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CORRELATION BETWEEN CLINICAL PROFILE, CD 4 COUNT AND TOTAL LYMPHOCYTE COUNT IN HIV INFECTED PERSONS

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ABSTRACT: BACKGROUND & OBJECTIVES: HIV infection can be monitored by laboratory and clinical markers of disease progression. In the absence of CD 4 count, the use of Total Lymphocyte Count has been advocated to predict CD 4 count and to stage HIV disease. This study was undertaken to show whether the TLC accurately predicts a low CD 4 count in HIV infected persons and its clinical correlation. **METHODOLOGY:** The data for the study was collected from HIV positive inpatients and outpatients of KIMS Hospital, Bangalore from March 2004 to March 2006. A total 50 patients were subjected to clinical examination and relevant investigations including CD 4 Count and TLC. **RESULTS:** In our study, males (39), outnumbered females (11). The major risk factor for HIV infection in these patients was unprotected, multiple sexual contacts. Fever, Anorexia, Weight loss, Lethargy, Cough, Diarrhea and Mouth ulcers were the common presenting symptoms. Tuberculosis, Chronic diarrhea, Oropharyngeal candidiasis were the most common opportunistic infections. Majority of the patients had CD 4 Count less than 350 cells/ μ l and were symptomatic. The Total Lymphocyte Counts of 1750 cells/ μ l and 2450 cells/ μ l correlated to CD 4 counts of 200 cells/ μ l and 350 cells/ μ l respectively. **INTERPRETATION & CONCLUSION:** There was a highly significant correlation between CD 4 Count and Total Lymphocyte Count. TLC can be used as an effective laboratory tool to monitor disease progression in HIV infected persons where CD 4 is not available and in resource poor countries.

KEYWORDS: TLC, CD 4 Count, HIV infection, Correlation.

INTRODUCTION: The Ministry of Health and Family Welfare and National AIDS Control Organization has estimated that the current number of people living with HIV infection in India is more than 5.13 million¹. It is estimated that 90% of HIV infected persons worldwide live in resource poor developing countries². The number of people living with HIV/AIDS continues to increase in several regions, most markedly in sub-Saharan Africa, with Southern Africa registering the highest prevalence. Asia and the Pacific as well as Eastern Europe and Central Asia continue to experience expanding epidemics, with the number of people living with HIV/AIDS growing year by year³.

The cost of antiretroviral therapy (ART) is dropping dramatically for low-income countries through various international initiatives. As availability of antiretroviral medications improves, it is important to develop feasible strategies for the clinical management of antiretroviral therapies in resource-limited settings¹. One of the major obstacles to the administration of ART is the absence of sophisticated and expensive laboratory equipment and infrastructure required for monitoring the efficacy of therapy¹⁻⁴. In industrialized nations, changes in CD 4 count and plasma viral load are used to determine the responses of the virus to antiretroviral therapy. Standard methods of CD 4 count and plasma viral load enumeration require highly trained personnel and tens of thousands of dollars of initial investment in laboratory instrumentation⁵. In few resource limited countries where such

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laboratory facilities exist, they are often concentrated in major cities and the assays are too expensive for the majority of patients to use for routine monitoring of ART.

Hence the WHO recommends the use of TLC to monitor the immune response to ART². TLC is an inexpensive and widely available laboratory parameter. TLC is easily obtained from the routine complete blood count (CBC) with differential by multiplying percentage lymphocytes by leukocyte count. In India, where the average annual income is < \$ 350 (US), the cumulative cost of monitoring SRT becomes a significant financial challenge⁶.

In light of its low cost and widespread availability, TLC has already been a useful tool in low-income countries for predicting immunosuppression and triggering opportunistic infection prophylaxis⁷⁻¹¹. Recent studies have also demonstrated that TLC alone and in combination with hemoglobin may be useful in determining when to initiate antiretroviral therapy¹²⁻¹⁴. However, there are fewer studies examining the change of TLC in patients on antiretroviral therapy¹⁵.

The purpose of this dissertation is to assess the capability and clinical utility of TLC change to serve as a surrogate marker for CD 4 count change in monitoring patients, which has important implications for resource-limited settings. We are correlating CD 4 Count to Total Lymphocyte Count, which is available in all resource limited settings as it is obtained by multiplying Total Leucocyte Count and percentage of Lymphocyte in Differential Count, to monitor disease progression in HIV infected persons.

This dissertation is also an attempt to look into the correlation between opportunistic infections and progression of the disease, which is monitored by change in CD 4 count and TLC. Since our study is being done in the resource poor set-up, the observations and conclusions can be used for monitoring progression of disease in HIV infected persons in our own set-up as well as other resource limited settings.

OBJECTIVE:

- To determine whether the Total Lymphocyte Count accurately predicts a low CD 4 count in HIV infected persons and its clinical correlation.

METHODOLOGY:

SUBJECTS AND METHODS:

Sources of data: In this study, all HIV positive patients with or without symptoms who visited KIMS Hospital as outpatients or as inpatients in the medical wards.

Place of study: The study was conducted in Kempegowda Institute of Medical Sciences and Research Centre, Bangalore.

Study Period: The study was conducted from March 2004 to March 2006.

Study Design: A Cross sectional descriptive study.

Inclusion Criteria:

- All HIV positive patients.

Exclusion Criteria:

- Cases on cytotoxic drugs.
- Cases with connective tissue disorders.

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Collection of Data: A total of 50 HIV positive patients who were admitted in KIMS Medical wards and those who visited Medical OPD were the study group.

A detailed history was obtained using a pre-tested proforma from all the patients, who were included in the study. Further, a detailed systemic examination followed by relevant investigations as mentioned in the proforma were conducted and results were noted.

METHODS: Venous samples were sent for evaluation of CD 4 count by Flow cytometry method.

Sample taken at the same time was also sent for evaluation of Total count and Differential count.

HIV infection was proved with: HIV antibody detection by ELISA method

Total lymphocyte count (TLC) was calculated by multiplying the differential count (DC) with Total leukocyte count (TC).

$$[TLC = TC \times DC]$$

Sensitivity and specificity of various Total lymphocyte count cut-off were computed for CD 4 count < 200 cells/ μ l and < 350 cells/ μ l. We evaluated changes in Total Lymphocyte count as a diagnostic monitoring marker of benchmark changes in CD 4 count that indicates favorable response to ART.

In the patients who were symptomatic the following investigations were done wherever necessary.

Collection and Processing of Specimens: Various samples e.g. Sputum, oral swab, blood, stool, urine, cerebrospinal fluid (CSF), lymph node aspirate were collected as per symptoms and clinical presentations. All the specimens were collected under universal aseptic precautions in suitable sterile containers¹⁶.

ETHICAL CLEARANCE: The ethical clearance was obtained from the Ethical Committee of Kempegowda Institute of Medical Sciences, Bangalore.

STATISTICAL METHODS: Analysis of variance has been used to find the significance of study parameters with variations with CD4 counts.

Pearson correlation co-efficient has been used to find the correlation of CD4 counts with study parameters.

Statistical software: The Statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS: Between March 2004 and March 2006, 50 HIV positive patients admitted in KIMS hospital or patients who were examined in the Medical OPD were selected to constitute the study population.

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Age in years	Male		Female		Total	
	No	%	No	%	No	%
≤30	4	10.3	4	36.4	8	16.0
31-40	17	43.6	5	45.5	22	44.0
41-50	11	28.2	2	18.2	13	26.0
51-60	6	15.4	-	-	6	12.0
>60	1	2.6	-	-	1	2.0
Total	39	100.0	11	100.0	50	100.0
Mean ± SD	41.38±10.10		35.45±8.85		40.08±10.07	

Table 1: Age distribution with sex

It is observed that 22 (44%) patients were in the age group of 31-40.

Risk exposure	Total (n=50)	Male (n=39)	Female (n=11)
A. Sexual contacts	48 (96.0%)	37(74.0%)	11(22.0%)
1. Hetero sexual	31 (64.6%)	27(72.9%)	4(36.4%)
2. Hetero sexual +STD	12(25.0%)	10(27.1%)	2(18.2%)
3. Homosexual +STD		-	-
4. Spouse of HIV +	5 (10.4%)	-	5(45.5%)
B. Drug addict	-	-	-
C. Blood transfusions	2(4.0%)	2 (4.0%)	-

Table 2: Distribution according to Risk exposure

In males, 27 (72.9%) had multiple heterosexual contact and 10 (27.1%) had history of multiple heterosexual contact and had also visited a STD Clinic. Of the 11 females, 5 (45.5%) of their spouses were HIV positive.

Presenting Symptoms	Number	%
1. Fever	37	74.0
2. Anorexia	34	68.0
3. Weight loss	25	50.0
4. Lethargy	20	40.0
5. Cough	19	38.0
6. Diarrhea	17	34.0
7. Malaise	10	20.0
8. Mouth ulcers	9	18.0
9. Lymphadenopathy	7	14.0
10. Breathlessness	2	4.0

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11. Chest pain	2	4.0
12. Genital ulcers	2	4.0
13. Skin infection	3	6.0

Table 3: Distribution according to Presenting Symptoms

Opportunistic Infection	Number (n=50)	%
A. Tuberculosis	28	56.0
1. Pulmonary	13	46.4
2. Extra pulmonary	15	53.6
3. Pulmonary + Extra Pulmonary	4	14.3
B. Chronic Diarrhea	17	34.0
C. Oropharyngeal Candidiasis	9	18.0
D. Pneumonitis	5	10.0
E. Skin infection	2	4.0
F. Genital warts	2	4.0
G. Pericardial Effusion	1	2.0
H. Herpes Zoster	1	2.0
I. Cryptococcal Meningitis	2	4.0
J. Pelvic Inflammatory Disease	1	2.0

Table 4: Distribution according to Opportunistic Infections

The common opportunistic infections in study group were Tuberculosis, Chronic diarrhea, Oropharyngeal candidiasis, Pneumonitis, Skin infection, Genital warts and Cryptococcal meningitis. Herpes zoster, Pelvic inflammatory disease and Pericardial effusion were other opportunistic infections seen in our study group

CD4 counts	Number (n=50)	%
<100	3	6.0
101-200	18	36.0
201-350	21	42.0
>350	8	16.0

Table 5: Distribution according to CD4 counts

The CD 4 counts were grouped under four different headings. They are <100 cells/ μ l, 101-200 cells/ μ l, 201-350 cells/ μ l and >350 cells/ μ l.

The lowest CD 4 count recorded was 83 cells/ μ l in 2 patients who had Extra pulmonary tuberculosis. The highest CD 4 count recorded was 742 cells/ μ l and the patient was asymptomatic.

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Study parameters (Mean $\pm$ SD)	CD4 Counts				Overall	P value
	<100	101-200	201-350	>350		
Total count	4833.33 $\pm$ 1484.36	4883.33 $\pm$ 2070.10	6103.81 $\pm$ 1814.14	9225.00 $\pm$ 4251.64	6087.60 $\pm$ 2787.01	0.001
Total Lymphocyte count	1780.67 $\pm$ 385.52	1774.44 $\pm$ 683.33	1799.48 $\pm$ 455.93	2490.75 $\pm$ 1082.33	1889.94 $\pm$ 698.74	0.073
Hemoglobin	9.86 $\pm$ 1.15	11.55 $\pm$ 1.76	12.10 $\pm$ 1.60	11.69 $\pm$ 2.15	11.70 $\pm$ 1.76	0.219
ESR	64.00 $\pm$ 36.16	58.88 $\pm$ 26.59	50.14 $\pm$ 25.42	71.00 $\pm$ 29.01	57.46 $\pm$ 27.18	0.298

Table 6: Mean pattern of study parameters with CD4 counts

Study parameters	Pearson correlation	P value
Total counts	0.638	P<0.001**
Total Lymphocyte counts	0.383	0.006**
Hemoglobin	0.240	0.093

Table 7: Pearson correlation of Study Parameters

** Highly significant

The Pearson Correlation of Total Counts and Total Lymphocyte Counts with CD 4 Counts were highly significant and that of Hemoglobin was significant.

The Total Count showed an upward trend with CD 4 count. The Pearson Correlation between TC and CD 4 Counts was 0.638, which is highly significant at $P < 0.001$ and shows a large correlation between the two variables.

In our study the Total Lymphocyte Count showed an upward trend with CD 4 count. The Pearson Correlation for TLC with CD 4 Counts was 0.383 (moderate correlation), which is highly significant at $P < 0.006$.

The Pearson Correlation for Hemoglobin with CD 4 Counts was 0.240, at $P < 0.093$ and shows a small correlation between the two variables. ESR also showed an upward trend with CD 4 Count.

DISCUSSION: Between March 2004 and March 2006, 50 HIV patients admitted in KIMS Hospital or those patients who were examined and treated on an Outpatient basis in the Medical OPD constituted the study population. Of the 50 patients 41 were Inpatients and 9 were Outpatients because majority of the patients were symptomatic and were in the advanced stages of HIV infection. Spouses of HIV positive patients constituted the major chunk of the asymptomatic patients.

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Age and Sex:

	Our study n= 50	NACO n= 2890	Kothari n= 100	A.R. Sircar n= 278
Male: female ratio (31-50years)	4:1	4:1	5:1	3:1

Table 8: Comparison of Male: female ratio among different studies

In our study, the majority, 35 (70%) patients were in the age group of 31-50 years, of which 28 were males and 7 were females. The Male to Female ratio was thus 4:1.

Risk Factors:

A	SEXUAL TRANSMISSION	Our Study n= 50	A.R. Sircar n= 278	A.D. Harris n= 150	Kothari n= 100	S.K. Sharma n=135	ACP & IDSA n= 600	NACO-2001 n= 2890
1.	Heterosexual	31(64%)	175(64%)	93(62%)	68(68%)	81(60%)	228(38%)	1878(65%)
2.	Heterosexual + STD	12 (25%)	27(10%)	34(23%)	27 (27%)	39(29%)	84(14%)	491(17%)
3.	Homosexual + STD	-	-	-	-	-	120(20%)	58(2%)
4.	Spouse of HIV +	5(10%)	25(9%)	12(8%)	-	13(7%)	72(12%)	173(6%)
5.	Bisexual	-	-	-	2(2%)	-	48(8%)	29(1%)
B.	Drug addicts	-	-	-	-	-	42(7%)	115(4%)
C.	Blood transfusions	2(4%)	43(17%)	10(7%)	3(3%)	5(4%)	6(1%)	144(5%)

Table 9: Comparison of modes of transmission of HIV in different studies

Heterosexual transmission was the commonest mode of spread of HIV infection in our study population which was similar to the findings in studies by A. R. Sircar et al (1998), A.D Harris (1990) and Kothari et al (2001). There were only 2 (4 %) patients who presented with a history of blood transfusion in our study, whereas 16.2% patients had history of blood transfusion in a study by A.R.Sircar et al (1998). IV

Presenting Symptoms:

Presenting symptoms	Our study n= 50	S.K Sharma study n= 135	YRG study n= 1315	ICMR bulletin n= 1200	A.R Sircar n= 278	Kothari n= 100
Fever	37(74%)	95(71%)	854(65%)	336(42%)	100(36%)	46(46%)
Anorexia	34(68%)	87(65%)	394(30%)	224(28%)	97(35%)	40(40%)
Weight loss	25(50%)	37(28%)	341(26%)	544(68%)	63(23%)	22(22%)

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Diarrhea	17(34%)	33(25%)	263(20%)	276(21%)	44(16%)	33(33%)
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Table-10: Comparison of presenting symptoms in different studies

Fevers, Anorexia, Weight loss, Lethargy, Cough, Diarrhea, Malaise, Mouth ulcers, Lymphadenopathy were the commonest clinical manifestations in our study population which were consistent with the findings in other studies.

Opportunistic infections:

Opportunistic infections	Our study n=50	S.K. Sharma n=135	Beck n=1534	Arthur Pitchenik n=306	Sara Chacko n=85
A. Tuberculosis	56.0%	71%	53%	56%	52%
B. Chronic Diarrhea	34.0%	26%	24%	37%	28%
C. Oropharyngeal Candidiasis	18.0%	39%	26%	37%	21%
D. Pneumonitis	10.0%	7.4%	22%	26%	12%
E. Skin infection	4.0%	3%	4%	6%	3%
F. Genital warts	4.0%	1%	11%	8%	5%
G. Pericardial Effusion	2.0%	3%	8%	4%	-
H. Herpes Zoster	2.0%	1%	9%	8%	1%
I. Cryptococcal Meningitis	4.0%	3.7%	15%	13%	8%
J. Pelvic Inflammatory Disease	2.0%	-	4%	3%	1%
K. Toxoplasmosis	-	37%	9%	22%	-

Table 11: Comparison of opportunistic infections in different studies

Tuberculosis was the most common opportunistic infection present in our study population, present in 28 (56%) patients. Contrary to the picture of AIDS in Africa and America our study as well as studies by A.R.Sircar et al (1998), Kothari et al (2001) and Sara Chacko et al (1994) have observed the absence of Kaposi's sarcoma, Toxoplasmosis, Lymphoma and Recurrent multidermatomal Herpes zoster.

CD 4 counts:

CD4 counts	Our study (n=50)	Beck (n=153)	John Hopkins study (n=5864)	YRG study (n=1315)
<100	3(6.0%)	12 (13%)	1055(18%)	105(8%)
101-200	18(36.0%)	53 (34%)	1584(27%)	395(30%)
201-350	21(42.0%)	78 (53%)	3225(55%)	905(62%)

Table 12: Comparison of distribution according to CD 4 counts in different studies

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The CD 4 counts were less than 200 cells/ μ l in 20 (40%) of the patients in our study.

The commonest opportunistic infections were Tuberculosis, Chronic Diarrhea and Oropharyngeal Candidiasis. There was a significant relationship between CD 4 count and the presence of TB as the CD 4 counts varied from less than 100 cells/ μ l to greater than 500 cells/ μ l. As the CD 4 count declines, incidence of extrapulmonary Tuberculosis increases over Pulmonary Tuberculosis.

In our study, Total count and Total Lymphocyte Count were showing positive trend to CD 4 counts and are statistically significant. The Total Counts of 4850 cells/ μ l and 9200 cells/ μ l showed similar observations to CD 4 counts of 200 cells/ μ l and 350 cells/ μ l. The Total Lymphocyte Counts of 1750 cells/ μ l and 2450 cells/ μ l showed similar observations to CD 4 counts of 200 cells/ μ l and 350 cells/ μ l respectively. The P value was highly significant with Pearson Correlation of 0.638 for Total Counts (P value < 0.001) and 0.383 for Total Lymphocyte Count (P value < 0.006). In the study done by Beck EJ et al at Academic Department of Public Health, St. Mary's Hospital Medical School, London, U.K. paired Total Lymphocyte Count and CD 4 counts were taken from 1534 patients. A significant correlation between the above two parameters was noted, especially for patients with symptomatic HIV disease. This demonstrates the suitability of the use of Total Lymphocyte Count in the absence of CD 4 count.

Studies	Different indices		
	TC	TLC	Hb
Our study	6087	1889	11.228
John Hopkins study	5267	1196	10.874
YRG study	5876	1252	11.160
Beck E.J study	6028	1300	11.279
KMC study (Manipal)	5276	1205	11.245
'p' value	0.001	0.074	0.219
Pearson Correlation	Highly significant	Highly significant	Significant

TABLE 13: Comparison of conclusions of different studies with CD 4 of 200 cells/ μ l.

The pattern of CD 4 counts over time is more important than any single CD 4 count value. CD 4 counts generally decrease as HIV progresses. Therefore it is more valuable to evaluate a series of CD 4 counts than any single CD 4 count.

As the CD 4 count is affected by the time of the day (lower in the morning), in acute illnesses, refrigeration of the blood sample (decreased CD 4 count), with rough handling or contamination of blood sample, so the serial recording of Total Lymphocyte Count can give an equally stable reflection of progression of disease and development of AIDS in HIV infected persons.

CONCLUSIONS:

- ⌚ Majority of the patients with opportunistic infections had a CD 4 count less than 350 cells/ μ l.
- ⌚ The Total Lymphocyte Counts of 1750 cells/ μ l and 2450 cells/ μ l correlated to CD 4 counts of 200 cells/ μ l and 350 cells/ μ l respectively.

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⌚ The correlation between Total Lymphocyte Count and CD 4 count were highly significant.

⌚ Thus, for the people who may not be able to afford the investigations and treatment, Total Lymphocyte Count can serve as a cost effective, affordable index to start ART and also to monitor ART in HIV infected persons.

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KEY TO MASTER CHART

- A = Anorexia
ALB = Albumin
ABD.KOCH = Abdominal Koch
- B = Breathlessness
BL.KOCH = Bilateral Koch's
BRONCHI. = Bronchitis
- C = Cough
C/S = Culture and Sensitivity
CP = Chest Pain
CSF = Cerebro-spinal Fluid
- D = Diarrhoea
- F = Fever
- GU = Genital Ulcers
- KLEB = Klebsiella
- L = Lymphadenopathy
LTH = Lethargy
- M = Malaise
MENG = Meningitis
MU = Mouth Ulcers
- N = Normal
NG = No Growth
- PC = Pus Cells
PER.EFU = Pericardial Effusion PLEU.EFU = Pleural effusion
PL.THICK = Pleural Thickening
PNEUM = Pneumonia
P.RUB = Pleural Rub
PUL.KOCH = Pulmonary Koch
- RT.EFU = Right sided Pleural effusion
TB.LYMPH = Tubercular Lymphadenitis
- WL = Weight Loss
+ = Positive
- = Negative

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