Diagnosis of Primary HIV-1 Infection

Eric S. Daar, MD; Susan Little, MD; Jacqui Pitt, RN; Joanne Santangelo, NP; Pauline Ho, MD; Nina Harawa, MPH; Peter Kerndt, MD; Janis V. Giorgi, PhD†; Jiexin Bai, MD; Paula Gaut, MD; Douglas D. Richman, MD; Susan Mandel, MD; and Stephen Nichols, MD, for the Los Angeles County Primary HIV Infection Recruitment Network*

Background: **The optimal approach for diagnosing primary HIV-1 infection has not been defined.**

Objective: **To determine the usefulness of symptoms and virologic tests for diagnosing primary HIV-1 infection.**

Design: **Prospective cohort study.**

Setting: **A teaching hospital in Los Angeles and a university research center in San Diego, California.**

Patients: **436 patients who had symptoms consistent with primary HIV infection.**

Measurements: **Clinical information and levels of HIV antibody, HIV RNA, and p24 antigen.**

Results: **Primary infection was diagnosed in 54 patients (12.4%). The sensitivity and specificity of the p24 antigen assay were 88.7% (95% CI, 77.0% to 95.7%) and 100% (CI, 99.3% to 100%), respectively. For the HIV RNA assay, sensitivity was 100% and specificity was 97.4% (CI, 94.9% to 98.9%). Fever, myalgia, rash, night sweats, and arthralgia occurred more frequently in patients with primary infection ($P < 0.05$).**

Conclusions: **No sign or symptom allows targeted screening for primary infection. Although assays for HIV RNA are more sensitive than those for p24 antigen in diagnosing primary infection, they are more expensive and are more likely to yield false-positive results.**

Ann Intern Med. 2001;134:25-29. www.annals.org

For author affiliations, current addresses, and contributions, see end of text.
†Deceased.
*For participants in the Los Angeles County Primary HIV Infection Recruitment Network, see Appendix.

See editorial comment on pp 75-77.

Primary HIV infection is characterized by diverse clinical symptoms (1, 2). Since patients with primary infection are just developing HIV antibodies, recognition of the syndrome in at-risk persons should prompt antibody testing as well as a virologic assay (3, 4). Diagnosing primary infection may decrease HIV transmission (5) and allow consideration of early treatment (2, 6). To date, the optimal patients to screen and the best algorithm for use of diagnostic tests have not been determined. The objectives of this study were to determine the sensitivity and specificity of virologic tests and specific clinical symptoms for diagnosing primary HIV infection.

Methods

Patients

Patients with potential exposure to HIV and compatible symptoms were referred from clinics, testing centers, emergency departments, and community physicians for primary infection screening at Cedars-Sinai Medical Center in Los Angeles, California, and the University of California, San Diego. In Los Angeles, 127 patients were screened between March 1993 and August 1995 (cohort 1) for enrollment in a placebo-controlled trial of zidovudine (7). A similar group of 255 patients was screened in Los Angeles between June 1996 and July 1999 (cohort 2), and 54 patients were screened between June 1996 and March 1999 in San Diego (cohort 3). Patients in cohort 2 received a standardized questionnaire regarding results of past HIV tests, presence of specific symptoms, and potential exposure to HIV during the preceding 2 months.

Virologic and Serologic Assays

The study was approved by local institutional review boards. All patients provided informed consent and received pre- and post-test counseling. Patients in cohorts 1 and 2 had real-time testing for HIV antibodies and p24 antigen by a polyclonal enzyme immunoassay (Abbott Laboratories, Abbott Park, Illinois); plasma was stored at −70 °C. In cohort 2, real-time plasma HIV RNA measurements were performed by branched-chain DNA (bDNA) assay, version 2.0 (lower limit of detection, 500 copies/mL), between June 1996 and July 1998 and by version 3.0 (lower limit of detection, 50 copies/mL) between August 1998 and July 1999 (Chiron Diagnostics, Emeryville, California). Stored samples from cohort 1 were retrospectively tested by bDNA assay,

version 3.0. After April 1998, p24 antigen was measured by using the monoclonal HIV AG-1 enzyme immunoassay (Abbott Laboratories), which replaced the previous assay. All samples with detectable p24 antigen were confirmed by neutralization (Abbott Laboratories). Samples positive for HIV antibody were confirmed by Western blot (Cambridge Biotech Corp., Rockville, Maryland). Samples that yielded indeterminate Western blots (<2 envelope bands, core bands, or both) were tested again in approximately 1 month to document seroconversion.

Patients in cohort 3 were screened in San Diego by using HIV antibody enzyme immunoassay (Abbott Laboratories). Plasma HIV RNA level was determined by using Amplicor HIV Monitor (Roche Diagnostic Systems, Indianapolis, Indiana), which had a lower limit of detection of 400 copies/mL. Samples that were positive for HIV RNA but negative for HIV antibody were tested for p24 antigen (Beckman Coulter, Fullerton, California).

Statistical Analysis

Primary infection was defined as a confirmed positive virologic test result with either a negative HIV antibody assay result or an indeterminate Western blot. Because there is no virologic gold standard, we assumed that levels of plasma HIV RNA had a sensitivity of 100% for diagnosing primary infection. False-positive HIV RNA measurements were defined as those that were negative on repeated testing and those obtained in patients who did not undergo seroconversion. In cohort 1, frozen plasma samples that were negative for HIV antibody and p24 antigen were retrospectively tested for HIV RNA. Follow-up was not available for these patients; therefore, before testing any samples, we determined that an HIV RNA level greater than 10 000 copies/mL would be considered a true-positive result. This HIV RNA level, in our experience, has not been seen in false-positive samples. Sensitivity and specificity, along with corresponding 95% confidence intervals, were determined by using tabulated exact binomial limits for sample sizes less than or equal to 100 (8) and by using the normal approximation to the binomial for sample sizes greater than 100.

Patients in cohort 2 were analyzed for predictors of primary infection on the basis of uniformly collected demographic characteristics, exposure history, and symptoms. All variables were compared by using a two-sample *t*-test. To assess the impact of several predictor variables, a stepwise discriminant analysis was performed by using variables that differed significantly between patients with and those without primary infection (significance was indicated by a P value < 0.05).

Role of the Funding Sources

The funding sources had no role in the collection, analysis, or interpretation of the data or in the decision to submit the paper for publication.

RESULTS

Demographic characteristics were similar across cohorts. Overall, 89% of patients were male, 74% were white, 13% were Hispanic, and 9% were African American. Regarding risk factors, 77% of patients were homosexual men, 18% were heterosexual women, and 4% used intravenous drugs. Patients were categorized as having 1) primary infection with undetectable HIV antibodies or an indeterminate Western blot (12.4%), 2) chronic infection with a positive Western blot (18.1%), or 3) no infection (69.5%).

Sensitivity and Specificity of Virologic Assays

The results of screening tests are summarized in **Table 1**. Patients with primary infection had undetectable HIV antibodies or indeterminate Western blot but a confirmed positive virologic test result. Two patients in cohort 1 were negative for HIV antibody and p24 antigen but positive for HIV RNA, with levels greater than 100 000 copies/mL. For the purpose of this analysis, they were considered to be true positive for primary HIV infection. Overall, p24 antigen testing had a sensitivity of 88.7% (95% CI, 77.0% to 95.7%). The mean concentration of HIV RNA in the five patients who had primary infection but undetectable p24 antigen was 251 189 copies/mL (range, 100 000 to 630 957 copies/mL). The overall specificity of tests for HIV RNA and p24 antigen was 97.4% (CI, 94.9% to 98.9%) and 100% (CI, 99.3% to 100%), respectively. Eight of 303 uninfected patients (2.6%) had false-positive results on HIV RNA testing (mean concentration, 269 copies/mL [range, 52 to 1950 copies/mL]).

Table 1. **Serologic and Virologic Results in Patients Screened for Primary HIV Infection**

Variable	Cohort 1 (n = 127)		Cohort 2 (n = 255)		Cohort 3 (n = 54)		Total (n = 436)*	
	Positive for HIV RNA	Positive for p24 Antigen	Positive for HIV RNA	Positive for p24 Antigen	Positive for HIV RNA	Positive for p24 Antigen	Positive for HIV RNA†	Positive for p24 Antigen
	← n/n (%) →							
Primary HIV infection								
Negative for HIV antibody on enzyme immunoassay	16/16 (100.0)	14/16 (87.5)	19/19 (100.0)	17/18 (94.4)‡	6/6 (100.0)	4/6 (66.7)	41/41 (100.0)	35/40 (87.5 73.2–95.8])‡
Indeterminate Western blot	6/6 (100.0)	5/6 (83.3)	6/6 (100.0)	6/6 (100.0)	1/1 (100.0)	1/1 (100.0)	13/13 (100.0)	12/13 (92.3 [64–99.8])
Chronic HIV infection	Not done	3/19 (15.8)	26/26 (100.0)	7/26 (26.9)	34/34 (100.0)	0/10 (0.0)§	60/60 (100.0)‖	10/55 (18.2)§
Uninfected¶	0/86 (0.0)	0/86 (0.0)	8/204 (3.9)	0/204 (0.0)	0/13 (0.0)	0/13 (0.0)	8/303 (2.6)	0/303 (0.0)

* HIV RNA assay had a specificity of 97.4% (CI, 94.9% to 98.9%); p124 antigen assay had a specificity of 100% (CI, 99.3% to 100%). Numbers in square brackets are 95% CIs.
† Because sensitivity for the HIV RNA assay was 100%, no confidence intervals are provided.
‡ p24 antigen assay was not performed in 1 patient at the time of the negative result on enzyme immunoassay.
§ p24 antigen assay was not performed in 24 patients.
‖ HIV RNA assay was not performed in 19 patients.
¶ All patients who were positive for HIV RNA but were classified as uninfected (false positives) had repeated HIV RNA assays at subsequent time points and were found to be negative. The patients had follow-up serologic examination that showed no evidence of seroconversion.

Predictors of Primary HIV Infection

Cohort 2 was divided into patients with primary infection and those who were uninfected or had chronic HIV infection (**Table 2**). The primary infection group involved 25 patients who had a negative antibody test result or indeterminate Western blot and 15 patients who had had a negative antibody test result in the preceding 3 months. Of these 15, 4 had had a negative Western blot in the previous 3 weeks and 11 had had a documented negative antibody test result or evolving Western blot in the previous 3 months. Those with primary infection were more likely to be homosexual; to have been exposed to an HIV-infected person; and to report fever, myalgia, arthralgia, rash, or night sweats ($P < 0.05$ for all comparisons) (**Table 2**). Combining fever, myalgia, and rash increased the predictive value of symptoms; however, no combination of symptoms identified more than 75% of patients with primary infection.

Discussion

Our study shows that no clinical symptoms have sufficient sensitivity or specificity for primary infection to allow targeted screening of at-risk persons. In addition, we show that while both p24 antigen and plasma HIV RNA assays are useful virologic tests for diagnosing primary infection, each has limitations. Assays for HIV RNA are likely to be more sensitive but are associated with lower specificity and can therefore yield more false-positive results.

Similar to our study, a study in India that screened patients in sexually transmitted disease clinics showed that certain symptoms occur with increased frequency in patients with primary HIV infection (9). Nevertheless, both this study and our study demonstrate that selective screening would miss a substantial number of patients

Table 2. **Clinical Predictors of Primary HIV Infection in Cohort 2**

Sign or Symptom	Patients with Primary HIV Infection (n = 40)*	Patients with No Primary HIV Infection (n = 164)†
	n (%)	
Fever	35 (87.5)‡	82 (50.0)
Myalgia	24 (60.0)‡	43 (26.2)
Rash	23 (57.5)‡	34 (20.7)
Night sweats	20 (50.0)‡	52 (31.7)
Arthralgia	11 (27.5)‡	21 (12.8)
Nasal congestion	7 (17.5)	62 (37.8)‡
Malaise	29 (72.5)	95 (57.9)
Headache	22 (55.0)	72 (43.9)
Sore throat	17 (42.5)	80 (48.8)
Lymphadenopathy	15 (37.5)	48 (29.3)
Oral ulcers	3 (7.5)	13 (7.9)
Thrush	2 (5.0)	7 (4.2)

* Involves patients who were screened between June 1996 and February 1999. Primary infection was defined as a negative result on enzyme immunoassay for HIV antibody, an indeterminate result at the time of screening, or symptoms that occurred in temporal relation to a negative or evolving result on Western blot in the preceding 12 weeks.
† Involves patients who were chronically infected or were uninfected.
‡ $P < 0.05$.

with primary infection. Thus, to maximize the number of identified infected patients, many seronegative persons must be screened.

Several groups have reported that assays for HIV RNA are more sensitive than those for p24 antigen in diagnosing primary infection. However, these studies often screened asymptomatic patients, such as those found to experience seroconversion during cohort studies (10) and those with indeterminate Western blots (11). This makes the findings less relevant for the prospective screening of symptomatic patients. Similar to our study, other studies have shown that the assay for p24 antigen is sensitive in symptomatic antibody-negative patients, in whom virologic testing is necessary for diagnosis (4, 7, 12). Overall, these studies and our own emphasize the importance of analyzing the sensitivity and specificity of virologic assays in the context of the patient's clinical and serologic status.

When selecting a virologic test, it is important to consider its cost in clinical practice. In our study, it cost approximately $6000 ($20 per test) to screen all seronegative patients in cohort 2 for p24 antigen. To identify a single patient missed by p24 antigen testing, we were required to perform HIV RNA assays on more than 100 patients at $100 per test, an additional cost of more than $10 000. Furthermore, false-positive results on HIV RNA assays require follow-up testing and extensive post-test counseling and are associated with substantial psychological distress. In addition, all screened persons are counseled about the possibility of false-negative results and the need for follow-up testing.

False-positive results on plasma HIV RNA assays (both bDNA and reverse transcriptase polymerase chain reaction) have been reported in case series (13), prospectively screened at-risk persons (14), and studies of assay performance (15). We found that during screening for symptomatic primary infection, false-positive results generally occurred at values less than 10 000 copies/mL. True-positive values, in contrast, consistently exceeded 100 000 copies/mL. However, a threshold for the interpretation of HIV RNA results in this setting will need to be defined in future studies.

Our study had several limitations. First, it was referral based and may be biased toward more symptomatic patients. However, patients were primarily referred from a broad range of ambulatory clinics, and the fact that recruitment was done at two centers may make our results more generalizable. Second, we do not address the potential role of other virologic tests, such as proviral DNA. Finally, studies involving presenting symptoms could be biased by poor patient reporting or recall, an effect we attempted to minimize by administering a standardized questionnaire.

Our study provides important information on optimal use of diagnostic tests in the evaluation of patients with suspected primary HIV infection. It could be argued that if resources for assays and pre- and post-test counseling are unlimited, then tests for HIV RNA may be the best screening tool. Alternatively, when testing patients for suspected primary symptomatic HIV infection, clinicians can be confident that more than 90% of those with negative results on assays for HIV antibody or indeterminate Western blots will be identified by the assay for p24 antigen alone.

Appendix

The following study sites and investigators participated in the Los Angeles County Primary HIV Infection Recruitment Network. All sites are located in Los Angeles, California.

Cedars-Sinai Medical Center: E. Daar (*principal investigator*), J. Pitt, P. Gaut, S. Nichols; Los Angeles County HIV Epidemiology/Project Open Window: P. Kerndt (*principal investigator*), N. Harawa, W. Senterfitt, E. Sey, C. Whitfield, Satellite Testing Office for Research and Education personnel; University of California, Los Angeles: R. Mitsuyasu (*principal investigator*), J. Giorgi, S. Chafey, C. Price, M. Carlson; Los Angeles County University of Southern California: M. Dube (*principal investigator*), J. Currier, C. Funk; Harbor/University of California, Los Angeles: G. Beale (*principal investigator*), S. Kruger, M. Witt, R. Lewis; AIDS Healthcare Foundation: C. Farthing (*principal investigator*), E. Graham; King Drew Medical Center: W. Jordan (*principal investigator*), L. Tolbert; Pacific Oaks Medical Group: D. Hardy (*principal investigator*), D. Morales; Jeffery Goodman Clinic: R. Bolan (*principal investigator*), E. Castrejon.

From Cedars-Sinai Burns & Allen Research Institute, University of California, Los Angeles, School of Medicine, Los Angeles County HIV Epidemiology Program, University of California, Los Angeles, Center for AIDS Research, and Los Angeles Free Clinic, Los Angeles, California; and University of California, San Diego, and San Diego Veterans Affairs Healthcare System, San Diego, California.

Note: The coauthors acknowledge the contribution of Janis V. Giorgi, PhD, who died on 30 May 2000. Dr. Giorgi, an internationally recognized cellular immunologist, will be remembered for her influence on her colleagues and for the profound impact of her work on the understanding of HIV immunopathogenesis.

Acknowledgments: The authors thank Dr. J. Elashoff for biostatistical support; C.-K. Chan, J. Gazola, S. Albanil, J. Vega, and N. Keating for technical assistance; Chiron Diagnostics for HIV RNA assays; and all patients who agreed to undergo screening for primary HIV infection in Los Angeles and San Diego.

Grant Support: In part by the Universitywide AIDS Research Program, University of California (PH97-SD-201, PH97-CS-202, General Clinical Research Center grant M01-RR00425); National Center for Research Resources, the Women's Guild (AI 43638, AI 27670, AI 38858, AI 36214 [Center for AIDS Research]); the National Institutes of Health (AI29164); the Research Center for AIDS and HIV Infection of the San Diego Veterans Affairs Medical Center; and the Women's Guild.

Requests for Single Reprints: Eric S. Daar, MD, Division of Infectious Diseases, Cedars-Sinai Medical Center, B217, 8700 Beverly Boulevard, Los Angeles, CA 90048; e-mail: Daar@CSHS.org.

Current Author Addresses: Drs. Daar, Ho, Bai, and Gaut and Ms. Pitt: Division of Infectious Diseases, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.
Dr. Little: University of California, San Diego, 2760 Fifth Avenue, Suite 300, San Diego, CA 92103.
Ms. Santangelo: University of California, San Diego, 9500 Gilman Drive #8208, San Diego, CA 92093-8208.
Ms. Harawa: HIV Epidemiology Program, Los Angeles County Department of Health Services, 600 South Commonwealth Boulevard, Suite 1920, Los Angeles, CA 90005.
Dr. Kerndt: Los Angeles County Department of Health Services STD Program, 2615 South Grand Avenue, Room 500, Los Angeles, CA 90007.
Dr. Richman: Department of Pathology and Medicine-0679, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0679.
Dr. Mandel: Los Angeles Free Clinic, 8405 Beverly Boulevard, Los Angeles, CA 90048.
Dr. Nichols: Department of Pathology, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.

Author Contributions: Conception and design: E.S. Daar, J. Pitt, P. Kerndt, J.V. Giorgi.
Analysis and interpretation of the data: E.S. Daar, S. Little, P. Ho, D.D. Richman.
Drafting of the article: E.S. Daar, D.D. Richman.
Critical revision of the article for important intellectual content: E.S. Daar, S. Little, J. Pitt, P. Ho, N. Harawa, P. Kerndt, J. Bai, P. Gaut, D.D. Richman, S. Mandel, S. Nichols.
Final approval of the article: E.S. Daar, S. Little, J. Pitt, P. Ho, N. Harawa, P. Kerndt, J. Bai, P. Gaut, D.D. Richman, S. Mandel, N. Nichols.
Provision of study materials or patients: E.S. Daar, S. Little, J. Pitt, N. Harawa, P. Kerndt, P. Gaut, D.D. Richman, S. Mandel.
Obtaining of funding: E.S. Daar, S. Little.
Administrative, technical, or logistic support: J. Pitt, P. Kerndt, J. Bai.
Collection and assembly of data: E.S. Daar, S. Little, J. Pitt, J. Santangelo, P. Ho.

References

1. **Vanhems P, Allard R, Cooper DA, Perrin L, Vizzard J, Hirschel B, et al.** Acute human immunodeficiency virus type 1 disease as a mononucleosis-like illness: is the diagnosis too restrictive? Clin Infect Dis. 1997;24:965-70. [PMID: 0009142802]

2. **Kahn JO, Walker BD.** Acute human immunodeficiency virus type 1 infection. N Engl J Med. 1998;339:33-9. [PMID: 0009647878]

3. **Daar ES, Moudgil T, Meyer RD, Ho DD.** Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med. 1991;324:961-4. [PMID: 0001823118]

4. **Schacker T, Collier AC, Hughes J, Shea T, Corey L.** Clinical and epidemiologic features of primary HIV infection. Ann Intern Med. 1996;125:257-64. [PMID: 0008678387]

5. **Jacquez JA, Koopman JS, Simon CP, Longini IM Jr.** Role of the primary infection in epidemics of HIV infection in gay cohorts. J Acquir Immune Defic Syndr. 1994;7:1169-84. [PMID: 0007932084]

6. **Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al.** Vigorous HIV-1-specific $CD4^+$ T cell responses associated with control of viremia. Science. 1997;278:1447-50. [PMID: 0009367954]

7. **Niu MT, Bethel J, Holodniy M, Standiford HC, Schnittman SM.** Zidovudine treatment in patients with primary (acute) human immunodeficiency virus type 1 infection: a randomized, double-blind, placebo-controlled trial. DATRI 002 Study Group. Division of AIDS Treatment Research Initiative. J Infect Dis. 1998;178:80-91. [PMID: 0009652426]

8. Exact confidence limits for p. In: Lentner C, Lentner C, Wink A. Geigy Scientific Tables. vol 2. West Caldwell, NJ: CIBA-Geigy; 1982:89-102.

9. **Bollinger RC, Brookmeyer RS, Mehendale SM, Paranjape RS, Shepherd ME, Gadkari DA, et al.** Risk factors and clinical presentation of acute primary HIV infection in India. JAMA. 1997;278:2085-9. [PMID: 0009403423]

10. **Phair JP, Margolick JB, Jacobson LP, Phillips J, Rinaldo C, Kaslow R, et al.** Detection of infection with human immunodeficiency virus type 1 before seroconversion: correlation with clinical symptoms and outcome. J Infect Dis. 1997; 175:959-62. [PMID: 0009086159]

11. **Henrard DR, Phillips J, Windsor I, Fortenberry D, Korte L, Fang C, et al.** Detection of human immunodeficiency virus type 1 p24 antigen and plasma RNA: relevance to indeterminate serologic tests. Transfusion. 1994;34:376-80. [PMID: 0008191559]

12. **Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S.** Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. J Clin Microbiol. 1994;32:292-300. [PMID: 0008150937]

13. **Rich JD, Merriman NA, Mylonakis E, Greenough TC, Flanigan TP, Mady BJ, et al.** Misdiagnosis of HIV infection by HIV-1 plasma viral load testing: a case series. Ann Intern Med. 1999;130:37-9. [PMID: 0009890848]

14. Program and Abstracts of the Sixth Conference on Retroviruses and Opportunistic Infections. Chicago, Illinois, 31 January–4 February 1999. Alexandria, VA: Foundation for Retrovirology and Human Health; 1999.

15. **Erice A, Brambilla D, Bremer J, Jackson J, Kokka R, Yen-Lieberman B, et al.** Performance characteristics of the bDNA 3.0 assay for quantitation of HIV-1 RNA in plasma [Abstract]. In: Seventh Conference on Retroviruses and Opportunistic Infections, San Francisco, California, 30 January–2 February 2000. Alexandria, VA: Foundation for Retrovirology and Human Health; 2000.