Original Article

Total lymphocyte count as a tool for timing opportunistic infection prophylaxis in resource-limited settings: a study from India

Sreenivasan Srirangaraj and Dasegowda Venkatesha

Department of Microbiology, Mysore Medical College and Research Institute, Mysore-570001, Karnataka, India

Abstract

Introduction: In resource-limited settings, due to the high cost of CD4 cell count testing, physicians must decide about opportunistic infection (OI) prophylaxis without a laboratory evaluation of HIV stage and level of immune suppression. This study aimed to evaluate the correlation of total lymphocyte count (TLC), an inexpensive laboratory parameter, to CD4 count, and to determine a range of TLC cut-offs for the initiation of OI prophylaxis that is appropriate for resource-limited settings.
Methodology: Spearman correlation between CD4 count and TLC was assessed in patients attending the Anti-Retroviral Therapy (ART) centre at Mysore, India. Positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of various TLC cut-offs were computed for CD4 counts < 200 cells/mm^3. Correlation and statistical indices were computed for all patients and for HIV patients with active tuberculosis.
Results: Good correlation was noted between the 106 paired TLC and CD4 counts (r = 0.3497).TLC < 1200cells/mm^3 had 88.14% sensitivity and 34.78% specificity for CD4 count < 200 cells/mm^3. In those patients with active tuberculosis, TLC< 2000cells/mm^3 had 95.24% sensitivity and 100% specificity for CD4 count < 200cells/ mm^3.
Conclusions: TLC could serve as a low-cost tool for determining when to initiate prophylaxis in resource-constrained settings.

Key words: total lymphocyte count; CD4 counts; opportunistic infection; tuberculosis

Introduction

In resource-limited settings, chemoprophylaxis for opportunistic infections (OIs) remains an important intervention that may improve quality of life, decrease morbidity, and lengthen survival of HIV-positive patients [1,2]. The enumeration of CD4^{+} T-lymphocytes in the peripheral blood is an essential tool for the laboratory monitoring of HIV-infected patients, both for the progression of disease and for the assessment of the outcome of the anti-retroviral treatment [3].

A CD4 count of less than 200 cells/mm^3 has been associated with increased risk of developing *Pneumocystis carinii* pneumonia (PCP) in HIV-positive patients [4] and is the recommended reference point for initiation of cotrimoxazole prophylaxis [5]; however , in resource-limited settings, CD4 count testing is very expensive and is not widely available because of a lack of sophisticated equipment.

Total Lymphocyte Count (TLC) is an inexpensive and widely available laboratory parameter, which is easily obtained from the routine complete blood count (CBC) with differential by multiplying percentage lymphocytes by leukocyte count [6]. The World Health Organization (WHO) has suggested that TLC in combination with clinical staging can be a useful marker of prognosis and survival in HIV-infected individuals, in areas where CD4 counts are not available [7].

There are very few studies examining the correlation of CD4 count to TLC in a cohort of HIV-positive patients from India [8].There is also little reported data regarding the correlation of TLC to CD4 counts in patients co-infected with HIV and tuberculosis (TB) [8,9].The need to assess TLC as a surrogate for CD4 count in patients with HIV and TB is underscored by the high prevalence of co-infection in resource-limited settings. Thus this study was conducted to evaluate the correlation of TLC to CD4 counts in HIV-positive patients attending the Anti-Retroviral Therapy (ART) centre in Mysore, Karnataka, which is one of the high-prevalence states in India [10] and discuss the role TLC could play in the management of OI prophylaxis in resource-limited settings.

Methodology

Study design and setting

From August 2006 to August 2007, 108 HIV-positive patients attending the ART Centre, K. R. Hospital, Mysore, India, were selected to participate in a prospective observational cohort study. K. R. Hospital, the largest government hospital in Mysore, provides comprehensive clinical care and counseling to HIV-positive patients from Mysore and surrounding districts through a dedicated ART centre. This study was approved by the ethics Committee of our institution.

After providing informed consent for HIV testing, individuals voluntarily attending the Integrated Counseling and Testing Centre (ICTC) at the Department of Microbiology, Mysore Medical College and Research Institute, Mysore (or any of the Government designated ICTCs), underwent pre-test counseling by male or female ICTC counselors, followed by HIV testing per strategy III of the National AIDS Control Organization (NACO) guidelines (for HIV testing) [11]. After post-test counseling, those found HIV positive were referred to the ART Centre, K. R. Hospital, Mysore Medical College and Research Institute, Mysore, where they underwent pre-ART counseling. After clinical evaluation, informed consent was taken from these patients and they were enrolled into the study if they satisfied the inclusion criteria.

Inclusion criteria

The inclusion criteria were as follows: individuals should be above 18 years of age, proven to be HIV-positive, and should not be on prior anti-retroviral therapy. Those individuals found to be HIV-seronegative and those on prior ART were excluded.

Those found eligible for ART per the WHO guidelines [7] were started on anti-retroviral therapy. CD4 counts and total lymphocyte counts obtained on the same day were determined by chart review and recorded as pairs. Chart review also included documentation of age, sex, and diagnosis of active pulmonary TB. Diagnosis of TB was based on consistent history, physical exam, and positive sputum tests for acid-fast bacilli or radiologic features compatible with pulmonary TB.

Standard treatment of TB consisted of isoniazid and rifampicin for six to nine months with ethambutol and pyrazinamide during the initial two months per the Revised National Tuberculosis Control Programme (RNTCP) guidelines [10].

The CD4/CD3 enumeration was done using the single-platform BD FACS Calibur™ machine (Becton, Dickinson and Company, San Jose, United States of America), by strictly following the manufacturer's instructions. Internal quality control was performed with process controls using the manufacturer's recommendations. External quality control was performed through an external quality assurance programme with the National AIDS Research Institute (NARI), Pune, India.

TLC was derived from the CBC by multiplying lymphocyte percentage by the white blood cell count. Correlation between CD4 count and TLC was assessed by computation of Spearman rank order correlation for all paired counts. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of various TLC ranges were also computed for CD4 count lower than 200 cells/mm^3. Assessment of correlation using the above methods was performed for all paired counts as well as for paired counts in patients with active TB.

All statistical analyses were performed using SPSS software (version 16.0, SPSS, Chicago, USA).

Results

During the study period, 108 patients generated 106 paired TLC and CD4 counts. The mean age was 34.69 years. Of the 108 patients, 71 (65.74%) were males and 37 (34.26%) were females. The mean CD4 count and mean TLC at baseline were 129.65 ± 76.84cells/mm^3 and 1262.96 ± 738.98 cells/mm^3, respectively. Seventy (64.8%) of the 108 patients were from rural areas and 96 (88.89%) had an income of less than US $1 per day. Ninety (83.4%) of the patients stated that they acquired HIV through heterosexual intercourse. In all, 25 patients had active tuberculosis.

All paired CD4 count and TLC (n = 106) showed good correlation by Spearman correlation analysis (r = 0.3497 [corrected for ties]; 95% confidence interval: 0.1647 to 0.5109; the two-tailed P value is 0.0002, which is considered significant). Detailed correlation analyses of different TLC values for CD4 count are shown in Table 1.

The significantly highest sensitivity value for CD4 < 200 cells/mm^3 was observed at TLC < 1000 cells/mm^3 (P 0.0062), whereas the highest positive predictive value was seen at TLV < 1300 cells/mm^3.

Table 1. Positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of TLC for CD4 count <200 cells/mm^3 in all paired counts (n = 106)

TLC(cells/mm^3)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
<2000	68.28	27.27	90.36	7.9
<1900	69.52	28.57	87.95	11.11
<1800	79.78	29.41	85.54	21.74
<1700	80.68	33.33	85.54	26.09
<1600	79.52	26.09	79.52	26.09
<1500	79.75	25.93	75.90	30.43
<1400	79.73	25.00	71.08	34.78
<1300	82.35	27.03	67.47	45.45
<1200	63.41	69.57	88.14	34.78
<1100	58.54	73.91	88.89	33.33
<1000	46.99	86.36	92.86	30.16

Table 2. PPV, NPV, sensitivity and specificity of TLC for CD4 count <200 cells/mm^3 in all paired counts (n = 25) from patients with active tuberculosis

TLC(cells/mm^3)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
<2000	100	80.00	95.24	100
<1900	82.61	0.00	90.48	0.00
<1800	82.61	0.00	90.48	0.00
<1700	82.61	0.00	90.48	0.00
<1600	80.95	0.00	80.95	0.00
<1500	80.00	0.00	76.19	0.00
<1400	80.00	0.00	76.19	0.00
<1300	84.21	16.67	76.19	25.00
<1200	83.33	14.29	71.43	25.00
<1100	87.50	22.22	66.67	50.00
<1000	92.31	25.00	57.14	75.00

Using a TLC cut-off of 1,200 cells/mm^3 or less would identify 88.14% of patients with a CD4 count of < 200 cells/mm^3. However, the specificity was less (34.78%).

Correlation analyses were also performed for patients with active TB. The Spearman correlation coefficient for all paired CD4 count and TLC (*n* = 25) in these patients was 0.3685 with a 95% confidence interval (-0.0436 to 0.6734); and the two-tailed P value was 0.0699. The result is marginally significant [12]. The positive predictive value, negative predictive value, sensitivity, and specificity of TLC for CD4 count < 200 cells/mm^3 are listed in Table 2.

TLC 1900-2000 cells/mm^3 would identify 95.24% of co-infected patients with a CD4 < 200 cells/mm^3 (the two-sided P value is 0.0004, which is considered extremely significant).

Discussion

In this study, we have demonstrated that TLC, a widely available and inexpensive parameter, can be used in place of CD4 count as a routine marker of immune status and trigger for OI prophylaxis.

In this cohort of Indian patients, there was a good correlation between TLC and CD4 count by Spearman rank order correlation (r = 0. 3497), indicating a moderately positive association between TLC and CD4 counts. However, Spearman correlations between TLC and CD4 count reported in North America (r = 0.77) [13], England (r = 0.76) [14] and India (r = 0.744) [8] were higher when compared with the results of this study. This difference could be due to the small size of the current study sample.

We found that a TLC < 1200 cells/mm^3 had a 63.41% PPV, 69.57% NPV, and was 88.14% sensitive, and 34.78% specific, for a CD4 count < 200 cells/mm^3. This shows that the WHO prescribed limit of TLC < 1200 cells/mm^3 [7] is a sensitive marker, when used as a surrogate for CD4 < 200cells/mm^3. However, it was not a specific marker.

Selecting TLC cut-offs for prophylaxis administration

The determination of which TLC cut-offs to use as markers for initiating cotrimoxazole prophylaxis should be made on a regional basis so that variations in OI incidence, antimicrobial resistance patterns, and availability and accessibility of the drug can be accounted for.

In general, higher TLC cut-offs that maximize sensitivity should be used in regions with high OI morbidity that is preventable by cotrimoxazole, and where incidence of these infections occurs at an earlier clinical stage. By maximizing sensitivity, patients most at risk for OI by the CD4 count threshold criteria of < 200 cells/mm^3 are most likely to receive prophylaxis [8]. As a guideline for initiating prophylaxis in this case, we would recommend the WHO prescribed TLC < 1200 cells/mm^3 for CD4 count < 200 cells/mm^3.

In general, lower TLC cut-offs that maximize positive predictive value should be used in regions where rates of antimicrobial resistance to cotrimoxazole and toxicity of the drug are increasing and/or where cotrimoxazole supply or ability to pay

for long-term prophylaxis is a problem. Some experts have suggested that widespread use of cotrimoxazole in sub-Saharan Africa may result in resistance in such pathogens as non-typhoidal *Salmonellae*, *Pneumococci* and even *Plasmodium falciparum*, because sulfadoxine-pyrimethamine is used as a first-line therapy for malaria in many African countries [15].

By maximizing positive predictive value, patients who receive cotrimoxazole are most likely to be at risk for OIs and would most benefit from prophylaxis. However, a number of patients with a CD4 count less than the threshold criteria, who are also at risk, would not receive prophylaxis as indicated by the lower sensitivities of lower TLC cut-offs relative to higher TLC cut-offs. As a guideline for initiating prophylaxis in this case, we would still recommend selecting a TLC < 1,200 cells/mm^3 for a CD4 count < 200 cells/mm^3.

TLC in patients with tuberculosis

Although TB is an AIDS-defining illness, consensus about starting cotrimoxazole prophylaxis in all HIV–TB co-infected patients has not been reached. Some clinicians cite the risk of developing cotrimoxazole resistance, cost to the patient, and excessive pill burden as reasons for using the CD4 count or TLC for determining prophylaxis in patients with TB [16].

In India, TB is the most common OI with an estimated 62% of HIV-positive patients affected [17]. Due to potential fluctuations in CD4 count related to TB-related immune activation [18] and anti-TB therapy [9], an additional objective of this study was to confirm the correlation of TLC to CD4 count in a cohort of patients co-infected with TB and HIV [9].

In a South African cohort of co-infected TB–HIV-positive patients on isoniazid, rifampicin, and pyrazinamide for one month, Martin *et al*. [12] found that a TLC of less than 1,400 cells/mm^3 had a positive predictive value and sensitivity between 80-85% for a CD4 count of less than 200 cells/mm^3. In an Indian study, Kumaraswamy *et al*. found that a TLC of less than 1600 cells/mm^3 had a positive predictive value and sensitivity of 77% and 86%, respectively, for a CD4 count of less than 200 cells/mm^3 [8]. But, in our study, we found that a TLC of less than 2000cells/mm^3 had a positive predictive value and sensitivity of 100% and 95.24 % respectively, for a CD4 count of less than 200cells/mm^3. However, more prospective longitudinal studies of CD4 count, TLC, and

incidence of tuberculosis among HIV-positive patients of different ethnic groups and regions are needed to establish optimal region-specific prophylaxis guidelines.

The main limitation of this study was the small size of the study sample, which was due to lack of any financial support and the high cost of conducting the CD4 testing.

In conclusion, this study has demonstrated that TLC could serve as a low-cost tool for determining when to initiate OI prophylaxis in resource-limited countries.

Acknowledgements

References

1. Dayton JM and Merson MH (2000) Global dimensions of the AIDS epidemic: implications for prevention and care. Infect Dis Clin North Am 14: 791-808.
2. Kaplan JE, Hu DJ, Holmes KK, Jaffe HW, Masur H, De Cock KM (1996) Preventing opportunistic infections in human immunodeficiency virus-infected persons: implications for the developing world. Am J Trop Med Hyg 55: 1-11.
3. Pattanapanyasat K and Thakar MR (2005) CD4+ T cell count as a tool to monitor HIV progression & anti-retroviral therapy. Indian J Med Res 121: 539-549.
4. Phair J, Munoz A, Detels R, Kaslow R, Rinaldo C, Saah A (1990) The risk of Pneumocystis carinii pneumonia among men infected with human immunodeficiency virus type 1. Multicenter AIDS Cohort Study Group. N Engl J Med 322: 161-165.
5. U.S.Public Health Service (USPHS) and Infectious Disease Societyof America (IDSA) (2010) USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. MMWR 48: 1-66.
6. Mahajan AP, Hogan JW, Snyder B, Kumarasamy N, Mehta K, Solomon S, Carpenter CC, Mayer KH, Flanigan TP (2004) Changes in total lymphocyte count as a surrogate for changes in CD4 count following initiation of HAART: implications for monitoring in resource-limited settings. J Acquir Immune Defic Syndr 36: 567-575.
7. World Health Organization (2006) Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach. Available: www.who.co.in. Accessed 4 March 2010.
8. Kumarasamy N, Mahajan AP, Flanigan TP, Hemalatha R, Mayer KH, Carpenter CC, Thyagarajan SP, Solomon S (2002) Total lymphocyte count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India and other resource-constrained countries. J Acquir Immune Defic Syndr 31: 378-383.
9. Martin DJ, Sim JG, Sole GJ, Rymer L, Shalekoff S, van Niekerk AB, *et al.* (1995) CD4+ lymphocyte count in African patients co-infected with HIV and tuberculosis. J Acquir Immune Defic Syndr Hum Retrovirol 8: 386-391.
10. Park K (2007) Park's Textbook of preventive and social medicine, 19th edition. Jabalpur: Banarsidas Bhanot 768 p.
11. National AIDS Control Organization (2001) HIV testing manual: Laboratory diagnosis, Biosafety and Quality Control. New Delhi: National AIDS Control Organization 162 p.
12. Chap T Le (2010) Introductory Biostatistics New Jersey: John Wiley & Sons 536 p.
13. Fournier AM, Sosenko JM (1992) The relationship of total lymphocyte count to CD4 lymphocyte counts in patients infected with human immunodeficiency virus. Am J Med Sci 304: 79-82.
14. Beck EJ, Kupek EJ, Gompels MM, Pinching AJ (1996) Correlation between total and CD4 lymphocyte counts in HIV infection: not making the good an enemy of the not so perfect. Int J STD AIDS 7: 422-428.
15. Badri M, Ehrlich R, Wood R, Maartens G (2001) Initiating co-trimoxazole prophylaxis in HIV-infected patients in Africa: an evaluation of the provisional WHO/UNAIDS recommendations. AIDS 15: 1143-1148.
16. Wiktor SZ, Sassan-Morokro M, Grant AD, Abouya L, Karon JM, Maurice C, Djomand G, Ackah A, Domoua K, Kadio A, Yapi A, Combe P, Tossou O, Roels TH, Lackritz EM, Coulibaly D, De Cock KM, Coulibaly IM, Greenberg AE (1999) Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1-infected patients with tuberculosis in Abidjan, Cote d'Ivoire: a randomised controlled trial. Lancet 353: 1469-1475.
17. Misra SN, Sengupta D, Satpathy SK (1998) AIDS in India: recent trends in opportunistic infections. Southeast Asian J Trop Med Public Health 29: 373-376.
18. Wallis RS, Vjecha M, mir-Tahmasseb M, Okwera A, Byekwaso F, Nyole S, Kabengera S, Mugerwa RD, Ellner JJ (1993) Influence of tuberculosis on human immunodeficiency virus (HIV-1): enhanced cytokine expression and elevated beta 2-microglobulin in HIV-1-associated tuberculosis. J Infect Dis 167: 43-48.

Corresponding author

Dr. S Srirangaraj
Department of Microbiology
Mahathma Gandhi Medical College and Research Institute
Pondicherry-607402, India
Tel: 0413-2615996; Mobile: 09787095587
Email: rangaraj1980@indiatimes.com

Conflict of interests: No conflict of interests is declared.