

# Potential Clinical Implications of Interlaboratory Variability in CD4<sup>+</sup> T-Lymphocyte Counts of Patients Infected with Human Immunodeficiency Virus

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The CD4<sup>+</sup> T-lymphocyte count is an important factor in the management of patients infected with human immunodeficiency virus. Previous studies have found significant variability among the counts determined by different laboratories. We conducted a study of lymphocyte phenotyping in four laboratories to assess this variability and its possible clinical implications. One laboratory was situated at the study site; the other three were selected randomly from a total of 11 commercial and hospital laboratories available locally. Blood specimens were obtained from 24 patients and were sent to the four laboratories for a complete blood count and a lymphocyte subset analysis. Using the Kruskal-Wallis test, we found that the laboratories' ranks of four individual components of the CD4 cell count differed significantly: total white blood cell count ( $P < .0001$ ), lymphocyte percentage ( $P = .003$ ), lymphocyte count ( $P = .002$ ), and CD4 percentage ( $P = .0004$ ). Of the 24 patients in this survey, 14 (58.3%) had CD4-count results with enough variation to have led to conflicting treatment recommendations; three of the 24 patients fulfilled the revised Centers for Disease Control and Prevention case definition of AIDS on the basis of results from some but not all laboratories. In addition, the laboratories disagreed on whether CD4 cell counts of nine patients (37.5%) had increased or decreased since the previous determination. We conclude that when strict thresholds of CD4 cell counts are used as a basis for treatment recommendations or for diagnosis of AIDS, interlaboratory variability may be sufficient to alter the decisions made.

The CD4<sup>+</sup> T-lymphocyte count is currently the most important laboratory parameter in the management and staging of patients infected with HIV. It is used for determining the risk of specific opportunistic infections [1], for guiding decisions about antiretroviral therapy and prophylaxis for opportunistic infections [2–4], and as a surrogate marker for drug efficacy in clinical trials. In conjunction with a positive HIV serology, a total CD4 cell count of  $<200/\text{mm}^3$  or a CD4 percentage of  $<14\%$  fulfills the 1993 Centers for Disease Control and Prevention (CDC) case definition of AIDS [5].

Despite the importance of this test, it has only recently become a component of routine clinical practice, and previous studies have indicated significant between-laboratory variability in the results obtained [6–8]. Individuals with HIV infection may receive care and undergo diagnostic testing in multiple settings—e.g., during a visit to their primary care provider, a meeting with a consultant, a hospitalization, or an evaluation for a research protocol. It is not uncommon for these different

settings to use different laboratories for determination of the CD4 cell count. We tested the hypothesis that interlaboratory variability in CD4 counts could be sufficient to influence the patient's management or the diagnosis of AIDS. We found that in a group of clinically stable HIV-infected patients, interlaboratory variability could result in the classification of the same patient in different therapeutic and diagnostic groups.

## Materials and Methods

Patients were considered eligible for this study if their most recent CD4 cell count had been  $>100/\text{mm}^3$  or if a lymphocyte subset determination was planned as part of their clinical care or their evaluation in a research protocol. Patients were excluded from consideration if their most recent count had been  $<100/\text{mm}^3$  since, at the time of the study, no therapeutic or diagnostic thresholds existed below this level. After informed consent had been obtained, blood specimens were drawn according to the procedures specified by the individual laboratories. Specimens were obtained at the same time from all patients, and a portion of each specimen was sent to each of the four participating laboratories.

To identify laboratories eligible for participation in the study, we called commercial laboratories listed in the Boston telephone directory as well as hospital laboratories in the Boston area and asked whether they performed CD4 cell counts. A total of 11 laboratories were eligible, of which five were commercial and six were hospital based. One laboratory chosen

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for this study was at the study site; the remainder of the eligible laboratories were randomly assigned numbers between 0 and 1.0, and the three laboratories with the highest numbers were chosen to participate.

Two of the four laboratories performed each component of the analysis (complete blood count, differential, and lymphocyte subset analysis) in the Boston area, while one transported specimens to Pennsylvania for testing. The fourth laboratory performed flow cytometry in Boston but had the complete blood count and differential performed in New Jersey. Automated WBC differentials were performed by all the laboratories; these percentages were used for calculation of the total CD4 cell count. All sites used the whole-blood lysis method for flow cytometry. Because of testing limitations imposed by one laboratory, specimens were collected on Monday and Wednesday mornings only.

Specimens were labeled with aliases, and laboratories were blinded to the purposes of the study. The total CD4 cell count was calculated as the product of the total WBC count, the percentage of WBCs that were lymphocytes (as determined by machine differential), and the percentage of lymphocytes that were CD4<sup>+</sup> (as determined by flow cytometry). When an absolute CD4 cell count was included in the laboratory report, the value was recalculated to ensure that there was no reporting error.

We examined total CD4 cell counts in light of three therapeutic and diagnostic thresholds: 500, 200, and 100 cells/mm<sup>3</sup>. We chose these values on the basis of the then-current criteria for consideration of the initiation of antiretroviral therapy, prophylaxis for *Pneumocystis carinii* pneumonia (PCP), and prophylaxis for *Mycobacterium avium* complex (MAC) infection, respectively [2–4]. In addition, we examined whether patients fulfilled the 1993 revised CDC case definition of AIDS, which is based on a CD4 count of <200 or <14% of the total number of lymphocytes. Finally, we compared the results obtained by the four laboratories with the patient's prior CD4 cell count to see whether the count had increased or decreased.

## Results

Twenty-four clinically stable patients (20 men and four women) participated in the study. None had a history of prior AIDS-defining opportunistic infections or neoplasms; 18 patients were currently receiving antiretroviral therapy (monotherapy or combination therapy with the nucleoside analogues zidovudine, didanosine, and zalcitabine), while six (patients 18, 19, 20, 21, 23, and 24) were not. The mean prior CD4 cell count for the whole cohort was 348/mm<sup>3</sup>, with a range of 110–900/mm<sup>3</sup> and a standard deviation of 230/mm<sup>3</sup>.

None of the specimens obtained was lost or found to be unsuitable for analysis by any of the laboratories. The results from each laboratory for each patient are displayed graphically

in figure 1. The mean absolute CD4 cell counts obtained from the four laboratories were 337, 335, 357, and 341/mm<sup>3</sup>, respectively. The range between laboratories (maximal value minus minimal value) per patient varied between 26 and 413 cells/mm<sup>3</sup>, with a mean range of 108/mm<sup>3</sup> and a standard deviation of 89.34/mm<sup>3</sup>. Three-quarters of the patients had CD4 cell count ranges of >50/mm<sup>3</sup>; for seven patients the range was 101–200/mm<sup>3</sup>, while for three it was ≥200/mm<sup>3</sup>. The higher the mean CD4 cell count, the higher the range (Spearman correlation, 0.002).

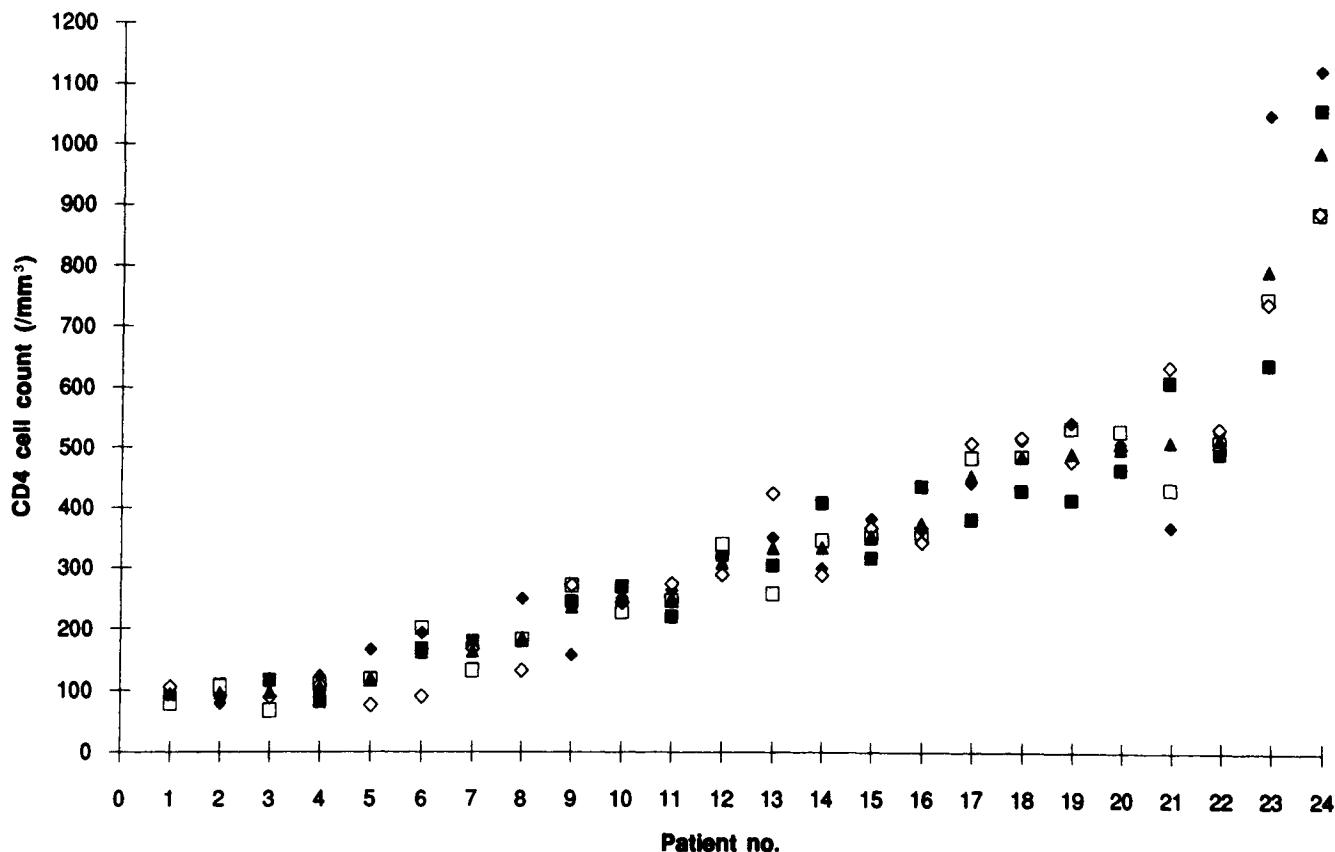
To test whether some laboratories usually obtained values higher or lower than those obtained by other laboratories for individual parameters, we ranked the four values for each patient and each measurement from lowest to highest (1 to 4). We then compared the ranks obtained for the 24 samples, using the Kruskal-Wallis test. Laboratories' ranks differed significantly for WBC count ( $P < .0001$ ), lymphocyte percentage ( $P = .003$ ), lymphocyte count ( $P = .002$ ), and CD4 percentage ( $P = .0004$ ). Laboratories that were systematically high for one of these components were low for others. As a result, total CD4 cell counts obtained by the four laboratories did not systematically differ as much as the individual components ( $P = .08$ ).

Of the 24 patients in this study, 14 (58.3%) had values reflecting a lack of agreement about treatment recommendations. One patient, whose CD4 cell counts ranged from 90 to 201/mm<sup>3</sup>, would have qualified for both MAC and PCP prophylaxis at one extreme and for neither intervention at the other. Moreover, three of the 24 patients met the 1993 revised CDC criteria for AIDS on the basis of results from at least one laboratory but not on the basis of values obtained at other laboratories.

Patients and clinicians often use an increase or decrease in CD4 cell count as a surrogate marker for the remission or progression of HIV-associated disease, regardless of clinical events. However, the four laboratories in this study disagreed about whether the counts of nine of these 24 clinically stable patients had increased or decreased; in four cases the conflicting results differed by ≥10% from baseline. The therapeutic and diagnostic implications of these disagreements are summarized in table 1.

## Discussion

Despite the availability of other markers (including p24 antigen,  $\beta_2$  microglobulin, neopterin, and—most recently—quantitative values for viral load), the CD4 cell count remains the most important surrogate marker for the staging and evaluation of patients with HIV infection. Depletion of CD4 cells appears to play a central role in the pathogenesis of HIV-related immunosuppression; hence the use of the CD4 count as an indicator of disease severity is biologically reasonable. The abnormality of the count in a significant proportion of the patients studied



**Figure 1.** Absolute CD4 cell counts determined at four laboratories (white and black squares and diamonds) for 24 patients infected with HIV. Patients are sorted by mean CD4 cell count (triangles), from lowest to highest.

makes it widely applicable throughout much of the course of HIV infection. Furthermore, this parameter is quantitatively meaningful: declining counts are associated with an increasing risk of opportunistic infections and death [1, 9–11]. The CD4 cell count is also used to diagnose idiopathic CD4<sup>+</sup> T lymphocytopenia; two tests showing a CD4 cell count of <300/mm<sup>3</sup>

or a CD4 percentage of <20% in the absence of HIV infection fulfill the CDC case definition for this condition [12].

However, CD4 cell counts may vary for a given patient independent of HIV disease progression or antiviral drug efficacy. Both biological and analytic factors may influence the total count. Biological factors that have been associated with alterations in the CD4 count include age, season, time of day, ethnic origin, splenectomy status, and drug use [13–16]. For pediatric patients and for adults who have undergone splenectomy, higher baseline CD4 cell counts without correspondingly better immune function make the thresholds for prophylaxis established for (nonsplenectomized) adults with HIV infection inapplicable [17]. For splenectomized adults, it has been suggested that the CD4 percentage more precisely reflects immune function than the absolute CD4 count. For children infected perinatally, even adjusted CD4 count thresholds have proven ineffective in reducing the incidence of PCP; thus it has been recommended that all infants born to HIV-infected mothers receive initial prophylaxis for PCP regardless of CD4 cell count [18].

In addition, analytic (i.e., between-laboratory) variability in CD4 cell counts may occur secondary to numerous technical factors: temperature of the specimen, interval from the drawing

**Table 1.** Therapeutic and diagnostic implications of disagreements among CD4 cell counts determined at four laboratories.

Decision in question	No. (%) of patients with conflicting results*
Recommendations for treatment	
Overall	14 (58.3)
Antiviral therapy	6 (25.0)
Prophylaxis	
<i>Pneumocystis carinii</i> pneumonia	2 (8.3)
Disseminated infection due to the <i>Mycobacterium avium</i> complex	5 (20.8)
Both	1 (4.2)
Diagnosis of AIDS	3 (12.5)
Change in count (increase vs. decrease)	9 (37.5)

\* Results that would lead to different decisions or conclusions.

of blood to analysis, anticoagulant used for collection, specimen handling, preparation of samples (whole-blood lysis vs. the Ficoll-Hypaque process), reagents and instruments used, and methods used for standardization of the flow cytometer. In a report of the results of the National Institute of Allergy and Infectious Diseases—Division of HIV/AIDS Flow Cytometry Quality Assessment Program, however, it was noted that a high proportion of interlaboratory variability remained unexplained. The authors suggested that each factor was likely to have contributed a small amount to overall variability [7]. Regardless of the type or source of variability, the problem is compounded by the fact that the total CD4 cell count cannot be determined directly. Since the CD4 count is calculated as the product of the total WBC count, the percentage of WBCs that are lymphocytes, and the percentage of lymphocytes that are CD4<sup>+</sup>, variability in any of these components affects the value obtained [13].

In the present study, a group of 24 clinically stable patients had CD4 cell counts determined as part of their routine clinical care or as part of their evaluation in a research protocol. One blood specimen was drawn from each patient, and a portion was sent to each of four laboratories. Despite their participation in quality assurance programs and use of state-of-the-art techniques, the laboratories obtained discordant results that had clinically significant implications in more than one-half of the cases.

One case offers a particularly striking example of the potential clinical significance of interlaboratory variability. This case involved an asymptomatic HIV-infected patient who was clinically stable, whose prior CD4 cell count was 510/mm<sup>3</sup>, and who was not receiving antiretroviral therapy. The four laboratories reported CD4 counts ranging from 370 to 640/mm<sup>3</sup>; one laboratory reported a CD4 percentage of 19%—below the threshold that some clinicians use for initiation of PCP prophylaxis.

Possible solutions to the problem of interlaboratory variability include repetition of the CD4 cell count, with averaging of the two values, as well as the use of the same laboratory for all determinations. Laboratories used for determination of CD4 cell counts should (1) be appropriately accredited by a recognized professional organization or governmental agency, (2) participate in quality assurance programs, and (3) use the CDC guidelines or other published guidelines for flow cytometry [13]. Since the determination of the CD4 percentage does not require the use of the complete blood count with differential [19], some authorities consider it a more reliable marker than the CD4 cell count; however, the CD4 percentage has not been formally incorporated into current treatment recommendations.

Fortunately, the results of recent multicenter proficiency testing indicate that rigorous quality assurance programs and training have increased the frequency of interlaboratory agreement. Citing the experience gained in the Army Lymphocyte Immunophenotyping Program, the CDC reported that analytic variability in CD4 cell counts was reduced from 29.4% in 1987 to

8.4% in 1991 [13]. However, as is shown in the present study, interlaboratory variability remains a clinically significant problem, especially affecting individual clinical decisions and the diagnosis of AIDS. Repetition of counts that are not consistent with prior trends is therefore prudent, particularly if decisions about treatment are to be based on the result [20]. Patients and practitioners should remain aware of this potential limitation in lymphocyte phenotyping as it is currently performed.

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#### References

- Masur H, Ognibene FP, Yarchoan R, et al. CD4 counts as predictors of opportunistic pneumonias in HIV infection. *Ann Intern Med* 1989; 111:223–31.
- Sande MA, Carpenter CJC, Cobbs CG, Holmes KK, Sanford JP. Antiretroviral therapy for adult HIV-infected patients: recommendations from a state-of-the-art conference. *JAMA* 1993;270:2583–9.
- Centers for Disease Control. Recommendations for prophylaxis against *Pneumocystis carinii* pneumonia for adults and adolescents infected with human immunodeficiency virus. *MMWR Morb Mortal Wkly Rep* 1992;41(RR-4):1–11.
- Centers for Disease Control and Prevention. Recommendations on prophylaxis and therapy for disseminated *Mycobacterium avium* complex for adults and adolescents infected with human immunodeficiency virus. *MMWR Morb Mortal Wkly Rep* 1993;42(RR-9):17–20.
- Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep* 1992;41(RR-17):1–19.
- Giorgi JV, Cheng HL, Margolick JB. Quality control in the flow cytometric measurement of T-lymphocyte subsets: The Multicenter AIDS Cohort Study experience. *Clin Immunol Immunopathol* 1990;55:173–86.
- Gelman R, Cheng SC, Kidd P, Waxdal M, Kagan J. Assessment of the effects of instrumentation, monoclonal antibody, and fluorochrome on flow cytometric immunophenotyping: a report based on 2 years of the NIAID DAIDS Flow Cytometry Quality Assessment Program. *Clin Immunol Immunopathol* 1993;66:150–62.
- Rickman WJ, Waxdal MJ, Monical C, Damato JD, Burke DS. Department of Army Lymphocyte Immunophenotyping Quality Assurance Program. *Clin Immunol Immunopathol* 1989;52:85–95.
- Yarchoan R, Venzon DJ, Pluda JM, et al. CD4 count and risk for death in patients with HIV receiving antiretroviral therapy. *Ann Intern Med* 1991;115:184–99.
- Crowe SM, Carlin JB, Stewart KI, Lucas R, Hoy JF. Predictive value of CD4 lymphocyte numbers for the development of opportunistic infections and malignancies in HIV-infected persons. *J Acquir Immune Defic Syndr* 1991;4:770–6.
- Fahey JL, Taylor JMG, Detels R, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type I. *N Engl J Med* 1990;322:166–72.
- Centers for Disease Control. Unexplained CD4<sup>+</sup> T-lymphocyte depletion in persons without evident HIV infection—United States. *MMWR Morb Mortal Wkly Rep* 1992;41:541–5.

13. Centers for Disease Control. Guidelines for the performance of CD4<sup>+</sup> T-cell determinations in persons with human immunodeficiency virus infection. *MMWR Morb Mortal Wkly Rep* 1992;41(RR-8):1-17.
14. Laurence J. T-cell subsets in health, infectious disease, and idiopathic CD4<sup>+</sup> T lymphocytopenia. *Ann Intern Med* 1993;119:55-62.
15. Malone JL, Simms TE, Gray GC, Wagner KF, Burge JR, Burke DS. Sources of variability in repeated T-helper lymphocyte counts from HIV type 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. *J Acquir Immune Defic Syndr* 1990;3:144-51.
16. Van Rood Y, Goulmy E, Blokland E, Pool J, van Rood J, van Houwelingen H. Month-related variability in immunological test results: implications for immunological follow-up studies. *Clin Exp Immunol* 1991;86:349-54.
17. Zurlo JJ, Wood L, Gaglione MM, Polis MA. Effect of splenectomy on T lymphocyte subsets in patients infected with the human immunodeficiency virus. *Clin Infect Dis* 1995;20:768-71.
18. Simonds RJ, Lindegren ML, Thomas P, et al. Prophylaxis against *P. carinii* pneumonia among children with perinatally acquired human immunodeficiency virus infection in the United States. *N Engl J Med* 1995;332:786-90.
19. Taylor JMG, Fahey JL, Detels R, Giorgi JV. CD4 percentage, CD4 number, and CD4:CD8 ratio in HIV infection: which to choose and how to use. *J Acquir Immune Defic Syndr* 1989;2:114-24.
20. Bartlett J. Laboratory tests. In: Bartlett J, ed. *Medical management of HIV infection*. Glenview, IL: Physicians and Scientists Publishing, 1994: 23-6.

## ARCANUM



A commonplace cure for a hangover is to take a little of “the hair of the dog that bit you.” What is the origin of this (dubious) cure? (See page 1285 for answers to arcanum and a commentary.)