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Risk stratification of HIV infection for patients needing molecular confirmation with the Abbott 4th generation Architect System

Richard J. Baltaro[a], Renuka Malenie[a], Heather Melbourne[b], Francisco Garcia[b], Edwin W. Gould[b], Andrew A. Renshaw[b,*]

[a] East Carolina University Brody School of Medicine and Vidant Medical Center, Greenville, NC, United States
[b] Homestead Hospital, Homestead, FL, United States

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ABSTRACT

Background: Some patients need their 4th generation HIV testing results confirmed with molecular testing after primary confirmatory testing which may not be immediately available. Further risk stratification of these patients pending the results of molecular testing may be of value not only for patient counseling but also for treatment of women in labor.

Objectives: To determine the risk of a positive test result on molecular testing for these patients.

Study design: The risk of a positive molecular test result for patients with a result needing molecular confirmation on a 4th generation HIV testing algorithm (Abbott Architect, Multispot/Geenius confirmatory test) was stratified based on the patient's white blood cell (WBC) count and the magnitude of Architect result Signal Cut Off ratio (S/CO).

Results: A total of 61,666 patients were tested with 658 (1.1%) positive results and 76 (0.12%) patients needing molecular confirmation. Patients with an S/CO of < 5 or an S/CO of 5–100 with a WBC $> 6.5 \times 10^9$ cells/l had a significantly lower risk of a positive molecular HIV test (0/48, 0%) than patients with an S/CO 5–100 with a WBC $< 6.0\,s \times 10^9$ cells/l (5/9, 56%, $p < .001$) or an S/CO > 100 (2/2, 100%, $p < .001$). Pregnant women had a significantly lower rate of positive test results (24/6924, 0.4%) than non-pregnant patients (634/54742, 1.1%, $p < 0.001$). All 12 cases needing molecular confirmation in pregnant women had negative NAT test results.

Conclusions: Patients who need their HIV results confirmed with molecular testing using a 4th generation algorithm that includes the Abbott Architect System can be further stratified into low, intermediate, and high risk groups based on additional laboratory information pending the results of molecular testing. This risk stratification may be of value for patient counseling and treatment of women in labor.

1. Background

The Clinical and Laboratory Standards Institute (CLSI) guidelines [1] published in 2011 recommend a 4th generation assay for HIV testing to reduce the "window period" to approximately 2 weeks [2] and facilitate early detection screening scenarios. Fourth generation assays have shown high sensitivity and specificity [3–8] including improved sensitivity compared with 3rd generation testing [9–12]. Nevertheless, a small percentage of patients tested with a 4th generation test will receive a result that needs to be confirmed with molecular testing (nucleic acid testing, NAT). This testing may not be immediately available, resulting in a period of time in which the true status of the patient is unknown. Counseling about the risk of HIV infection during this waiting period may be appropriate.

Screening is also recommended for pregnant women at the time of delivery who have no prenatal care. These patients are at risk for vertical transmission of the virus to the fetus, and several interventions can reduce the risk of this transmission from 25% to less than 2% [13]. Current recommendations from the U.S. Preventive Services Task Force, the American College of Obstetricians and Gynecologists, and the British HIV Association include routine prenatal HIV testing as well as a rapid (< 1 h) testing on all mothers in labor whose HIV status is not known [13–16]. Patients whose rapid tests need molecular confirmation are currently recommended to receive antiviral therapy, though some studies suggest that pregnant women are more prone to false positive results and may have twice as many results that need molecular

* Corresponding author at: Department of Pathology, Baptist Hospital, 8900 N Kendall Dr, Miami, FL, 33176, United States.
E-mail address: andrewr@baptisthealth.net (A.A. Renshaw).

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confirmation than other patients [17–21]. Further risk stratification may be of particular utility in this setting [13].

The Architect 4th generation HIV test (Abbott, Chicago IL) currently uses a signal cut off ratio (S/CO or sample RLU (relative light unit)/Cutoff RLU) of 1.0; all specimens with a signal above that range must be both repeatedly tested and verified using a primary confirmatory rapid assay. Specimens that are not reactive on repeat testing or non reactive or indeterminate on that second rapid assay are reported provisionally as needing molecular confirmation and must undergo qualitative NAT testing. Overall the reported rate of subsequent positive NAT testing in this group ranges from 42 to 57% [4,21]. Whether these patients can be further stratified into higher and lower risk groups for a subsequent positive NAT test results prior to NAT testing is not well characterized. Previous reports have shown that the majority of false positive test results have an S/CO of less than 15 [6]. While 0% of low risk patients and 10% of high risk patients have been reported to have a positive NAT when the S/CO is < 15, as many as 37% of patients with acute infection may be positive [6]. The majority of patients with an S/CO > 15 have had positive NAT testing [6]. Subsequent studies have suggested that the optimal S/CO cutoff may be 2.5 [22]. Whether additional improvements in stratification could be achieved using additional laboratory data has not been well studied.

2. Objectives

We sought to determine if more accurate risk stratification could be achieved in patients needing confirmation of their HIV result using both the S/CO from the Architect machine and additional laboratory information.

3. Study design

All patients ages 18 and older who presented to the Homestead Hospital Emergency Department (Homestead, FL) between 5/2016 and 7/2018 or the East Carolina University Brody School of Medicine and Vidant Medical Center Emergency Department (Greenville, NC) between 5/2016 and 7/2018 were evaluated. Those that needed blood work and did not opt-out of routine HIV testing were screened. Although Homestead Hospital is located in a community with a known high rate of HIV infection, screening was offered to all patients regardless of their individual risk status. Testing was performed using the 4th generation Abbott Architect Assay System. All reactive specimens (S/CO > = 1.0) had repeat testing. If repeatedly reactive, these specimens were verified using either the Multispot or Geenius assay (BioRad, Hercules, CA). If these assays were negative or indeterminate, the specimen was deemed as needing confirmation and sent to a reference laboratory for NAT testing. White blood cell counts were obtained from the medical record. The normal range at Homestead Hospital was 3.4–11.0 × 10^9 cells/l. The normal range at Vidant Medical Center was 4.5–11.0 × 10^9 cells/l.

Statistical analysis was performed using a Fishers exact test or two tailed Chi square test with Yates correction as appropriate. A significance level of 0.05 was used.

4. Results

At Homestead Hospital there were 27,288 patients screened with 274 (1.0%) positive results and 30 (0.1%) results that needed molecular confirmation, while at East Carolina University Brody School of Medicine and Vidant Medical Center there were 34,378 patients screened with 384 (1.1%) positive results and 46 (0.13%) results that needed molecular confirmation. There was no significant difference in either the positive rate (p = 0.19) or rate of molecular confirmation (p = 0.46) between the two sites, so the data from both sites was analyzed together, resulting in 61,666 patients, 658 (1.1%) positive results, and 76 (0.12%) patients needing molecular confirmation.

Table 1
Risk Stratification for All Patients Who Need Molecular Confirmation of an HIV Result[a].

Risk	Cases # (%)	S/CO	WBC (x 10^9cells/l)[a]	NAT Positive # (%)
Low	48	< 5	NA	0 (0)
Low	11	5–100	> 6.5	0 (0)
Intermediate	9	5–100	< 6.5	5 (56)
High	2	> 100	NA	2(100)

NA = not applicable.
[a] 76 patients from 61,666 patients tested, of which 6/76 patients excluded due to incomplete data, including one patient with a positive NAT.

The majority of patients screened were female (40,214, 65.2% female). Eight of 76 (10.5%) cases needing molecular confirmation were positive on NAT testing.

S/CO and WBC information was available on 70 of the 76 patients needing molecular confirmation (92.1%). These 70 patients could be divided into three risk groups based on the S/CO and WBC, see Table 1. The risk of a positive NAT in the low risk groups (, those patients with an S/CO < 5 or those with and S/CO between 5–100 and a high WBC, 0/59, 0%) was significantly lower than that in the intermediate (, S/CO between 5–100 and a low WBC, 5/9, 56%) and high risk groups (, S/CO > 100, 2/2, 100%) (p < .001 for each). The two patients with a positive NAT test and an S/CO > 100 both had WBCs < 6.5 × 10^9 cells/l, so every patient needing molecular confirmation of an HIV result who was positive on NAT had a WBC < 6.5 × 10^9 cells/l.

The results for pregnant women are shown in Table 2. There was a significantly lower rate of positive test results (24/6924, 0.4%) than in the non pregnant patients (634/54742, 1.1%, p < 0.001). In contrast, there were more cases that needed molecular confirmation (12/6924, 0.2%) than in non-pregnant patients (64/54742, 0.1%, p = .25), but this difference was not significant. All 12 cases needing molecular confirmation were low risk (as defined above and in Table 1, see Table 2), and none had a positive NAT test result.

5. Discussion

Recommended HIV testing algorithms include 3 categories of HIV testing, including a 4th generation HIV antibody and antigen combination (combo) assay, an HIV-1/HIV-2 differentiation assay, and nucleic acid testing (NAT) [1]. The 4th generation assays include in a single test immunoassays for both IgG and IgM antibodies for both HIV-1 and HIV-2 along with a p24 antigen assay. The inclusion of the p24 antigen assay is designed to reduce the “window period” to approximately 2 weeks [2] and facilitate early detection in screening scenarios.

There are conflicting results concerning the specificity of 4th generation testing. At least one study suggests that the 4th generation Architect is more specific than the 3rd generation Centaur assay [11], but the specificity of some 4th generation tests has been lower than others [9]. The Alere (Waltham MA) Determine test may also not be as specific [5,23]. There are also a variety of immediate tests available to verify the results of a screening 4th generation test, although the Multispot assay (Bio-Rad) [24] for HIV-1/HIV-2 differentiation test is no

Table 2
Risk Stratification for Pregnant Patients who need molecular confirmation of an HIV Result[a].

Risk	Cases # (%)	S/CO	WBC (× 10^9cells/l)	NAT Positive # (%)
Low	10	< 5	NA	0 (0)
Low	2	5-100	> 6.5	0(0)
Intermediate	0	5-100	< 6.5	0
High	0	> 100	NA	0

NA = not applicable.
[a] 12 cases from 6924 patients tested.

longer available and has been replaced with the Geenius (Bio-Rad) assay which has similar performance [25] and is the only FDA approved confirmatory differentiating rapid assay.

Our results support those of prior studies [4,6,21,22] which show that the signal cut off ratio (S/CO or sample RLU/Cutoff RLU) in the Abbott Architect System can be of use in stratifying the risk of a positive NAT test in patients who need molecular confirmation. While overall the reported rate of subsequent positive NAT testing in these patients ranges from 42 to 57% [4,21] the majority of false positive test results have a S/CO of less than 15 [6] and the optimal cutoff point may be as low as 2.5% [22]. Our results show a lower rate of HIV positivity on NAT testing, with an overall rate of NAT positivity of 10%. Nevertheless, a subset of both acute and non-acute HIV infected specimens will also have a signal of less than 15, and as many as 37% of acute infections in high risk settings may have an S/CO of < 15, though very few of these patients will have an S/CO of < 2.5 [6]. Our study supports these prior results, and suggests that virtually all false positive results have an S/CO of < 100, while the majority of false positive results have an S/CO of < 15. Nevertheless, we found that a more accurate stratification of the risk for the patient could be achieved using slightly different S/CO (5 and 100) ratios in conjunction with the patient's WBC. Based on Figure 2 in Marson et al. [6] approximately 4/62 (6.5%) acute HIV infections and 2/357 (0.6%) non-acute HIV infections had S/CO levels < 5, suggesting a false negative rate based on this prior studies data of less than 6.5% if patients with S/CO values < 5 are assumed to be negative. The actual negative predictive value will depend on the ratio of acute HIV infection, non-acute HIV infection, and non-infected patients with S/CO values between 1 and 5. Based on our false negative rate of 0% for an S/CO threshold of 5, we believe that the negative predictive value of this threshold in this patient population is very high. Certainly counseling these patients will likely be different if the risk of a true HIV infection is closer to 0% than the 42–57% [4,21] reported in the literature.

There are several possible reasons why the WBC may be useful in this setting. It appears that acute HIV infection that produces a low S/CO is associated with a low WBC. The exact reason for this correlation is not clear. We did not find that further stratification of the WBC into specific types of cells (i.e. absolute lymphocyte count) improved the predictive value of this test (data not shown). Additionally, patients with a false positive result may have an elevated WBC as part of their inflammatory state. The choice of a threshold of 6.5×10^{9} cells/l was based on review of the data; it is possible that other thresholds may perform better in different data sets. Further evaluation appears warranted.

The current study focuses on the performance of 4th generation testing in screening in the emergency department in high risk communities. Screening of pregnant women with no prenatal care at the time of delivery is also recommended. If a patient is HIV positive, several interventions, including treatment with antiviral drugs, C-sections, and breast feeding guidelines are recommended to reduce the risk of this transmission from 25 to less than 2% [13,16] and are effective even when initiated at the time of delivery or within 24–48 h after delivery. Patients whose rapid tests need confirmation are recommended to receive antiviral therapy [26].

Nevertheless, there remain barriers to universal implementation of these recommendations [27–34]. Some studies suggest that pregnant women are more prone to false positive results and may have twice as many results needing confirmation as other patients [17–21]. The rate of positive molecular tests for results for pregnant women who need molecular confirmation has been reported to be as low as 13% [35]; in this series it was 0%. It may become increasingly difficult to make clinicians take the results of a preliminary positive HIV test that needs molecular confirmation seriously if the experience continues to show that virtually all of these cases are false positive results. As noted by others, "false positive tests results [may be] accompanied by irritated customers (physicians and patients) but also by additional work in the laboratory," [8] as well as additional work and unnecessary expense in the pharmacy as well. Counseling may also be complicated by the uncertainty of an unconfirmed result [13]. Our study suggests that it may be possible to further stratify the risk of HIV infection in these women using available laboratory information. Further studies concerning the performance of these risk stratification algorithms appears warranted.

In conclusion, we have shown that 0.1% of tests performed using the 4th generation HIV Abbott Architect Assay need molecular confirmation. For these patients, the risk of a subsequent positive NAT result can be stratified based on the S/CO result as well as the patients WBC. In this series none of the pregnant women with a preliminary positive HIV test result needing confirmation had a positive NAT test. This risk information may be of value in counseling patients regarding their test results.

CRediT authorship contribution statement

Richard J. Baltaro: Supervision, Writing - review & editing. **Renuka Malenie:** Data curation, Writing - review & editing. **Heather Melbourne:** Project administration, Supervision, Writing - review & editing. **Francisco Garcia:** Data curation, Writing - review & editing. **Edwin W. Gould:** Supervision, Writing - review & editing. **Andrew A. Renshaw:** Conceptualization, Data curation, Writing.

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