidad y especificidad en este subgrupo de población.*

Palabras claves: Hemoglobina, VIH, conteo total de linfocitos, marcador sustituto

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INTRODUCTION

Human immunodeficiency virus (HIV) chiefly affects and multiplies within CD_4 cells, mainly CD_4+ T_H cells. Hence, CD_4 cell counts are said to corroborate with the disease progression (1, 2). CD_4+ T_H lymphocytes activate all other cell lines of immunity by means of various releasing factors. Therefore, a fall in CD_4 cell count affects other cell lines in the immune system. This lapse in the immunity is the cause of opportunistic infections which form the basis of the World Health Organization (WHO) clinical classification. The patients in WHO clinical stage 4 qualify for initiation of highly active antiretroviral therapy (HAART), irrespective of blood counts. We have considered only patients in WHO clinical stages 1, 2 or 3, who may be deprived of HAART in a resource-limited setting. The use of HAART is limited by the cost of the therapy and also the risk of development of drug resistance. Hence, HAART is initiated when CD_4 count < 200/mm³ as per Centers for Disease Control and Prevention (CDC) guidelines or when total lymphocyte count (TLC) < 1200/mm³ as per the WHO. Many studies have found a strong correlation between TLC and CD_4 counts (3−6), while a few studies had contrary findings (7−9). Eventually, haemoglobin (Hb) concentration was proved to be an independent prognostic marker of AIDS (3, 10). The aim of this study was to test the cheaper surrogate markers of CD_4 counts as TLC and Hb independently as well as to better the conventional TLC surrogate marker by designing a novel algorithm in a subset of the Indian population. Our algorithm differs from other studies in using several Hb cut-offs in this Indian population subset.

SUBJECTS AND METHODS

After approval from the Institutional Ethics Committee of Seth GS Medical College and KEM Hospital, 203 consecutive treatment-naïve seropositive adult patients attending the virology clinic at a tertiary healthcare centre in Mumbai were included. At presentation, patients were staged clinically as per the revised WHO clinical staging recommendations. This was done on the basis of general examination and previous reports of patients from their respective referral centres. Patients already on HAART and in WHO clinical stage 4 were excluded since these patients were recommended to be put on HAART irrespective of their CD_4 counts (7). The remaining patients were investigated for their complete blood count including the total lymphocyte count and haemoglobin concentration and CD_4 cell counts by flow cytometry. Two algorithms were used to predict CD_4 count < 200/mm³ (Figure). In the first algorithm, only TLC

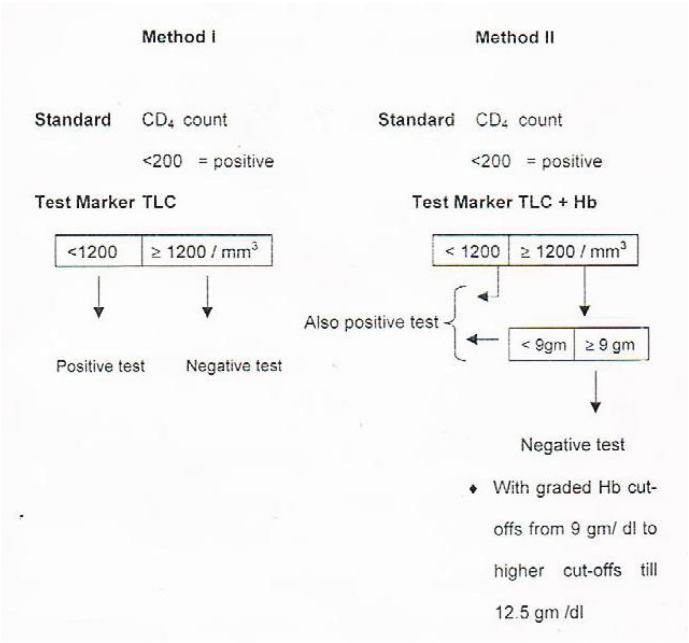

Figure: The surrogate marker algorithms.

was used as a marker, and a correlation between CD_4 counts and TLC was performed. Then, we used a single TLC cut-off of 1200/mm³ to predict an outcome of CD_4 count < 200/mm³. Hence, TLC < 1200/mm³ was defined as a positive test outcome and TLC ≥ 1200/mm³ was labelled a negative test outcome. These data were then analysed to obtain descriptive statistics. Next, we performed a correlation between CD_4 count and Hb. In the second algorithm, we intended to better the sensitivity of the earlier algorithm using Hb as a parameter. To facilitate the same, the positive outcome of this new algorithm was now determined as a summation of the group of positive test outcomes with TLC < 1200/mm³ in the first algorithm and also another group of patients with TLC ≥ 1200/mm³ but with Hb less than a cut-off value. This Hb cut-off was serially graded as 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5 g/dL.

As an example, if an Hb cut-off of 9 g/dL was selected as per the second algorithm, the new set of positive outcomes to determine CD_4 count of 200/mm³ would be a total of the patients with TLC < 1200/mm³ and patients with TLC ≥ 1200 but Hb concentration < 9 g/dL. A descriptive analysis was

done with this new algorithm with various serial Hb cut-off values.

The data from a total of 203 treatment-naïve seropositive patients were analysed using SPSS version 10 with level of significance being 5% and power of 80%. Pearson's correlation coefficient was used to find the correlation between TLC and CD_4 and then between Hb and CD_4 counts. Then a 2 × 2 contingency table was used to determine the descriptive analysis like the sensitivity, specificity, positive predictive value, and to compare the two algorithms.

RESULTS

The mean age of the 203 participants was 33.35 (± 8.15) years. Sixty-six (33%) were females and the remaining 134 (67%) were males. The mean CD_4 count was 224.35/mm^3 (± 174.3) and that of TLC was 1839.22/mm^3 (± 741.16). On comparing the CD_4 and TLC alone, the Pearson's coefficient of correlation (r) was found to be 0.130 and coefficient of variance was 0.017, *p*-value being 0.0671 (two-tailed). The mean haemoglobin value was 9.78 (± 1.62) g/dL. The Pearson's coefficient of correlation (r) was 0.273 and coefficient of variance was 0.075 with *p*-value being 0.0001 (two-tailed).

The data were analysed using the 2 × 2 contingency table. The results of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TLC alone, as well as TLC combined with Hb concentration to predict a CD_4 count < 200/mm^3 using both the algorithms are shown in the Table. The sensitivity of the first algorithm using TLC alone was 23.27% and specificity was 86.90% and that of the second algorithm using Hb cut-off of 10.5 g/dL was 80.17% and specificity was 40.47%. The PPV remained low in both cases (71.05% and 65.03%, respectively).

DISCUSSION

Highly active antiretroviral therapy is to be initiated in an HIV-positive patient with a CD_4 count < 200/mm^3. This cut-off is selected since HAART is limited by the cost of the therapy and also the risk of development of drug resistance if initiated prematurely, and risks of disease complications if HAART is started very late. These financial issues are compounded in resource-limited settings where CD_4 cell counts are cost-prohibitive. Hence, a cheaper surrogate for CD_4 cell count would benefit the resource-limited settings. With conflicting views in different studies regarding the use of only TLC as a CD_4 count surrogate marker, we came across another reliable, cheap and easily available marker such as Hb concentration (3−10). We designed an algorithm combining both these surrogate markers to determine a CD_4 count < 200/mm^3.

In this study, the sensitivity of the first algorithm using TLC < 1200/mm^3 alone to determine CD_4 count < 200/mm^3 was 23.27% and specificity was 86.90%. This would deprive many (76.73%) deserving patients from receiving HAART. There is a need to improve the sensitivity of this marker. Most studies used various graded TLC cut-offs, though we did not use the same in this study (3, 7, 11).

Table: Performance of surrogate marker with serially graded haemoglobin cut-off level

n = 203 **CD_4 count < 200/mm^3**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
First method: WHO criterion TLC < 1200/mm^3	23.27	86.90	71.05	45.06
Second method: combined with Hb				
a) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 9 g/dL)	49.13	73.80	72.15	51.23
b) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 9.5 g/dL)	54.31	61.90	66.31	49.52
c) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 10 g/dL)	64.28	57.14	66.67	52.17
d) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 10.5 g/dL)	80.17	40.47	65.03	59.64
e) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 11 g/dL)	83.62	28.57	61.78	55.81
f) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 11.5 g/dL)	89.65	17.85	60.11	55.56
g) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 12 g/dL)	92.24	11.90	59.11	52.63
h) TLC < 1200/mm^3 (as in first method) and (TLC ≥ 1200/mm^3 but Hb < 12.5 g/dL)	99.13	8.34	59.89	87.5

PPV = positive predictive value, NPV = negative predictive value, WHO = World Health Organization, Hb = haemoglobin, TLC = total lymphocyte count

In a study by Spacek *et al*, a similar TLC and Hb combined surrogate marker was used to determine CD_4 count < 200/mm^3 (7). This study used two different TLC cut-offs (1200 and 2000/mm^3) as against a single TLC (1200/mm^3) cut-off used in our study. Spacek *et al* also included patients who were on HAART, measured their serial cell counts and used four different methods with frequent use of flow cytometry, which we thought was not practical to implement in a resource-limited setting where frequent use of flow cytometry is cost-prohibitive. Hence, we chose to include only treatment-naïve patients, with flow cytometry being used only once for CD_4 cell counts and we designed a very simple, easy to understand algorithm.

The other issue was to select a suitable trade-off for optimum sensitivity and specificity. A test with poor sensitivity will deprive many needy patients of HAART and one with a very high sensitivity will be cost prohibitive and also raise the issue of drug resistance. In the study by Spacek *et al*, a single Hb cut-off of 12 g/dL was used. We instead selected serial graded Hb cut-offs in the second algorithm from which we recommend using a cut-off of 10.5 g/dL in this population subset to optimize the sensitivity (80.17%), specificity and PPV. This finding is in concert with the study by Spacek *et al* which states that the sensitivity of the marker can be improved by adding Hb as a parameter and that each setting must define its own algorithm to cater to a different subset of population. As a corollary, even in an economically sound setting, CD_4 count could be done selectively for patients with Hb < 10.5 g/dL (or a cut-off suitable for that subset of population) without performing the TLC, thereby reducing investigational costs.

We conducted this study in a specific subset of the population. There are many confounding factors such as malnutrition, concurrent subclinical infections, gender and physiological variations which affect both TLC and Hb (12). The incidence of anaemia depends on nutritional status, ethnicity, gender and pregnancy. Hence, there could be a variation in these surrogate parameters among the developing and developed countries. Though we cannot generalize the findings of this study since it is limited to this population subset, we do recommend that the sensitivity of the conventional surrogate marker can be improved with newer cost-effective algorithms. Similar algorithms should be designed for a larger sample size and different population subsets. More efforts are needed to scale-up access to CD_4 count. Also, we do understand that CD_4 count itself is a surrogate marker for the viral load (13).

CONCLUSION

Total lymphocyte count alone does not seem to correlate with the CD_4 count. Haemoglobin does seem to be an important predictor of disease activity. Adding Hb to TLC does improve the sensitivity of the marker to predict the CD_4 count < 200/mm^3, ensuring initiation of HAART and thereby reducing investigational cost. A trade-off of a Hb cut-off of 10.5 g/dL is suggested to be used to optimize the use of this surrogate marker in this population subset. The sensitivity of using TLC alone was 23.27% and specificity was 86.90%, but while also using Hb cut-off of 10.5 g/dL, sensitivity was 80.17% and specificity was 40.47%. The PPV remained low in both cases (71.05% and 65.03%, respectively). There is a glaring need to scale-up access to CD_4 count availability to accurately initiate and monitor HAART.

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