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This study had two objectives: first, to determine whether there is evidence for loss of human immunodeficiency virus type 1(HIV-1) antibody in serreactive individuals Second, if true seroreversion occurs, to determine its incidence relative to errors in the testing process. A retrospective cohort study reviewing the results of 5,446,161 HIV-1 antibody tests performed on 2,580,974 individuals(US ARMY HIV data system) from 1985 thru 1992. For all patients with one or more seroreactive sample followed by one or more nonreactive sample, we examined the available records and retested the samples. Serum samples had been obtained from active-duty and retired military personnel, their dependents, and applicants to the military. Review of this database demonstrates no evidence for true seroreversion of HIV-1 antibody status. We conclude that if seroreversion occurs at all, it is exceedingly rare. In fact, most(if not all) cases of apparentseroreversion represent errors of attribution or testing.

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Absence of True Seroreversion of HIV-1 Antibody in Seroreactive Individuals

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Objectives.—First, to determine whether there is evidence for loss of human immunodeficiency virus type 1 (HIV-1) antibody in seroreactive individuals. Second, if true seroreversion occurs, to determine its incidence relative to errors in the testing process.

Design.— A retrospective cohort study reviewing the results of 5 446 161 HIV-1 antibody tests performed on 2 580 974 individuals (the US Army HIV Data System) from 1985 through 1992. For all patients with one or more seroreactive sample followed by one or more nonreactive sample, we examined available records and retested the samples.

Participants.—Serum samples had been obtained from active-duty and retired military personnel, their dependents, and applicants to the military.

Results.—Of 4911 individuals reported to be seroreactive for HIV-1 by two independent samples, only six were potential seroreverters. Review of the six cases revealed that five actually were HIV-seroreactive patients who had samples from nonreactive individuals mistakenly attributed to them, while the sixth had a testing error proven by retesting the discrepant specimen. Errors in the testing process were identified (n=23) or suspected (n=3) in another 26 individuals who had not had independent confirmation of reactivity by a second sample. The cumulative error rate was 12.4 per 1 million patients tested. An additional group of 31 uninfected infants appeared to seroverert due to loss of antibody acquired from their HIV-1–infected mothers.

Conclusions.—Review of this database demonstrates no evidence for true seroreversion of HIV-1 antibody status. We conclude that if seroreversion occurs at all, it is exceedingly rare. In fact, most (if not all) cases of apparent seroreversion represent errors of attribution or testing.

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HUMAN immunodeficiency virus type 1 (HIV-1), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), can be transmitted by sexual intercourse, by sharing of needles among intravenous drug users, by transfusion of blood or blood products, and transplacentally or perinatally from mother to fetus.[1,2] Infection with HIV-1 is commonly identified by the presence in the serum of antibodies to specific viral structural antigens. A highly sensitive enzyme-linked immunosorbent assay (ELISA) and a more specific Western blot are the most popular antibody tests, while a recombinant enzyme immunoassay (EIA) and/or a radioimmunoprecipitation assay are generally used only if diagnostic uncertainty persists.[3] The development of antibodies to HIV-1 typically occurs 3 to 12 weeks after infection, although significant delays in seroconversion have been reported.[4,5]

While hundreds of thousands of seropositive patients have experienced progressive immunosuppression and/or life-threatening opportunistic infections, two reports[6,7] in the medical literature have attempted to document a total of five seroreverters who would appear to have lost previously detectable HIV-1 serum antibodies. One was an isolated report of the wife of a hemophiliac who was transiently reactive by ELISA alone[6]; the other four cases were culled by the Multicenter AIDS Cohort Study from their 1000 reportedly seroreactive patients.[7] However, none of the cases had documented seroreactivity by a second independent sample prior to apparent seroreversion, and neither report incorporated a thorough search for potential errors that could explain the findings. Subsequently, Holmberg et al[8] examined three cohorts involving a total of 660 seroreactive patients and identified 16 potential seroreverters. Again, seroreactivity was not documented by two independent specimens, but a systematic review identified 11 clerical errors and eight testing errors responsible for the 16 cases.

Because of the rarity of seroreversion (if it indeed occurs), we felt that a directed analysis of a much larger database was necessary to determine the potential for true seroreversion and to estimate its incidence relative to the testing of samples and the recording of data. This is important in helping to define the parameters of the natural history of HIV infection, as well as in determining blood bank policy regarding donations from individuals with a history of seroreactivity.

METHODS

Data

The US Department of the Army began testing all new recruits for HIV-1 in October 1985. All active-duty army personnel have been tested annually, beginning in January 1986. Applicants for

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the army, army reserves, and the National Guard have also been tested. Some military dependents and retirees have been tested at their request or at the discretion of their physicians. Through June 1992, a total of 5 446 161 samples obtained from 2 580 974 individuals had been tested. All test results have been retained in the computer-based US Army HIV Data System (USAHDS), facilitating this analysis.

Our testing algorithm for distinguishing seroreactivity has been described.[9,10] The algorithm includes an ELISA (Abbott Laboratories, North Chicago, Ill, or DuPont Laboratories, Wilmington, Del), a Western blot (DuPont Laboratories, Wilmington, Del), a highly sensitive protein-based EIA (Recombigen HIV-EIA, Cambridge Bioscience, Worcester, Mass), and/or a radioimmunoprecipitation assay. It is important to note that in August 1987, an improved Western blot became available, increasing the sensitivity and specificity of the testing process. In rare instances when results were inconclusive after all four types of testing, the tests were repeated and/or additional samples were obtained.

A retrospective computer-directed review of the USAHDS database selected all patients reported to have had a reactive test result followed at a later date by a nonreactive result. All serum samples had been stored in test tubes at −20°C at the Walter Reed Army Institute of Research, Washington, DC. Samples identified in the computer search were retrieved for retesting and tube labels were reviewed. Labels for samples collected before August 1987 bore the testee's name and social security number (SSN), except for military dependents. For dependents, their own name was accompanied by the SSN of the soldier through whom they were eligible for care. Use of the soldier's SSN with the name of their dependent is standard throughout the military for identification purposes. Beginning in August 1987, names and SSNs were replaced on the labels by five-digit sample codes. Also beginning in 1987, two-digit family member prefixes were recorded in the database with the SSNs, decreasing confusion of samples within families by giving each member a unique identifier.

Written records of ELISA and Western blot results were reviewed and compared with results recorded in the computer database. Photographs of the Western blots were reviewed and compared with the written and computerized results. Individual patient records were reviewed, when available, for reports of test results, evidence of active HIV infection, and other illnesses that could be responsible for false-reactive results. In several cases, physicians who cared for study cases were contacted to provide additional information regarding patients' clinical status.

Table 1.—Potential Seroreverters

Patient No.	Sex	Type of Error	Date	Test Results* ELISA	WB	EIA
1	F	Attribution	6/86	+	+	+
			6/86	+	+	+
			7/86†	−	−	−
			7/86†	−	−	−
			7/86†	−	−	−
2	M	Attribution	6/86	+	+	+
			6/86	+	+	+
			7/86‡	−	−	−
3	M	Attribution	4/86	+	+	+
			5/86	+	+	+
			5/86†	−	−	−
			5/86†	−	−	−
			8/86†	−	−	−
4	F	Attribution	6/86	+	+	+
			7/86	+	+	+
			7/86†	−	−	−
			7/86†	−	−	−
5	F	Testing	9/86	+	+	+
			9/86	+	+	+
			12/86; retest 6/89	−; +	ND; +	ND; +
			6/87	+	+	+
			7/87	+	+	+
			9/87	+	+	+
6	M	Undetermined	10/91; retest 7/92	+; +	+; +	+; +
			1/92; retest 7/92	+; +	+; +	+; +
			3/92; retest 7/92	−; −	−; −	−; −
			5/92; retest 7/92	+; +	+; +	+; +
			9/92	+	+	+

*ELISA indicates enzyme-linked immunosorbent assay; WB, Western blot; EIA, enzyme immunoassay; +, reactive; −, nonreactive; and ND, not done.
†Sample found to have been drawn from a different family member.
‡Sample found to have been drawn from an unrelated individual.

Definitions

For the purposes of the study, we used the following definitions:

Potential Seroreverter.—An individual having two recorded reactive samples followed by a recorded nonreactive sample.

True Seroreverter.—An individual having two reactive samples confirmed by retesting and confirmed to belong to that individual, followed by a nonreactive sample confirmed in the same manner.

Attributional Error.—A case in which a sample from one patient was inappropriately attributed to another patient, most often ascertained by reviewing the labels on the test tubes containing sera.

Transcriptional Error.—A case in which a test result was erroneously transcribed, either on paper or into the computer database. These were ascertained by the direct comparison of Western blot photographs and written and computerized results.

Testing Error.—A case in which retesting of a sample resulted in a different result than was originally obtained.

RESULTS

Of the almost 2.6 million individuals tested by June 1992, a total of 4911 (0.19%) were reactive on two independent samples. Sixty-three individuals had a nonreactive test result when retested at a later date, including 31 uninfected infants who transiently carried antibodies acquired from their HIV-infected mothers. The other 32 individuals (31 adults, one child) included 26 who had only a single reactive result recorded prior to a nonreactive test. Thus, in this large database, only six patients met our definition of potential seroreverter.

We carefully evaluated each of the six potential seroreverters by the methods described. All were adults; three were women and three were men. Five have since died of complications from AIDS or intravenous drug use, while the clinical status of one individual is unknown. Review of labels on the samples revealed that for four of the six individuals, the sole nonreactive sample had been drawn

Table 2.—Errors in Patients With Nonreactive Samples After a Single Reactive Sample

Type of Error	No. of Patients
Attribution	11
Transcription	6
Testing	6
Undetermined	3
Total	**26**

Table 3.—All-Cause Errors in Testing

Year	No. of Tests	No. of Patients Tested	No. of Errors	Errors per 1 000 000 Patients Tested
1985 and 1986	541 947	509 670	17	33.4
1987	948 374	803 804	8	10.0
1988	798 397	714 939	4	5.6
1989	955 664	841 818	1	1.2
1990	1 021 243	882 563	0	0
1991	881 592	760 455	1	1.3
1992	298 944	288 839	1	3.5
Total	**5 446 161**	2 580 974*	**32**	12.4

*Does not correspond to sum of values in column because numerous patients were tested in more than 1 year.

from a different patient. An explanation for the attributional error was readily available in every case. For three of the four, after each had confirmed antibody reactivity, other family members were tested; none of the family members were reactive and their results were erroneously recorded under the name of the infected individual (Table 1, patients 1, 3, and 4). Patient 2 was the only case of the four not resulting from intrafamilial misattribution; a nonreactive result from an unrelated individual with a similar surname was misattributed to patient 2 the month after he had been diagnosed with infection on the basis of two reactive samples.

For patient 5, a testing error was identified. Her initial reactive specimen had been confirmed by a second reactive sample 2 weeks later. A third sample taken 3 months after the first was reportedly nonreactive by ELISA, and in accord with the algorithm, it had not been subject to further testing. However, when we tested this sample it was strongly reactive by EIA and Western blot. Furthermore, samples obtained 6, 7, and 9 months after the discrepant specimen were all strongly reactive. In fact, the patient has since died of complications of AIDS.

Patient 6 had two reactive samples followed by a nonreactive sample. He demonstrated progression of disease, with a declining CD4 cell count, and has since died from complications relating to recurrent intravenous drug use. Two more strongly reactive samples were obtained 2 and 6 months after the nonreactive specimen. Retesting of the first four samples confirmed the original results. Review of the laboratory records at the site of his medical care corroborates that samples were obtained in October 1991, and January, May, and September 1992; however, there is no record of an HIV antibody test having been requested and/or obtained in March 1992, when the USAHDS database attributed a nonreactive sample to this individual. The clinicians who had seen the patient in March 1992 did not recall having requested an HIV-1 antibody test on him at that time. Their clinic records and the patient's personal file did not show that an antibody test was planned, requested, or obtained.

We also evaluated each individual who had a single reactive sample followed by one or more nonreactive samples (Table 2). Although this group of individuals had not met our criteria for HIV-1 infection, we analyzed their cases in order to facilitate comparisons with previous reports that did include such patients. In this group, we found errors of attribution (n=11), transcription (n=6), and testing (n=6). Again, attributional errors were primarily intrafamilial (nine of 11). The six transcriptional errors were equally divided between erroneous entrance of results on written records and erroneous transfer of results from written records into the computer. For half the testing errors, the more accurate Western blot that had become available since the initial tests was a factor in the reversal. For three of the 26 patients, our systematic review did not identify a specific error. The first was a 43-year-old man who initially had an HIV-1 antibody test performed because of a history of intermittent adenopathy and recurrent pharyngitis. The test was reactive by ELISA, strongly reactive by EIA, and moderately reactive by Western blot. Retesting of this sample demonstrated strong reactivity by EIA and Western blot. However, two other samples drawn 12 and 15 days after the initial sample were completely nonreactive both initially and when retested. The tube labels all bore the same identity. The patient remains alive and without evidence of HIV-1 infection. The other two individuals were tested at a time when names no longer appeared on the samples. Both were male active-duty US Army personnel stationed in Germany at the time of testing, rendering retrieval of clinical data difficult. Unfortunately, they have been lost to follow-up. One had a strongly reactive sample, followed by nonreactive samples 2, 3, and 7 months later; results were confirmed by retests. The other had a moderately reactive sample followed by nonreactive samples 2 weeks and 3 months later, also confirmed by retests. Overall, 32 patients with suspected or proven errors were identified. This yields a cumulative error rate of 12.4 per 1 million patients tested from 1985 through 1992 (Table 3).

COMMENT

This is by far the largest evaluation to date of potential HIV-1 seroreversion. Of nearly 2.6 million individuals tested, only six could legitimately be considered potential seroreverters. For five of the six, a readily identifiable error was responsible for the apparent seroreversion. Although a specific error was not identified in the sixth case, seroreversion was effectively ruled out by both clinical progression and subsequent antibody tests. In fact, the pattern of strongly reactive specimens 2 months before and 2 months after the lone, entirely nonreactive sample clearly suggests that the discrepant sample was obtained from a different individual. Unfortunately, retrospective delineation of an attributional error occurring after 1987 is difficult, because of the elimination of patient names and SSNs from tube labels.

Most (if not all) of the errors identified are human errors. This is readily apparent for attributional and transcriptional errors, but also may be true for what we have classified as testing errors. A change in a result on retesting may indicate a technical problem with the original test kit, but this should be detected by the mandated testing of known controls. Human error could be responsible for contaminating samples, improperly combining serum and reagents, or incorrect initial recording of results. Since even human error is uncommon, it can be best controlled by repeating all procedures twice. The effectiveness of such a policy is readily evidenced by the elimination of most cases of false seroreversion simply by requiring that patients have two independent samples verified as seroreactive before they are considered infected. Double entry of results, both on paper and into a computer database, with comparison of entries, should eliminate the great majority of transcriptional errors. Testing errors can be controlled by simply repeating the tests. The abil-

ity to limit errors is evidenced by the marked decline in the error rate during the course of this study (Table 3), particularly the drop in attributional errors after the 1987 introduction of family member prefixes. Even if careful methods including duplication are used, errors will occur, but one error in hundreds of thousands of tests may be an acceptable rate.

The Multicenter AIDS Cohort Study reported four cases of apparent seroreversion among 1000 seroreactive men.[7] In every case, seroreactivity had not been confirmed by a second sample prior to the purported seroreversion. Therefore, their patients are similar to those in our Table 2. Although the investigators seem to have effectively ruled out errors of attribution, our study would suggest that errors in the testing process or in the transcription of results may be responsible for their findings. More importantly, it elucidates the importance of not labeling an individual as seroreactive until he or she has had seroreactivity confirmed by a second sample. In fact, we had three patients with a single reactive specimen for whom our systematic review failed to distinguish a specific error. Whereas previous investigators may have considered them to be potential seroreverters, we believe that it is not accurate to label them as seroreverters due to the lack of confirmation of initial seroreactivity.

The impact of this approach on estimated prevalence of potential seroreversion is significant. In the Multicenter Cohort,[7] the incidence would drop from 0.004 to 0. The risk of error and the consequences of misdiagnosis are too great when seroreactivity is determined based on the results of a single specimen. In Holmberg et al,[8] it is not possible to distinguish how many of their 16 cases had confirmed seroreactivity, but presumably it would have been very few. They calculate an error rate of 0.0115, based on 19 errors after 1658 tests performed. Using similar calculations, we are able to derive an error rate of 0.00000588, based on 32 known or presumed errors after 5 446 161 tests performed. Moreover, this rate would drop to about one in 1 million if two samples were required to confirm seroreactivity.

Although the prevalence of disease was much lower in our population than in either of the two previously reported cohorts, and this is in part responsible for our lower error rate, we believe our remarkably low rate also attests to the quality of our testing program. As Table 3 indicates, the error rate also progressively declined, demonstrating the benefits of quality improvement. Investigators in the future may well find differences in the relative frequencies of the categories of errors depending on the quality of the components of their programs; however, the types of errors are likely to be similar to those we have identified. Utilization of the USAHDS database had other advantages and disadvantages. One benefit is that a large number of tests were performed in diverse settings and for diverse indications, facilitating generalizability of our results to other populations. Another is that uniform techniques and interpretations were applied to a very large number of antibody tests, providing a wealth of data that might otherwise be difficult to compile. On the other hand, the fact that a number of our cases had been tested in sites ranging from Germany to South Korea sometimes rendered clinical information and follow-up difficult to obtain.

We excluded infants from this study because they are known to transplacentally acquire maternal antibody, which gradually disappears over the first 9 to 15 months of life when they are not infected. However, we believe they may provide further evidence that the unresolved discrepancies in our three patients, and in those reported from the Multicenter Cohort, are due to errors rather than true seroreversion. Individuals who serorevert, for whatever reason, could reasonably be expected to have a slow decline in antibody strength over many months, similar to uninfected infants. The three patients we classified as undetermined had abrupt changes in reactivity status, becoming entirely nonreactive only 12, 18, and 69 days after clearly reactive tests. Clearance this rapid lacks biologic plausibility. It is the type of dramatic change we have seen for cases in which errors were made, as opposed to infants, who seem to be the only true seroreverters. Human leukocyte antigen haplotyping would have been useful in further confirming attributional errors; unfortunately, only serum was saved from each sample of blood obtained and cells were not available to perform this procedure.

We have demonstrated that after the performance of several million HIV-1 antibody tests, there is no evidence that an individual who has been confirmed to be seroreactive by two separate samples can subsequently become nonreactive. This has several ramifications. First, it is important to obtain two distinct specimens before labeling an individual with HIV-1 infection; this will overcome the great majority of inadvertent errors that may occur during the testing process. Second, once an individual has been confirmed as reactive, there is virtually no chance that a properly performed test thereafter will be nonreactive. In fact, if such a result is obtained, those performing the test should look for errors of attribution, transcription, and testing, which are immeasurably more likely than seroreversion. Finally, our results have implications for blood banks, which perform HIV-1 antibody tests primarily to ensure a safe blood supply; as such, they should continue to discard blood if the individual has even a single reactive ELISA. Common practice is to then perform a Western blot and to notify the donor if that is reactive. In such cases, we encourage blood banks to refer the individual to a physician or health department, as many undoubtedly already do, to be retested for confirmation of reactivity. Blood banks should never accept donations from individuals with a history of two previous reactive HIV-1 antibody tests; even if a nonreactive test is obtained at the time, that history would indicate that the nonreactive result is likely to be erroneous. Our study provides compelling evidence to dispel the notion that infected individuals may lose antibodies to HIV-1.

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