Avidity Index for Anti-HIV Antibodies: Comparison between Third- and Fourth-Generation Automated Immunoassays

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The development of assays for detecting recent HIV infections has become crucial for analyzing trends in infection in different populations, both for surveillance and prevention activities. The anti-HIV avidity index (AI), measured with third-generation immunoassays (which detect anti-HIV antibody), has been shown to be an accurate tool for discriminating recent HIV infections (<6 months) from established infections (≥6 months). We compared a third-generation immunoassay (AxSYM HIV 1/2 gO; Abbott Diagnostics) to a fourth-generation immunoassay (Architect HIV Ag/Ab Combo; Abbott Diagnostics; which detects anti-HIV antibody and p24 antigen) in terms of AI performance in distinguishing between recent and established HIV infections. A total of 142 samples from 75 HIV-infected individuals with an estimated date of seroconversion were assayed. The two assays showed the same accuracy in identifying a recent infection (91.5%), using an AI cutoff of 0.80, although Architect HIV Ag/Ab Combo was slightly more sensitive (98.7% versus 93.4%; P > 0.05) and yet less specific (93.4% versus 7.4%; P > 0.05). The correlation between assays was high (r = 0.87). When 20 specimens falling in the gray zone around the cutoff point (0.75 ≤ AI ≤ 0.84) were excluded, the accuracy of AI with Architect HIV Ag/Ab Combo was 94.7%, and the concordance between the two assays was 99.2%. The anti-HIV AI is a serological marker that accurately discriminates recent from established HIV infections. It can be successfully applied on fully automated fourth-generation HIV Ab/Ag immunoassays, which have several advantages, including increased throughput, high reproducibility, no need for specific technical skills, and easy comparability of results obtained in different settings.

In recent years, serological assays for detecting recent HIV infection have been developed, primarily for epidemiological purposes, although they also have a potentially important role in individual diagnosis (6, 9). These assays allow the incidence of HIV infection to be estimated in cross-sectional surveys, which are simpler to conduct, less expensive, shorter in time, and less resource intensive than the longitudinal studies usually used to investigate incidence.

One of these assays is the avidity index (AI) for antibodies against HIV, which is based on evidence that the antibody avidity/affinity for the antigen is low in the early phase of infection (0 to 12 months from infection) and increases with time until complete antibody maturation (3, 17). In previous studies using a third-generation commercial enzyme immunoassay (EIA) for HIV antibody detection (13), the AI was fairly accurate in discriminating recent from established HIV infections among persons infected with B or non-B HIV subtypes (15, 16). However, third-generation EIAs will soon be replaced by fourth-generation EIAs, which, in addition to anti-HIV antibodies, detect HIV antigens. The objective of the present study was to compare a third-generation EIA to a fourth-generation EIA in terms of AI performance in distinguishing between recent and established HIV infections, using serum samples from individuals with a known time of HIV seroconversion.

MATERIALS AND METHODS

Study population. We conducted the present study using serum samples from 75 HIV-positive persons who had been diagnosed with HIV infection at the Microbiology and Virology Unit of the Hospital Spedali Civili in Brescia, Italy, and who since 2000 have been monitored at this unit. In particular, the serum samples were taken from: (i) persons with AIDS known to be HIV positive for at least 10 years (47 samples from 47 individuals) and thus considered to have “established infections” and (ii) persons who had not developed AIDS and for whom it was possible to estimate the date of HIV seroconversion (midpoint in time between the date of the last documented HIV-negative test and that of the first HIV-positive test). The latter category included 95 serial serum samples from 28 HIV-positive persons (average of 3.4 serial serum samples available [range, 1.0 to 8.0]). Persons who had seroconverted in the 6 months prior to taking the serum sample were considered to have “recent infections,” whereas the remaining persons were considered to have “established infections (non-AIDS).”

Males accounted for 73.3% of the study participants. The median age was 39 years (interquartile range [IQR], 33 to 45 years). Seven participants were from countries other than Italy. For all participants, HIV infection was diagnosed based on the criteria reported below (see “Laboratory methods,” below). The basic condition for performing AI on serum samples was confirmed positivity to anti-HIV antibodies.

Laboratory methods. HIV was diagnosed with first-line tests: two third-generation HIV EIAs (AxSYM HIV 1/2 gO [Abbott Diagnostics, Wiesbaden, Germany] and Vitros Anti-HIV 1 + 2 [Ortho Clinical Diagnostics, Raritan, NJ]) and, in the past 4 years, a fourth-generation HIV EIA (Architect HIV Ag/Ab Combo [Abbott Diagnostics, Wiesbaden, Germany]). All first-line positive diagnoses were confirmed with Western blot (New LAV Blot HIVI [Bio-Rad, Marnes-La-Coquette, France]). When the result of the fourth-generation EIA was positive but the Western blot result was negative, an additional quantitative test for the HIV-1 gag p24 antigen (Innotest HIV Antigen mAb [Innogenetics, Gent, Belgium]) was performed using the following methods.

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AVIDITY INDEX FOR ANTI-HIV ANTIBODIES

TABLE 1. Classification of 142 serum samples based on the HIV AI (cutoff of 0.80) measured with two commercial EIAs, compared to classification based on the estimated seroconversion date or AIDS diagnosis

<table>
<thead>
<tr>
<th>Parameters based on HIV AI</th>
<th>Recent infection (≤6 mo)</th>
<th>Established infection non-AIDS (&gt;6 mo)</th>
<th>Established infection AIDS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of sera</td>
<td>66</td>
<td>29</td>
<td>47</td>
<td>142</td>
</tr>
<tr>
<td>No. of samples identified (% correctly classified) by:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI AxSYM</td>
<td>56 (84.8)</td>
<td>27 (93.1)</td>
<td>47 (100)</td>
<td>130 (91.5)</td>
</tr>
<tr>
<td>AI Architect</td>
<td>59 (89.4)</td>
<td>24 (82.8)</td>
<td>47 (100)</td>
<td>130 (91.5)</td>
</tr>
<tr>
<td>No. of samples misclassified by both assays</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>No. of GZ by AxSYM</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>No. of GZ by Architect</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

* GZ, gray zones (AI between 0.75 and 0.84).

The median AI values obtained with AxSYM HIV 1/2 gO were compared to those obtained with Architect HIV Ag/Ab Combo, using the Mann-Whitney test. For each sample, the correlation between the AI values obtained with the two assays was calculated with the parametric test of the Pearson correlation coefficient (r). For the qualitative AI results (i.e., classification as a recent or an established infection), the concordance between the two assays was calculated with the nonparametric tests of concordance and Cohen's kappa coefficient (κ). Data analysis was performed using SPSS 17.0.

RESULTS

The median age of the 47 persons with AIDS was 39 years (IQR, 33 to 45 years); 36 were males. For the 28 participants for whom the date of seroconversion was estimated, the median age was 36 years (IQR, 30 to 41 years); 19 were males. In this group, the median interval between the date of the last HIV-negative test and that of the first HIV-positive test was 13 days (IQR, 7 to 46 days).

Based on the estimated seroconversion date, 66 (46.5%) serum samples were recent infections, and 76 (53.5%) were established infections (including the persons with AIDS). In Table 1, this classification is compared to that based on the AI using the two EIAs. In Fig. 1, the distribution of the AI values obtained with AxSYM HIV 1/2 gO (Fig. 1A) and Architect HIV Ag/Ab Combo (Fig. 1B) by classification (recent infection, established infection [non-AIDS], or established infection AIDS, based on the seroconversion date or AIDS diagnosis) is shown. No correlation was observed between plasma viral load and AI results with either assay.

Performance of the AI with AxSYM HIV 1/2 gO. The median AI was significantly lower for recent infections compared to established infections (0.54 ± 0.21 and 0.97 ± 0.08, respectively; P < 0.0001). The sensitivity of the AI in correctly classifying recent infections was 84.8% (56/66), the specificity was 97.4% (74/76), the false recent rate was 2.6% (2/76), and the accuracy was 91.5% (130/142); 95% confidence interval = 86.9 to 96.1). When the 12 (8.4%) samples with the AI in the gray zone are excluded, the sensitivity was 88.3% (53/60), the specificity was 98.6% (69/70), and the accuracy was 93.8%; however, none of the increases were significant (P > 0.05).

Performance of the AI with Architect HIV Ag/Ab Combo. The median AI was significantly lower for recent infections compared to established infections (0.52 ± 0.22 and 1.02 ± 0.12, respectively; P < 0.0001). The sensitivity of the AI in correctly classifying recent infections was 89.4% (59/66), the specificity was 93.4% (71/76), the false recent rate was 6.6%
and the accuracy was 91.5% (130/142; 95% confidence interval = 86.9 to 96.1). When the 10 (7.0%) samples with the AI in the gray zone are excluded, the sensitivity was 91.7% (55/60), the specificity was 97.2% (70/72), and the accuracy was 94.7%; none of the increases were significant. A total of 34 antibody-positive samples were collected within 30 days of the estimated seroconversion date and tested for p24; of these, 9 were p24 positive. All nine samples were correctly classified by the AI as recent infections. These nine p24-positive samples were included in the calculations of sensitivity and specificity because they were also antibody positive.

**Comparison between AxSYM HIV 1/2 gO and Architect HIV Ag/Ab Combo.** The distribution of the AI values obtained with AxSYM HIV 1/2 gO and Architect HIV Ag/Ab Combo by classification is shown in Fig. 1. The distribution of AI values was similar for the two assays. Specifically, all specimens obtained from persons with AIDS had an AI of >0.80 and were correctly classified as established infections, whereas six serum samples were misclassified as recent infections by both assays (Table 1); of these samples, four were drawn from two individuals.

The nonparametric analysis of the AI values obtained with the two assays showed an $r$ correlation coefficient of 0.87 ($P < 0.0001$) (Fig. 2). Regarding the qualitative AI (i.e., classification as recent or established infection), there was concordance between the two assays for 132 of the 142 samples (concordance rate of 92.9%; $\kappa = 0.86$, $P < 0.0001$). The AI was in the gray zone for 12 samples with AxSYM HIV 1/2 gO and 10 samples with Architect HIV Ag/Ab Combo. When the 20 samples in the gray zone according to one of the assays were excluded, the concordance rate was 99.2%, $\kappa = 0.98$, $P < 0.0001$), and a discordant result was found for only one sample.

**DISCUSSION**

This is the first study to compare a third-generation and a fourth-generation EIA for HIV in terms of the performance of the AI and to evaluate a fourth-generation assay in terms of the AI. Moreover, recent infections were distinguished from established infections based on the estimated date of seroconversion. Both assays showed good sensitivity (i.e., classification as recent infections) and specificity (i.e., classification as established infections). Although a high proportion of samples were correctly classified as established infections with both assays, this proportion was slightly higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9). The proportion of sera correctly classified as recent infections was somewhat higher with AxSYM HIV 1/2 gO. With regard to persons with AIDS, all samples were correctly classified as established infections with both assays, whereas with other assays the classification of persons with AIDS is not consistently accurate (9).
sons, suggesting that in a few cases individual variability in the immunological response can lead to a rapid or slow maturation of the antibodies (11, 16, 17).

The gray zone is commonly used in commercial assays that measure the avidity of antibodies against infectious diseases, such as cytomegalovirus infection and toxoplasmosis (4, 7), with the scope of defining an area of uncertain results. In the present study, the proportion of sera included in the gray zone was small (≤9%), and it was consistent with the proportions reported for other widely used avidity assays (4). When these gray zone samples were excluded from the analysis, no significant change in the overall accuracy was observed with either assay, and the concordance between assays became almost absolute (99.2%), with only one discordant sample out of the gray zone. However, if the proportion of samples in the gray zone were higher (i.e., >15%), the overall classification of samples could be inaccurate. To address this issue, the gray zone samples could be assayed once or twice again, and the AI values could be averaged. Alternatively, an additional serum sample could be collected after some days, which would allow a clear crossing of the antibody avidity cutoff to be detected; this strategy has been recommended when testing for AI for other infectious agents (4, 7).

The originality and advantage of the AI approach is that, since the AI is a continuous variable, the window period (or mean recency period, i.e., the time interval between seroconversion and the assay value that defines the progression from recent to established infection) can be established a priori. We selected an a priori window period of 6 months to identify recent infections and then identified the best AI cutoff value at 6 months from seroconversion.

The study population showed a number of advantages. In particular, all participants without AIDS had a recent documented HIV-negative test result, which allowed the date of seroconversion to be estimated, and only a short time (about 2 weeks on average) had elapsed between the last negative HIV-test result and the first positive result, which allowed the date of seroconversion to be estimated rather accurately. Information on antiretroviral treatment was available for every participant, allowing us to exclude samples taken during treatment and thus to avoid treatment-induced problems on the determination of the AI.

The main limitation of the present study is the lack of individual information on the HIV subtype, which has been shown to be associated with different patterns of immune response to HIV, affecting the results of some assays for the identification of recent HIV infection (1, 11). However, a study conducted among persons infected with non-B HIV subtypes showed no influence of HIV subtype on AI accuracy (16).

Because fourth-generation EIAs detect not only HIV antibodies but also p24 antigen, they have an epidemiological advantage over third-generation EIAs in that the estimate of HIV incidence is more accurate; in fact, this estimate is based on recent infections identified by positivity to the p24 antigen only plus those identified by the low avidity of antibodies (8, 10). However, when doing so, the 6-month window period is calculated from the date of estimated antigen positivity instead of the estimated seroconversion date.

In conclusion, the AI for anti-HIV antibodies appears to be an accurate serological marker for discriminating recent from established HIV infections also using a fourth-generation assay. At present, given that no commercial HIV avidity assay on an automated platform is available, the described procedure must still rely on a manual preanalytical preparation of samples. However, similar problems arise with other laboratory methods for identifying recent HIV infections (1, 11). That the testing procedure itself is fully automated has several advantages, such as increased throughput, higher reproducibility, no need for specific technical skills, and easy comparability of results obtained in different settings. The importance of this latter feature must be stressed, given that harmonizing data generated for the detection of recently acquired HIV infections in different European countries represents a priority for the control of HIV spread within and across Europe.

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REFERENCES