Correlation of CD4+ T cell Count with Total Lymphocyte Count, Haemoglobin and Erythrocyte Sedimentation Rate Levels in Human Immunodeficiency Virus Type-1 Disease

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Abstract

Background: Studies in human immunodeficiency virus (HIV) infected adults have demonstrated association of total lymphocyte count (TLC) $<1200/\text{mm}^3$ and subsequent disease progression or mortality. The association of other surrogate makers such as haemoglobin (Hb), and erythrocyte sedimentation rate (ESR) with CD4 count and disease progression has also been suggested. This study was carried out to determine the relationship of CD4-positive T lymphocyte counts with TLC, Hb and ESR in HIV-infected individuals.

Methods: The study population comprised of 215 antiretroviral treatment naïve HIV-1 infected adults. The CD4 positive T cell counts, TLC, Hb and ESR of study participants were measured. Spearman's rank order correlation and Receiver Operating Characteristic were used for statistical analyses.

Result: The sensitivity, specificity, positive and negative likelihood ratios for cut-off value of TLC $<1200/\text{mm}^3$ for predicting CD4 counts <200 cells/ mm^3 and <350 cells/ mm^3 were 9.4%, 100%, not measurable and 1.1, and 6.1%, 98.8%, 5.13 and 0.95, respectively. The association of Hb (<10 , 11, 12 g/dl and <10 , 12, 14 g/dl for CD4 counts <200 cells/ mm^3 and <350 cells/ mm^3 , respectively), and ESR (<10 , 20 and 30 mm fall after 1 hour) with these two CD4 counts cut-off values were suboptimal.

Conclusion: This study reveals the poor association of TLC, Hb, and ESR with CD4 counts in HIV infected adults, thus highlighting the need to review the utility of these surrogate markers, for predicting CD4 counts in people living with HIV/AIDS.

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Key Words : Human immunodeficiency virus; Total lymphocyte count; Haemoglobin; Erythrocyte sedimentation rate

Introduction

As per the 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among adolescents and adults [1], the clinical importance of CD4 counts in HIV-disease staging has been emphasized.

The World Health Organization (WHO) currently recommends initiation of antiretroviral therapy (ART) in people living with HIV/AIDS (PLHA) with CD4 T-lymphocyte counts <350 cells/ mm^3 irrespective of the WHO clinical staging [2]. In India, however, as per the National AIDS Control Organization (NACO) recommendations, initiation of ART should be considered if CD4 cell counts are less than $350/\text{mm}^3$ and in those with symptomatic HIV disease and CD4 cell counts between $200\text{--}350/\text{mm}^3$ [3].

Obtaining CD4 counts requires the use of expensive

tools, which are not readily available in resource-limited settings. The identification of laboratory tests that help the clinician to predict progression is useful not only to monitor the patients' disease evolution but also to define the appropriate time to initiate treatment.

According to the WHO guidelines [4], in the absence of CD4 counts, total lymphocyte count (TLC) $<1200/\text{mm}^3$, though a less useful substitute, can be used for starting ART in individuals with symptomatic HIV disease. There are studies in HIV-infected adults that have demonstrated association of TLC $<1200/\text{mm}^3$ and subsequent disease progression or mortality [5-7] as well as those which propose that the rate of TLC decline should be used in disease monitoring [7,8]. However, it is important to note that the WHO continues to recommend CD4 count as the main laboratory measurement for making decisions about when to start,

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stop, and change ART [4].

There are studies which have investigated the association of other surrogate markers such as haemoglobin (Hb), and erythrocyte sedimentation rate (ESR) with CD4 count and disease progression. In a study from Africa, ESR has been demonstrated to have a strong negative association with CD4 count [9]. While the rates of decrease in Hb have been reported to correlate with falling CD4 counts [5,6], there have been suggestions that increases in haemoglobin are predictive of treatment success when combined with an increase in TLC [8].

This study aimed to find out the relationship of CD4 counts with TLC, Hb and ESR and whether these parameters can be used as substitute surrogate markers for CD4-positive T lymphocyte counts in HIV-infected individuals.

Material And Methods

The study was a cross sectional pilot study, wherein the participants were HIV-1 positive adults admitted to, or attending outpatient departments at a tertiary care hospital between May and July 2009 (n=215). Those HIV-1 infected individuals who were previously ART-experienced were excluded from the study.

The blood and serum samples were collected after obtaining informed consent. The TLCs of study participants were measured using the Beckman Coulter AcT Diffuse II Haematology Analyzer. Haemoglobin was estimated using the cyanmethemoglobin method. ESR was measured using Wintrobe's method. CD4+ TLCs were obtained using FACS Counter (Becton, Dickinson and company).

The study participant data on the three surrogate markers TLC, Hb and ESR, were divided into 3 categories based on the CDC Classification System for HIV-Infected Adults and Adolescents, namely, Category 1: CD4 cells > 500 cells/mm³; Category 2: CD4 cells 200-499 cells/mm³; Category 3: CD4 < 200 cells/mm³ [1]. Simultaneously, the same participant data were divided into three additional CD4 cell count-based categories, namely Category 1: CD4 cells > 500 cells/mm³; Category 2: CD4 cells 350-499 cells/mm³; Category 3: CD4 < 350 cells/mm³.

The observations were statistically analyzed to calculate Spearman's rank order correlation with online software [10]. The cut-off points with best sensitivity and specificity combination were determined using online software [11] for receiver operating characteristic (ROC). Sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) with 95% confidence intervals were calculated.

Results

The study population comprised of 215 HIV-1 infected adult individuals of whom 179 were males and 36 females. The median age of the study population was 35 years (range: 24-60 years).

The scatter diagram in Fig. 1 depicts the distribution of the CD4+ T lymphocytes when plotted against the TLCs, Hb levels and ESRs of the study participants.

Spearman's rank order correlation was applied as a statistical test of significance on the different study population categories based on their respective CD4+ lymphocyte counts. It was observed that there was no correlation between TLC, Hb and ESR with any of the study subgroups, or the entire study population (Table 1).

Mean CD4, sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) with 95% confidence intervals for different levels of TLC, Hb and ESR cut-off values among the study specimens while predicting CD4 cell-counts <200 cells/mm³ and <350 cells/mm³ are shown in Table 2, 3, respectively.

Discussion

Spacek et al [12] (2003) studied the ability of TLC and Hb to predict CD4 count. A total of 3,269 individuals from the Johns Hopkins HIV Observational Cohort were evaluated retrospectively. While the cut off values for TLC below 1200 cells/mm³ and haemoglobin below 12 g/dl significantly predicted CD4 cell counts below 200 cells/mm³, for TLC alone, the sensitivity and specificity were reported as 70.7 and 81.7%, respectively. This

Fig. 1 : Scatter diagram showing the distribution of the CD4+ T lymphocytes plotted against the total lymphocyte counts (TLC), Haemoglobin (Hb) levels and Erythrocyte sedimentation rates (ESR) of the study participants.

study concluded that TLC <1200 cells/mm³ was associated with CD4 count <200 cells/mm³ and in addition, suggested that adding Hb to TLC will increase sensitivity, thereby reducing the risk of false-negative results. In a similar study, Badri et al [13] (2003) followed 266 patients at a hospital in South Africa to determine the usefulness of TLC versus CD4-count and viral load

Table 1

Table showing Spearman's rank order correlation between CD₄⁺ T cell counts based groups and total lymphocyte counts (TLC), haemoglobin (Hb) levels and erythrocyte sedimentation rates (ESR) in the study population

CD ₄ count (cells/mm ³) with TLC	Spearman's rank order correlation
Total (n=215)	+ 0.250471
SubGroups	
CD ₄ <200 (n=63)	+ 0.157
CD ₄ 200-500 (n=116)	- 0.141937
CD ₄ <350 (n=131)	+ 0.275
CD ₄ 350-500 (n=48)	- 0.310917
CD ₄ >500 (n=36)	+ 0.027298
CD ₄ count (cells/mm ³) with Hb	
Total (n=215)	+ 0.253945
SubGroups	
CD ₄ <200 (n=63)	+ 0.271842
CD ₄ 200-500 (n=116)	+ 0.037105
CD ₄ <350 (n=131)	+ 0.332325
CD ₄ 350-500 (n=48)	- 0.026005
CD ₄ >500 (cells/mm ³) (n=36)	- 0.035673
CD ₄ count (cells/mm ³) with ESR	
Total (n=205)	- 0.217
SubGroups	
CD ₄ <200 (n=58)	- 0.094739
CD ₄ 200-500 (n=113)	+ 0.237
CD ₄ <350 (n=125)	- 0.279330
CD ₄ 350-500 (n=46)	+ 0.231680
CD ₄ >500 (n=34)	- 0.090200

Table 2

Table showing Mean CD4, sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) with 95% confidence intervals for different levels of cut-off values for total lymphocyte counts (TLC), haemoglobin (Hb) levels and erythrocyte sedimentation rates (ESR) among the study specimens while predicting CD4⁺ T cell counts <200 /mm³

	Mean CD4 (cells/mm ³)	Sensitivity (%)	Specificity (%)	PLR	NLR
TLC (cells/mm³)					
1200	151.56	9.4 (4.8-18.9)	100 (97.5-100)	*	1.1 (1.02-1.19)
1500	241.08	18.8 (11-29.9)	96 (91.6-98.2)	4.72 (1.85-12.02)	0.85 (0.75-0.96)
1850	251.57	35.9 (25.3-48.1)	84.8 (78.2-89.6)	2.36 (1.43-3.88)	0.76 (0.62-0.92)
Hb (g/dL)					
>10	268.83	20.3 (12.3-31.7)	92.7 (87.4-95.9)	2.79 (1.32-5.89)	0.86 (0.75-0.98)
>11	282.7	31.3 (21.2-43.4)	82.8 (75.9-87.9)	1.82 (1.09-3)	0.83 (0.69-0.99)
>12	281.26	54.7 (42.6-66.3)	72.2 (64.6-78.8)	1.97 (1.39-2.76)	0.63 (0.47-0.84)
ESR (mm fall after 1 hour)					
>10	312.63	89.7 (79.2-95.2)	19 (13.5-26.2)	1.11 (0.96-1.25)	0.54 (0.24-1.24)
>20	294	81 (69.2-89.1)	48.9 (41-56.9)	1.59 (1.29-1.94)	0.39 (0.22-0.68)
>30	296.31	55.2 (42.5-67.3)	66.7 (58.7-73.8)	1.66 (1.19-2.29)	0.67 (0.49-0.92)

* Not measurable since the divisor is zero

measurements for monitoring patients on ART and found a significant correlation between changes in TLC and changes in CD4 count. Subsequently, Mahajan et al [14] (2004) examined the longitudinal variation between changes in TLC and concomitant changes in CD4 count following the initiation of ART. The study found that patients with a baseline CD4 count <250 cells/mm³ who had increases in TLC during the first two years on ART also experienced increases in CD4 count $>95\%$ of the time, concluding that positive trends in TLC in patients receiving ART suggest positive change in CD4 count as well. However, decreases in TLC did not predict the direction of CD4 change very well and patients who had decreases in TLC during the first two years on treatment had corresponding decreases in CD4 count only 43-63% of the time. The authors suggested that, when patients have drops in TLC, clinicians should determine whether these declines correlate with other clinical indications of deteriorating immune status such as development of an opportunistic infection. Overall, the direction of change in TLC was reported as a strong marker for a concomitant change in CD4 count with sensitivity and specificity ranging between 86-94 and 80-85%, respectively. Based on such clinical studies of CD4 count and TLC, the WHO recommended in its 2003 guidelines and 2006 newsletter that health facilities without the ability to perform CD4 measurement should use TLC to guide decisions on when to start ART in patients who are mildly symptomatic [4,15].

The data regarding relationship of ESR levels with CD4 cell decline has been conflicting. Vazquez et al [16] (2001) analyzed the clinical, immune and virologic implications of an elevated ESR in HIV-positive patients in Madrid. After conducting a retrospective crossover study of 350 HIV-positive patients, the relationship between the ESR (cut-off point: 20 mm/h), the clinical

Table 3

Table showing Mean CD₄, sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) with 95% confidence intervals for different levels of cut-off values for total lymphocyte counts (TLC), haemoglobin (Hb) levels and erythrocyte sedimentation rates (ESR) among the study specimens while predicting CD₄+ T cell counts < 350/ mm³

	Mean CD ₄ (cells/mm ³)	Sensitivity (%)	Specificity (%)	PLR	NLR
TLC (cells/mm³)					
1200	151.56	6.1 (3.1-11.6)	98.8 (93.6-99.8)	5.13 (0.65-40.28)	0.95 (0.9-0.99)
1500	241.08	11.5 (7.1-18)	90.5 (82-95.1)	1.2 (0.53-2.71)	0.98 (0.89-1.07)
1850	251.57	25.2 (18.5-33.3)	84.5 (75-90.7)	1.62 (0.91-2.9)	0.89 (0.77-1.01)
2100	266.57	35.9 (28.2-44.4)	72.6 (62.3-81)	1.31 (0.86-1.99)	0.88 (0.74-1.06)
2400	288.56	57.3 (48.7-65)	52.4 (41.8-62.7)	1.2 (0.92-1.57)	0.81 (0.61-1.08)
Hb (g/dL)					
>10	268.83	12.7 (8.1-19.4)	92.6 (84.8-96.6)	1.71 (0.7-4.17)	0.94 ((0.86-1.03)
>12	282.7	40.3 (32.4-48.8)	72.8 (62.3-81.3)	1.48 (0.98-2.24)	0.82 (0.68-0.99)
>14	281.26	79.1 (71.5-85.1)	33.3 (24-44.2)	1.19 (0.99-1.42)	0.63 (0.39-0.98)
ESR (mm fall after 1 hour)					
>10	312.63	87.2 (80.2-91.1)	21.3 (13.7-31.4)	1.1 (0.97-1.26)	0.6 (0.32-1.12)
>20	294	64 (55.3-71.9)	50 (39.3-60.7)	1.28 (0.99-1.65)	0.72 (0.52-0.99)
>30	296.31	43.2 (34.9-51.9)	67.5 (56.6-76.8)	1.33 (0.91-1.93)	0.84 (0.67-1.04)

status (symptomatic or asymptomatic), the immune status (CD₄, cut-off point: 200 cells/mm³) and viral status (viral load, cut-off point: 3 log) of the patients was analyzed. In 71% cases, the ESR levels were normal, while it was >20 mm/h in 29% cases. There was no significant relation either between ESR and clinical status, or between ESR and the CD₄ level. On adjusting for factors such as age, sex, gammaglobulin, hematocrit and co-infection with hepatitis C or B virus, the authors did not find a relation between the ESR and the clinical, immune or viral status of the seropositive patients. In contrast, Ndakotsu et al [9] (2008) in a study involving 104 consecutive ART naïve HIV-infected adults and 51 controls in Nigeria concluded that ESR may be useful in monitoring HIV/AIDS disease [9]. Recently, Morpeth et al [17] (2007) evaluated the performance characteristics of WHO staging criteria, anthropometrics and simple laboratory measurements for predicting CD₄ counts <200 cells/mm³ among 202 HIV-infected adult patients in Tanzania. It was concluded that the presence of mucocutaneous manifestations, TLC <1200 cells/mm³, or ESR ≥120 mm/h was a strong predictor of CD₄ count <200 cells/mm³ and enhanced the sensitivity of the 2006 WHO staging criteria for identifying patients likely to benefit from ART.

As discussed earlier, the utility of Hb as a surrogate marker for predicting CD₄+ T cell counts has been suggested [12]. Anastos et al [5] (2004) reported Hb < 10.6 g/dL to be consistently independently associated both with death and with AIDS defining illness and suggested further study to investigate the usefulness of haemoglobin level as an indication to provide ART in resource-limited settings. Moore et al [18] (2007) found that though TLCs appeared to be useful in predicting

the eligibility for ART based on CD₄ T cell counts, use of Hb values marginally improved the accuracy.

Of late, however, data has started emerging which is in disagreement with the above stated previous studies thus questioning the utility of the TLC cut off value < 1200/mm³ for predicting CD₄ counts < 200 cells/mm³ in a resource limited condition. Gupta et al [19] (2007) assessed the sensitivity, specificity and PPV of TLC for predicting low CD₄ counts in antepartum and postpartum women in Pune, India. In this study, CD₄, TLC, and haemoglobin were measured at third trimester, delivery and 6, 9, and 12 months postpartum in a cohort of 779 HIV-infected women. Sensitivity, specificity and PPV using TLC <1200 cells/mm³ for predicting CD₄ <200 cells/mm³ was reported as 59, 94, and 47%, respectively. The authors opined that the WHO-recommended TLC cut-off of <1200 cells/mm³ is not optimal for identifying antepartum and postpartum Indian women who require ART. Similarly, in a study conducted by Gitura et al [21] (2007) at Nairobi, the classification utility of TLC cut-off value of 1200 cells/mm³ for patients as having a CD₄ counts < 200 cells/mm³ was reported as suboptimal, with 37% sensitivity, 99% specificity and 56% NPV. Additionally, an optimal TLC cut-off of 1900 cells/mm³ cut-off was found to be of greatest utility for this study population to classify patients as either above or below the CD₄ count cut-off value of 200 cells/mm³, with sensitivity, specificity, PPV and NPV of 81.1%, 90.3%, 90.8% and 80.2%, respectively. A study of 2019 HIV-infected subjects in Ethiopia by Daka et al [22] (2008) revealed sensitivity, specificity, positive and negative predictive values of TLC < 1200/mm³ to predict CD₄ count < 200 cells/mm³ to be 41%, 83.5%, 87.9% and 32.5%, respectively, once again highlighting low

sensitivity and specificity of TLC as a surrogate measure for CD4 count.

In the current study, we analyzed a total of 215 HIV-1 infected study participants. Our findings reveal that the utility of a cut off TLC value of 1200/mm³ for correlating with CD4 cell count < 200/mm³ was not adequate. The sensitivity, specificity, PLR and NLR at this TLC cut off value was 9.4%, 100%, not measurable and 1.1, respectively. On raising the TLC cut off value to 1500- and 1800-cells/mm³, there was a marginal improvement in the sensitivity to 18% and 35.9% respectively, which thus continued to be suboptimal, and were associated with a lowering in specificity of association to 96% and 86.8%, respectively (Table 2). Similar analyses to investigate the association of cut off levels of Hb (<10, 11, 12 g/dl), and ESR (<10, 20 and 30 mm fall after 1 hour) in our study subjects showed that their relationship with CD4 counts < 200 cells/mm³ were not optimal (Table 2). The results were similar when the relationship of CD4 counts <350 cells/mm³ were compared with TLC, Hb and ESR levels (Table 3). With cut-off value of TLC ranging between 1200-2400 cells/mm³ at this CD4 count cut-off level, the sensitivity, specificity, PLR and NLR changed from 6.1 to 57.3%, 98.8 to 52.4%, 5.13 to 1.2%, and 0.95 to 0.81%, respectively. A similar trend was observed between CD4 count at this cut-off level when compared with Hb and ESR levels. Thus, our study analyzing the relationship of TLC, Hb and ESR with CD4 counts at two cut-off levels (<200 cells/mm³ and <350 cells/mm³) was in agreement with recent studies from India and abroad [19-21] highlighting the need to review the utility of these surrogate markers, namely TLC, Hb, and ESR for predicting CD₄ counts in PLHA.

While there are studies from the Americas, Europe and Africa [5-13,16-18,21-22] that have attempted to define the association and cut-off levels for various haematological parameters viz. TLC, Hb and ESR in HIV-1 infected individuals towards prediction of CD4 lymphocyte counts, the same for Indian subcontinent in sparse [14,19,20].

The current study is a pilot study carried out at a tertiary care centre to investigate the relationship of the above stated parameters. While this study demonstrates that in HIV-1 infected individuals the association of TLC, Hb and ESR in predicting of CD4 lymphocyte counts is poor, the same needs to be validated with large scale, multi-centric studies in India so as to investigate the utility of such cheaper laboratory test modalities, namely TLC, Hb, and ESR, if any, in predicting HIV-1 disease progression and initiating antiretroviral therapy in resource limited settings.

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Conflicts of Interest

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