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Research Article

An Accurate and Rapid Laboratory Testing Algorithm for the Diagnosis of HIV Infection for Decentralisation: Use of A Rapid Fourth Generation Test as a Second-line Test

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Abstract

For the accurate and early diagnosis of Human Immunodeficiency virus (HIV), the classical laboratory testing algorithm that included Western Blot (WB) as the confirmatory test has been replaced by another one, the Centers for Disease Control and Prevention (CDC) algorithm that includes testing the fourth generation enzyme immunoassay (EIA) reactive samples with a rapid HIV-1/2 antibody differentiating second-line test, followed by HIV-1 nucleic acid amplification test (NAT) in the case of non-reactive or indeterminate second test result. The aim of the study was to evaluate the utility of a fourth generation rapid test as an alternative for antibody differentiation test in the second step of a three step diagnostic algorithm. The specificity and sensitivity of new version Alere HIV Combo test as the second test in the algorithm was evaluated by using 130 EIA negative and 123 4th generation EIA repeteadly reactive and WB positive sera, respectively. All of 130 sera that were negative by 4th generation EIA were non-reactive by Alere HIV Combo test resulting in the specificity of 100%. The sensitivity of the rapid test was found to be 100% because 123 sera that were 4th generation EIA repeatedly reactive and WB positive were all found to be reactive. Alere HIV Combo, a fourth generation rapid test with 100% sensitivity and specificity, can reliably be considered as a second-line test in the algorithm in which the non-reactive samples should be tested by HIV-1 NAT.

Key words: HIV Algorithm; HIV-1/2 Differentiating Test; NAT; Second-line Fourth Generation Rapid Test;

Introduction

Early and accurate diagnosis of HIV infection is mandatory for reducing the number of new infections. In parallel with the improvements in the diagnostic techniques, classical laboratory HIV testing algorithm has been updated by the CDC. In the updated algorithm, WB that was used as the confirmatory test in the previous algorithm has been withdrawn due to its low sensitivity in detecting acute cases and replaced by a second-line, rapid HIV-1/2 differentiating test. Moreover, specimens that are non-reactive with the differentiating test but repeatedly reactive with the first screening EIA test have been proposed to be tested with HIV-1 NAT in order to avoid missing the acute cases [1, 2].

The Turkish national guideline for the diagnosis of HIV infection has implemented the updated protocol recommended by the CDC [3]. Although the diagnostic accuracy of the algorithm is high, it has a disadvantage that there are limited commercially available tests for the second step that can differentiate HIV-1 and HIV-2 antibodies, leading to the inaccessibility of the test and/or high cost. In the guideline suggested by the World Health Organization (WHO) for the diagnosis of HIV infection, a series of three tests have been proposed. It has been reported in the guideline that the combinations of rapid diagnostic tests (RDTs) or RDTs and EIAs can reliably be used rather than conventional EIA/Western blot combination, especially in resource limited settings. As long as chosen correctly, RDTs can be included in the algorithm leading to more rapid and cost-effective diagnosis. Minimum requirements for RDTs and EIAs as second-line/third-line assay are ≥99% and 100% clinical sensitivity, respectively, and 99% clinical specificity of each [4]. A combination of WHO and CDC algorithms that includes a fourth generation RDT as second line test followed by HIV-1 NAT as a third step for non-reactive samples may result in a rapid, reliable, and cost-effective diagnostic approach. The fourth generation RDT that will be used as a second line test replacing HIV-1/2 differentiating test should fulfill the minimum requirements suggested by WHO so that the diagnostic accuracy is increased and the turnaround time of the algorithm is decreased.

In order to provide a rapid diagnostic approach that would prevent the transmission of HIV infection much more effectively, the present study was planned to question the utility of a fourth generation rapid test as a second-line test in the HIV laboratory testing algorithm in Turkey, a country where the incidence of acute retroviral syndrome and the seroprevalence of HIV infection are not known exactly.

Materials and Methods

The specificity and sensitivity of the new version Alere™ HIV Combo test (Alere Medical Co. Ltd., Matsudo-chi, Japan) was calculated using the sera samples that were previously confirmed to be HIV-1 negative (fourth generation ELISA test negative) and HIV-1 positive (fourth generation ELISA repeatedly reactive and Western Blot positive), respectively. The samples were tested by the rapid diagnostic test according to the manufacturer's instructions.

Results

In total, 253 sera were included in the study. Of 253 sera, 130 were 4th generation EIA negative and 123 were 4th generation EIA repeatedly reactive and WB positive.

All of 130 sera which were previously tested negative by the 4th generation EIA were found to be negative by the RDT, yielding 100% specificity. The sensitivity of the RDT was determined to be 100% because all of 123 sera that were 4th generation EIA repeatedly reactive and WB positive were found to be reactive by the RDT.

Discussion

Individuals recently infected with HIV who are estimated to account for 5-20% of HIV infections are more likely to transmit the infection because acute infections are commonly associated with high viral load. Because of the critical role of these newly infected people in the further transmission of the infection, updated laboratory HIV testing algorithm has concentrated on not to miss any of acute cases [1-5].

In the diagnostic algorithm suggested by the CDC, the second step that included WB test following the repeatedly reactive EIA test has recently been replaced by HIV-1/2 antibody differentiation immunoassay in order to differentiate HIV-1/2 rapidly and shorten the turnaround time. There is another recently proposed algorithm that suggests the application of HIV-1 NAT test in the second step followed, if necessary, by HIV-1/2 differentiating test. In this algorithm where HIV-1 NAT is used as the second test instead of an HIV-1/HIV-2 antibody differentiation immunoassay, specimens that are negative on HIV-1 NAT test should be repeated using HIV-1/HIV-2 antibody differentiation immunoassay or the United States Food and Drug Administration (FDA)-approved HIV-1 supplemental antibody test. In case HIV-1 supplemental antibody test is nonreactive or indeterminate, an HIV-2 antibody test should be performed. Inability to distinguish acute HIV-1 infection from the established one and the increased turnaround time are the two important limitations of the proposed algorithm [1, 2].

In low prevalence settings, positive predictive value of first-line fourth generation EIAs is low leading to considerable false positive test results. Therefore, any testing algorithm that uses a fourth generation EIA as the first-line test should include a more specific second/third-line assay(s) in order to confirm the diagnosis and rule out false reactivity. Moreover, second and third-line assays should be sensitive so that they do not yield false negative or indiscriminate results leading to failure or increased turnaround time in the diagnosis of the acute infection [4].

Rapid and reliable diagnosis of HIV infection is critical not only for the patient care but also nationwide for preventing the further transmission of the infection [1-4]. Third and previous generation rapid point-of-care tests were designed to detect HIV-1/2 antibodies. However, individuals with an acute or primary infection whose viral RNA is commonly high, thus highly infectious, do not produce antibodies till three weeks after the infection, leading to false negativity with these tests due to the window period. On the other hand, these individuals are positive for p24 antigen in the second week following the infection. Inclusion of the detection of p24 antigen in addition to the antibodies via the fourth generation point of care tests not only led to rapid diagnosis but also reduced the test-negative window to two weeks resulting in the earlier diagnosis of the infection compared to the previous generation tests. The first 4th generation rapid test, "Alere Determine HIV-1/2 Combo test" was approved by the FDA in 2013. The test provided better diagnostic results than previous generation point of care tests. In a recent study, the overall sensitivity and the specificity of Alere Determine HIV 1/2 Combo test was reported to be 95% and 100% where as those of another fourth generation RDT (SD Diagnostics Bioline HIV Ag/Ab Combo) were found to be 91% and 100%, respectively. In our previous study, the overall sensitivity of Alere Determine HIV-1/2 Combo test was found to be 97.6% [6]. Although by using Alere Determine HIV-1/2 Combo test we detected an acute case that was reported as indeterminate with WB [unpublished data], Alere Determine HIV-1/2 Combo test was disappointingly reported in various studies to have low sensitivity for detecting acute infections [7-10]. In a recent study, the sensitivity of Alere Determine HIV 1/2 Combo for the detection of acute infection was reported to be 76.9% [11].

To improve the sensitivity for the detection of p24 antigen, Alere released a new test, CE Marked Alere HIV Combo™ test, with a better performance. The sensitivity and specificity of the reformulated fourth generation rapid test for the detection of established and acute/early primary infection were compared to those of Alere Determine HIV-1/2 Combo test [12]. CE Marked Alere HIV Combo™ test was reactive for all of the six samples of patients with acute/primary infection whereas Alere Determine HIV-1/2 Combo test was weakly reactive only for three samples. In another study including 57 antibody-negative pre-seroconversion plasma samples of VOICE (a randomized, Phase 2B placebo-controlled trial for evaluating the safety and effectiveness of a pre-exposure prophylaxis for HIV prevention) participants with HIV RNA > 20 copies/mL, CE-Marked Alere™ HIV Combo was reactive for 16 (28%) of the samples that account for 21% more acute case detection than Alere Determine HIV-1/2 Combo test. Moreover, only 7 and none of 16 samples were confirmed by WB and Geenius™ HIV-1/2 Supplemental Assay, respectively [13].

Our purpose in this study was to detect the sensitivity and the specificity of a fourth generation rapid test, CE-Marked Alere™ HIV Combo Rapid Test, using sera samples that were fourth generation EIA negative and fourth generation EIA repeatedly reactive and WB confirmed HIV-1 positive. In the present study, CE-Marked Alere™ HIV Combo Rapid Test was analyzed as an alternative for the second step of the algorithm suggested by the CDC in which the non-reactive specimens will be tested by third line test HIV-1 NAT. Both the sensitivity and specificity of the test were found to be 100%. As a limitation of the present study, the sensitivity of the test for the detection of acute infection was not detected. However, the performance of CE-Marked Alere™ HIV Combo Rapid Test in detecting the acute cases has already been documented in previous studies [12, 13].

In an approach to combine the algorithms proposed by WHO and CDC, it is thought that the use of a fourth generation RDT with sensitivity and specificity greater than 99% as a second-line test in countries where HIV prevalence is not exactly known or is low will increase the positive and negative predictive values of the first-line screening test with a rapid turnaround time in a cost-effective manner. Quantitative HIV RNA test that will be implemented to the algorithm as a third step for the specimens that are reactive in the first step but non-reactive in the second has been shown to detect HIV-1 RNA at low copies increasing the sensitivity of the algorithm for the detection of acute cases and enabling the detection of the initial RNA count for the further evaluation of the effectiveness of the treatment [14].

Conclusions

As a conclusion, a fourth generation EIA test followed by a fourth generation RDT and HIV RNA test may help the urgent diagnosis of HIV infection with a total turnaround time of approximately six hours. The rapid and cost-effective algorithm proposed in this study is thought to avoid the ignorance of any acute infection and be practical especially for underdeveloped countries and the countries where the exact prevalence of HIV-1 is unknown and HIV-2 prevalence is low. None of the testing algorithms available is perfect for the diagnosis of HIV. As recommended by WHO [16], countries should adopt the best rapid testing strategy that is appropriate according to their local needs and validate the algorithm before they use. The three step algorithm proposed in the present study should be validated.

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