# Total Lymphocyte Count: A Surrogate Marker for Predicting CD4+ Count in Enrolled Process for Antiretroviral Therapy in Resource-Limited Settings

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***Objective:*** *The purpose of the present study was to evaluate the correlation between total lymphocyte count (TLC) and CD4+ count and TLC cut-off for predicting CD4+ count < 200 cells/mm³.*
***Material and Method:*** *The 176-naïve HIV-infected patients from the OPD Khon Kaen Hospital, were recruited for CD4+ count by flow cytometer and TLC by automated cell counter. All results were analyzed by STATA statistical software for sensitivity, specificity, PPV, and NPV.*
***Results:*** *From 176 patients, 61 (34.7%) had CD4+ > 200 cells/mm³ and 115 (65.3%) had CD4+ < 200 cells/mm³. The ROC curve between CD4+ count and TLC showed TLC < 1,800 cells/mm³ could predict CD4+ count < 200 cells/mm³ with 78.26% sensitivity, 73.77% specificity, 84.91% PPV, and 64.29% NPV. The sensitivity was further decreased and specificity was increased when TLC was lower than cut-off.*
***Conclusion:*** *The presented finding indicates that the appropriated TLC for predicting CD4+ count. < 200 cells/ mm³ was TLC < 1,800 cells/mm³. TLC can also be used as a surrogate marker for starting ARV therapy in resource limited setting.*

***Keywords:*** *Total lymphocyte count, Flow cytometer, CD4+ count*

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CD4+ cell count is commonly used as a surrogate marker for disease progression of HIV-infected patients. Depletion of CD4+ cell count has been shown to be associated with an increased risk of developing opportunistic infections (OIs) and with mortality. The number of CD4+ cell count and CD4+ percentage are widely used as laboratory parameters for the introduction of primary prophylaxis and antiretroviral (ARV) therapy[1]. In Thailand, the accurate determination of CD4+ cell count was done by flow cytometer, an expensive technique that is available in regional hospitals or university hospitals. In 2002, the ministry of public health launched the pilot antiretroviral therapy project for HIV-infected patients. The enrolled community or district hospitals send blood samples to regional hospitals for measurement of CD4+ cell counts. The patients who have a CD4+ cell count lower than 200 cells/mm³ received ARV drugs free of charge. However, high unit cost and high workload are the main problems for the hospitals, which performed CD4+ cell count by flow cytometer. WHO has suggested that total lymphocyte count (TLC) could serve as a potential marker for immunosuppressive patient where CD4+ count is unavailable because TLC is easily obtained from routine complete blood count by multiplying the percentage of lymphocytes by white blood cell count[2-4]. The TLC could serve as a surrogate marker of CD4+ count in resource limited settings (RLS).

Several studies from different regions of the world have demonstrated a good correlation between absolute lymphocyte count (total lymphocyte count) and CD4+ cell count[5,6]. The objective of the present study was to evaluate the correlation of total lymphocyte

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count (TLC) to CD4+ cell count and to determine absolute lymphocyte count cutoff for predicting CD4+ cell count lower than 200 cells/mm$^3$ in HIV-infected patients.

## Material and Method

Subjects for the present study were recruited from the out patient department, Khon Kaen Hospital, Thailand between 2003 and 2004. The 176-naïve HIV-infected patients were included for CD4+ cell count, and absolute lymphocyte measurement. Total lymphocyte count (TLC) was measured by a 5-part diff (STK-S Beckman Coulter) automated blood-analyzer. The CD4+ cell count was done by FACScan (Becton-Dickinson, USA) with 'lyse-no-wash' and tricolor monoclonal antibody technique on the same day with TLC measurement. All results from TLC and CD4+ cell count were analyzed by STATA statistical software. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) with 95% confidence interval (95% CI) of various cut-points of the absolute lymphocyte count to predict a CD4+ cell count < 200 cells/mm$^3$ were calculated. ROC curve was plotted to determine TLC cutoff correlated with CD4+ cell count < 200 cells/mm$^3$.

## Results

The data of TLC and CD4+ cell count from 176 patients were analyzed to determine the correlation between CD4+ cell count and TLC. CD4+ cell count was divided into two groups, above and below 200 cell/ mm$^3$. From 176 patients, 61 (34.7%) had CD4+ cell count > 200 cells/ mm3 and 115 (65.3%) has CD4+ cell count < 200 cells/mm$^3$. The sensitivity, specificity, PPV and NPV of various TLC ranges for CD4+ cell count < 200 cells/mm$^3$ are listed in Table 1. The maximum sensitivity was found at the highest TLC range (< 2400 cell/mm$^3$) while the maximum positive predictive value was found at the lowest TLC range (< 1000 cells/ mm$^3$). An ROC curve between CD4+ count and TLC show TLC < 1,800 cells/mm$^3$ can predict CD4+ count < 200 cells/ mm$^3$ (Fig. 1). An TLC cutoff at 1,800 cells/mm$^3$ or less would identify the patients who have CD4+ cell count < 200 cells/mm$^3$ with sensitivity 78.26% (72.17-84.35%), specificity 73.77% (67.27-80.27%), positive predictive value 84.91% (79.62-9-.19%) and negative predictive value 64.29% (57.21-71.36%).

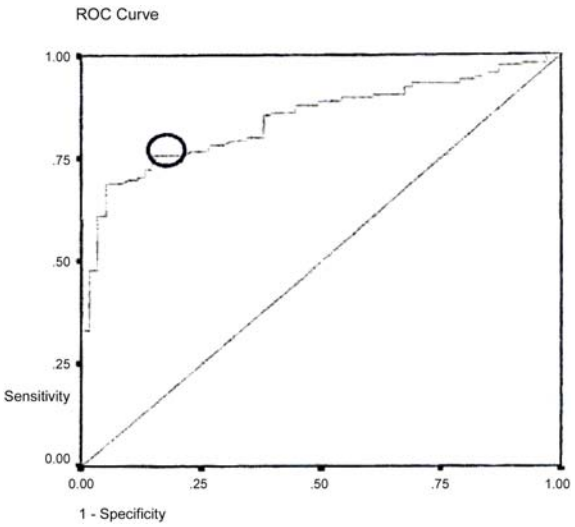


**Fig. 1** The ROC curve showing the optimum total lymphocyte count cutoff for predicting CD4+ count < 200 cell/mm$^3$ (n = 115)

## Discussion

CD4+ lymphocyte is the principle target cell of HIV viral infection. The number of CD4+ cell count remains a useful marker of disease progression and

**Table 1.** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of TLC for CD4+ cell count < 200 cells/mm$^3$ (n = 115)

| TLC (cells/mm$^3$) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| <1,000 | 25.22 | 100.00 | 100.00 | 41.50 |
| <1,200 | 40.00 | 98.36 | 97.87 | 46.51 |
| <1,400 | 58.26 | 96.72 | 97.10 | 55.14 |
| <1,600 | 70.43 | 86.89 | 91.01 | 60.92 |
| <1,800 | 78.26 | 73.77 | 84.91 | 64.29 |
| <2,000 | 81.74 | 62.30 | 80.30 | 64.41 |
| <2,200 | 87.83 | 50.82 | 77.10 | 60.89 |
| <2,400 | 89.53 | 40.98 | 74.10 | 67.57 |

widely used as indicators for starting antiretroviral therapy or primary prophylaxis for OIs. However, accurate determination of CD4+ cell count needs to be done by flow cytometry, an expensive technique that is not available in the majority of general hospital laboratories[1,5,8]. In rural areas, CD4+ cell count testing for starting ARV therapy is limited by high cost. Several studies have suggested that TLC can be used to predict the CD4+ cell count in HIV/AIDS patients[5-6,8-12].

The present study demonstrated that TLC, a widely available and inexpensive parameter, could be routinely used as a surrogate marker for the CD4+ cell count of immunosuppressive. The determination of TLC cutoff to predict CD4+ cell count < 200 cells/$mm^3$ was done by ROC curve. The authors found that at TLC < 1800 cells/$mm^3$, the prediction for CD4+ cell count < 200 cells/$mm^3$ was possible with 78.26% sensitivity, 73.77% specificity, 84.91% PPV and 64.29 % NPV. Then, TLC < 1800 cells/$mm^3$ cutoff is a reasonable surrogate for evaluating the CD4+ cell count < 200 cell/$mm^3$. At this level, sensitivity, specificity, PPV and NPV are comparable to a recent study by Kumarasamy et al[4].

Due to a constant reduction in antiretroviral drug prices and the support for ARV therapy project from the Thai government, more HIV infected-patients have gained access to ARV therapy. However, the cost for CD4+ cell count testing by flow cytometry is relatively high for the low social-economic patients and for hospital expense. The present finding indicates that in addition to the use of CD4+ cell count for initiating antiretroviral therapy, TLC can also be used as a surrogate marker for starting therapy at a low cost. In screening process for recruitment of the patient to start ARV therapy in community and provincial hospitals where flow cytometry equipment is not available, TLC could be done to predict CD4+ cell count < 200 cells/$mm^3$. If TLC is lower than 1800 cells/$mm^3$, CD4+ cell count by flow cytometer should be performed by regional or university hospitals.

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## *ค่าสมบูรณ์ลิมโฟซัยต์: ค่าตัวแทนในการทำนายจำนวน CD4+ ในกระบวนการคัดกรองผู้ที่ได้รับยาต้านไวรัส ในพื้นที่ที่มีทรัพยากรจำกัด*

***วัฒนชัย สุแสงรัตน์, มัณฑนา มิตรชัย, นิรมล ลี้รัตนเพชร***

***วัตถุประสงค์:*** *ประเมินหาความสัมพันธ์ของค่าสมบูรณ์ลิมโฟซัยต์กับจำนวน CD4+ และหาค่าสมบูรณ์ลิมโฟซัยต์ที่สามารถทำนายจำนวน CD4+ น้อยกว่า 200 เซลล์/ลบ.มม.*

***วัสดุและวิธีการ:*** *ผู้ติดเชื้อเอชไอวีที่ไม่เคยได้รับยาต้านไวรัสมาก่อนจำนวน 176 ราย จากคลินิกผู้ป่วยนอก โรงพยาบาลขอนแก่นตรวจหาจำนวน CD4+ ด้วยเครื่องโฟลซัยโตมิเตอร์ และหาค่าสมบูรณ์ลิมโฟซัยต์ด้วยเครื่องนับจำนวนเซลล์อัตโนมัติ ข้อมูลที่ได้ถูกนำมาวิเคราะห์ด้วยโปรแกรมสถิติ STATA เพื่อหาค่าความไว ความจำเพาะ ค่าทำนายผลบวกและค่าทำนายผลลบ*

***ผลการศึกษา:*** *กลุ่มผู้ป่วย HIV 176 ราย ร้อยละ34.7 มีค่า CD4+ น้อยกว่า 200 เซลล์/ลบ.มม. ร้อยละ 65.3 มีค่า CD4+ มากกว่า 200 เซลล์/ลบ.มม. จากกราฟ ROC ระหว่างค่าสมบูรณ์ลิมโฟซัยต์ กับ จำนวน CD4+ พบว่าค่าสมบูรณ์ลิมโฟซัยต์ ที่ 1,800 เซลล์/ลบ.มม. สามารถทำนายค่า CD4+ น้อยกว่า 200 เซลล์/ลบ.มม. โดยมีความไว ร้อยละ 78.26 ความจำเพาะร้อยละ 73.77 ค่าทำนายผลบวกร้อยละ 84.91 ค่าทำนายผลลบร้อยละ 64.29 โดยความไวจะลดลง ขณะที่ความจำเพาะเพิ่มขึ้น เมื่อค่าสมบูรณ์ลิมโฟซัยต์น้อยกว่าค่าที่กำหนดข้างต้น*

***สรุป:*** *จากข้อมูลที่ศึกษา พบว่าค่าสมบูรณ์ลิมโฟซัยต์ที่ 1,800 เซลล์/ลบ.มม. สามารถทำนายค่า CD4+ น้อยกว่า 200 เซลล์/ลบ.มม.ได้ ดังนั้นในพื้นที่ที่มีทรัพยากรจำกัด สามารถนำค่าสมบูรณ์ลิมโฟซัยต์ เพื่อช่วยคัดกรองก่อนเข้ารับการรักษาด้วยยาต้านไวรัสได้*