Screening and Diagnosis of HIV-infection in Indonesia: One, Two or Three Tests?

Agnes R Indrati*, Reinout van Crevel**, Ida Parwati*, Anna Tjandrawati*, Noormartany*, July Kumalawati***

* Department of Clinical Pathology, University of Padjadjaran/Hasan Sadikin Hospital. Jl. Pasirkaliki no. 190, Bandung 40151, Indonesia. ** Health Research Unit, Medical Faculty, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia. *** Department of Clinical Pathology, University of Indonesia/Cipto Mangunkusumo Hospital. Jakarta, Indonesia

Correspondence mail to: agnesariantana_sppk@yahoo.co.id

ABSTRACT

Aim: to examine among high-risk populations or patients with signs or symptoms suggesting HIV-infection, two tests or even one single test might be sufficiently accurate for diagnosis of HIV in a hospital setting in Indonesia.

Methods: we retrospectively examined the rate of false-positive results of initial HIV-tests for all subjects tested in the referral hospital for HIV in West-Java, Indonesia, between 2006 and 2008. We also calculated the positive and negative predictive value of single test results and dual-testing, based on sensitivity and specificity of commonly used methods and prevalence data from Indonesia.

Results: among 3121 subjects, 803 were tested positive (25.7%). The initial rapid HIV-tests did not show a single false positive result, and no discrepancy was found between the second and third supplemental tests. Based on their high accuracy, most rapid tests carry a low risk of false-positive results among risk groups. Dual testing algorithms almost eliminate the risk of false-positive HIV-results, and are probably as accurate as three tests, even in low prevalence settings.

Conclusion: based on expected prevalence rates and the accuracy of methods used in Indonesia, one or two tests are usually accurate for HIV-diagnosis, especially for high risk populations. The possible implications and optimal conditions for more simple testing algorithms warrant further investigation.

Key words: initial HIV-tests, HIV-diagnosis.

INTRODUCTION

Indonesia has one of the most rapidly growing HIV-epidemics in Asia.1 Unfortunately, relatively few people are HIV-tested in Indonesia. Increasing the number of people tested for HIV is an essential step towards HIV-prevention and treatment as knowing one’s HIV-serostatus is both the entry point for HIV-treatment, and a tool to reduce risk-behavior and HIV-transmission.2 Many studies have addressed barriers to HIV-testing, but so far, few have examined the role of the tests or testing algorithms used.

The accuracy of HIV-tests has improved considerably, but there is still a risk of false-positive results. Because of the risk of false-positive results, a single positive test is usually followed by one or more supplementary tests (except for serosurveys). The guideline of the Indonesian Ministry of Health recommends one or two supplementary tests for diagnosing purposes, depending on the HIV-prevalence in the population tested and clinical manifestations of the subject tested. Serial testing diminishes the risk of false-positive results, but it requires people to return for the result of confirmation tests, which may delay or reduce uptake of HIV-treatment and other interventions. This definitely seems to be the case in Indonesia; 54% or less of individuals tested get their result (STBP-IBSS, Ministry of Health 2007).

Among high-risk populations or patients with signs or symptoms suggesting HIV-infection, two tests or even one single test might be sufficiently accurate for diagnosis of HIV. We examined this hypothesis in a hospital setting in Indonesia. We measured the...
accuracy of a single positive test among more than 3000 Indonesian subjects who were tested for HIV between 2006 and 2008 and calculated the predictive value of rapid HIV-tests, alone or in combination with a second test, using reported sensitivity and specificity rates of commonly used tests.

METHODS

Accuracy of a Single Positive HIV-test in Daily Practice

We retrospectively examined the rate of false-positive results of a first HIV-test, and possible discrepancy with the second and third test for all subjects tested from 2006 until 2008 in Hasan Sadikin hospital, the referral hospital for HIV in West Java (population 40 million). This hospital is one of the hospitals initially selected by the Indonesian Ministry of Health to provide voluntary counseling and testing (VCT) and HIV-treatment. All laboratory HIV-testing in this hospital is conducted at the department of Clinical Pathology. The majority of samples for HIV-testing are referred from the HIV-AIDS policlinic as part of VCT and medical check-ups (MCU). Samples also come from the hospital-based methadone program, the emergency room, in-wards, and since 2007 from Banceuy prison in Bandung. External quality control of HIV, HBV and HCV serology at the National Serology Reference Laboratory, Australia, has shown a 100% accuracy. After the first screening test, all positive results are followed by two additional tests, in accordance with national guidelines. The first method used is a rapid test, SD HIV 1/2 3.0 (Standard Diagnostics, Kyonggi-do, Korea) or Determine HIV 1/2 (Abbott,Tokyo). For the second and third test, an enzyme linked immune sorbent assay (Virolisa HIV 1/2, Index Union Diagnostics(Korea) and an electrochemiluminescence immunoassay (ECLI;Elecsys HIV Combi,Roche Diagnostics, Mannheim, Germany) are used.

Predictive Value of HIV-tests Used in Indonesia

Besides evaluation of routinely collected laboratory data, we also calculated the predictive value of different methods for HIV-diagnosis as recommended by the Indonesian Ministry of Health (Table 1). A distinction was made between rapid tests (which are used as initial HIV-test) and other methods (ELISA, ECLIA). The sensitivity and specificity of each respective method, as shown in Table 1, were used to calculate the likelihood ratio for a positive test (LR+) and likelihood ratio for negative test result (LR-) using established formulas. The LR+ equals the probability of finding a positive HIV-test result in someone who is HIV-infected divided by the probability of finding a positive HIV-test result in someone who is not HIV-infected. Similarly, the LR- equals the probability of finding a negative test result in someone who is HIV-infected divided by the probability of finding a negative test result in someone who is not HIV-infected. The chance of a false positive test result equals 100-post-test probability (%) after a positive test result, while the chance of a false-negative result equals the post-test probability (%) after a negative test result. The formulas used for calculating the predictive value of diagnostic tests are depicted in Table 2.

For single tests, the risks of false-positive and negative test results were calculated using reported HIV-prevalence rates in Indonesia. For two serial tests, the risk of a false-positive result (both tests being positive) was calculated using previously determined sensitivity and specificity for dual testing.

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>A</td>
<td>Determining HIV 1/2 (Abbott)</td>
<td>Rapid</td>
<td>99.33</td>
</tr>
<tr>
<td>B</td>
<td>SD HIV 1/2 3.0 (Standard Diagnostics)</td>
<td>Rapid</td>
<td>98.86</td>
</tr>
<tr>
<td>C</td>
<td>Acon HIV 1/2</td>
<td>Rapid</td>
<td>99.34</td>
</tr>
<tr>
<td>D</td>
<td>HIV 1/2 oncoprobe</td>
<td>Rapid</td>
<td>97.39</td>
</tr>
<tr>
<td>E</td>
<td>Hexagon HIV Human</td>
<td>Rapid</td>
<td>97.37</td>
</tr>
<tr>
<td>F</td>
<td>Advance Quality Rapid HIV test Intec</td>
<td>Rapid</td>
<td>98.68</td>
</tr>
<tr>
<td></td>
<td>Virolisa HIV 1/2 (Index Union Diagnostics)</td>
<td>ELISA</td>
<td>99.40</td>
</tr>
<tr>
<td></td>
<td>Elecsys HIV Combi (Roche Diagnostics)</td>
<td>ECLI</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Vidas (BioMerieux)</td>
<td>ELISA</td>
<td>98.68</td>
</tr>
<tr>
<td></td>
<td>Axsym (Abbot)</td>
<td>ELISA</td>
<td>100</td>
</tr>
</tbody>
</table>

Sensitivity and specificity are expressed as %. A-F correspond with tests A-F in Figure 2.
RESULTS

Accuracy of a Single Positive HIV-test in Daily Practice

Between 2006 and 2008, a total number of 4727 tests were performed for 3121 subjects. Three hundred seventy-seven subjects were tested in 2006, 1020 in 2007 and 1724 in 2008, accounting for a 4.6-fold increase in two years. Overall, 803 out of 3121 (25.7%) subjects were HIV-positive, 37% in 2006, 25% in 2007, and 24% in 2008. The positive predictive value of the first screening test was 100%; no single false-positive test was found (Figure 1). There were also no discrepancies between the two confirmatory tests.

Predictive Value of Single Test Results

The predictive value of a single HIV-test depends on the sensitivity and specificity of the test used, and on the prevalence of HIV (Table 2). The rapid tests, most often used as the first method, have a sensitivity and specificity ranging from 97-100% (Table 1). Three commonly used ELISA’s and one ECLIA have slightly higher sensitivity (98.7 - 100%), but more variable specificity, ranging from 93.7% - 100% (Table 1). Figure 2 shows the risk of a false-positive (2a) and false-negative (2b) test results for rapid tests and different HIV-prevalence rates. As can be seen, the lower the HIV-prevalence or ‘pre-test-probability’, the higher the risk of a false-positive result. With a very high HIV-prevalence, or a very high ‘pre-test probability’ in an individual patient, there is a high risk of a false-negative result (Figure 2b).

Table 2. Formulas to calculate the predictive value of a single HIV test

<table>
<thead>
<tr>
<th>Formulas</th>
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<tbody>
<tr>
<td>Sensitivity/(1-specificity) = LR+</td>
<td></td>
</tr>
<tr>
<td>(1-sensitivity)/specificity = LR-</td>
<td></td>
</tr>
<tr>
<td>Prevalence*(1-prevalence) = pre-test odds</td>
<td></td>
</tr>
<tr>
<td>Pre-test odds x LR = post test odds</td>
<td></td>
</tr>
<tr>
<td>Post-test odds/(post-test odds+1) = post-test probability</td>
<td></td>
</tr>
</tbody>
</table>

Example

presumed pre-test likelihood (prevalence in particular setting): 10%
rapid test ‘Determine’ (sensitivity 99.33%, specificity 99.44%)
LR+: 177.38; LR-: 0.0067
Pre-test Odds: 10/90 = 0.11
Post test odds : 19.7 (positive result); 0.00074 (negative result)
probability after positive result: 95.2%
probability after negative result: 0.07%
risk of false-positive result: 4.8%
risk of false-negative result: 0.07%

* one can also use the ‘pre-test likelihood’ for a particular patient, or prevalence in a specific setting / patient category. LR = likelihood ratio

When a single test is used for HIV-diagnosis, and based on reported HIV-prevalence rates in different settings in Indonesia, the risk of a false-positive result is high among the general population, but very low among subjects with a high chance of being HIV-infected. Clearly, a single positive HIV-test among the general population in Indonesia (e.g. a healthy blood donor) should be interpreted with caution, unless the test has a 100% specificity (Figure 2a). For test ‘B’ (Table 1), one of the rapid tests used in Hasan Sadikin Hospital, there is never any risk of false-positive results, as this test has a specificity of 100%. With the other rapid test used (‘A’), the risk would depend on the HIV-prevalence. With method ‘A’ the risk of a false positive test result would be 21.6% among pulmonary tuberculosis patients in Indonesia.
(estimated HIV-prevalence 2%), 6.9% among incoming prisoners (HIV-prevalence 7%), and 1.6% among adult meningitis cases (reported HIV-prevalence 25%). Finally, among clients in a methadone program (reported HIV-prevalence 75%), 0.18% would be false-positive. For rapid tests with a lower specificity, the risk of false-positive results would be higher (data not shown).

The risk of a false-negative test result would generally be very small, ranging from one per 100,000 in the general population to one in 2000 prisoners. However, if the HIV-prevalence is very high (e.g., among clients in a methadone program, or among patients with an opportunistic infection), one in 50 (2%) or more negative rapid HIV-test results may be false-negative.

**Predictive Value of Two Tests Combined**

When two serial HIV-tests are used, the risk that both tests are false-positive is very low. Among 66 combinations of two HIV-tests recently evaluated using 513 HIV-positive and 621 HIV-negative samples, 64 combinations had a specificity of 100%, and therefore, a risk of false-positive results of 0%. Only two test-combinations (3% of all evaluated) had less than 100% specificity. Both combinations, each containing a first-generation ELISA, had a combined sensitivity of 99.0% and specificity of 99.8%, similar to many of the newer rapid tests (Table 1).

**DISCUSSION**

We examined the risk of a false-positive HIV-test result using empirical data, and the calculated predictive value of various methods used in Indonesia. The initial (first) HIV-test showed no single false-positive result among 3121 subjects tested over a three-year period. This finding is in line with a recent study among 1222 pregnant women admitted to a labor ward in India which revealed no false-positive results, and with a large survey in nine African countries, which showed a concordance between point-of-care rapid testing and reference laboratory retesting of 98.7%. The high accuracy of single HIV-tests in our study and these published reports is due to the high sensitivity and specificity of rapid tests used, and the high ‘pre-test probability’ of HIV-infection in many patient settings, including ours. The calculated risk of false-positive rapid tests used in Indonesia varies between different methods, but is generally low, especially in settings with a moderate or high HIV-prevalence.

Not surprisingly, when two tests are combined, the estimated risk of a false-positive result is almost eliminated. Our hospital data showed no single false-positive result, neither with a single, nor with two (or three) tests. From the literature, only 3% of 64 test combinations recently evaluated with a panel of 1150 blood samples carried any (generally very small) risk of false-positive results. When tests are combined, they can be performed one after another (‘serial’) or at the same time (‘parallel’). In serial testing, a first positive result is followed by a second test, but a first negative result is not. Serial testing increases the specificity of results (lowering the risk of a false-positive result), while parallel testing increases its sensitivity (lowering the risk of a false-negative result). For asymptomatic patients or settings with an HIV-prevalence below 10%, the Indonesian guidelines recommend the use of two supplementary tests after an initial positive test, in order to avoid the risk of false-positive results. The Indonesian guideline stands alone in this recommendation; neighboring countries with lower HIV-rates like Vietnam or Laos use a two-test algorithm. The latest guidelines of the WHO recommend the use of two serial or parallel tests, only followed by a third test ‘tie-breaker test’ if the first two test show opposite results. A recent study evaluating two- and three-test algorithms showed that two-dual test algorithms perform equally well to a three-test ‘tie-breaker’ algorithm.

Changing the three-test algorithm into dual testing, or even one test (in high-prevalence settings) has important economic and clinical implications. A recent study examined the cost-effectiveness of a single (rapid) test algorithm, and a serial and parallel (two-test) algorithm. Compared with a single-test algorithm, the cost of one averted false-positive result would be $480 for parallel and $120 for serial testing, in a setting with 25% HIV-prevalence (similar to the HIV-prevalence among subjects tested in our hospital), parallel testing would reduce the risk of a false-negative result, but at a cost of $4441 (compared to serial testing) and $5966 (compared to single testing) per false negative result. Obviously, a change in the Indonesian guidelines, allowing for single-test strategies in high-prevalence settings or for individuals with a high ‘pre-test probability’, or dual-test strategies for individuals with a low risk of being HIV-infected would significantly reduce costs.

More simple algorithms might also help to increase the number of people starting HIV-treatment. Current estimates of the number of people at high risk of HIV-infection in Bandung who received the HIV test result are 37%. Higher costs associated with serial testing may be a barrier for subjects to access HIV-care. In addition, serial testing requires people to return for the confirmation of results, which may not always happen.
People counseled and tested may also feel unhappy to discuss the result with a different health care provider or counsellor than the one who did the pre-test counseling. Therefore, and based on the very low (empirical and theoretical) risk of a false positive HIV-test among people who are currently tested for HIV in Indonesia, one might consider immediate post-test counseling. Single rapid tests, which usually have a turn-around time of 30-60 minutes, could be followed directly by post-test counseling. Most likely, this would improve the efficiency and quality of counseling, and obviate the need to return for a second time for all individuals whose initial test is negative. If CD4-testing is available, the second visit might be used to discuss the need for antiretroviral treatment based on a CD4 cell-count. In Africa single (rapid) test algorithms have been implemented successfully for ‘point-of-care’ testing in over 600 sites in nine different countries, without compromising the accuracy of testing.11

We were unable to evaluate the risk of false-negative tests, but in patients with a very high apriori-risk (e.g. methadone clients in Indonesia) or clear clinical signs of AIDS, the risk of a false-negative result may be high. In such cases, dual (parallel) testing will eliminate the risk of a false-negative result. Under all circumstances, both the accuracy of the methods used, and quality control of HIV-testing are crucial. In addition, in the absence of a clinical indication or a CD4 cell-count, HIV-treatment should not be started based on a single positive HIV-test.

CONCLUSION

In summary, our results indicate that the low chance of a false-positive HIV-test result in Indonesia may not warrant the use of a serial three-test algorithm. In many settings in Indonesia, dual-test or even single rapid-test algorithms may be equally accurate and much more cost-effective. Single tests would allow for immediate post-test counselling, which might help to increase the number of individuals tested and treated for HIV. For screening in low-prevalence settings (e.g. regular pulmonary TB-patients) two tests may be accurate, and care should be taken with the interpretation of single positive test results in individuals who have no apparent risk for HIV-infection.

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