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BRIEF REPORT

Do I Have HIV or Not? Lack of RNA Detection and the Case for Sensitive DNA Testing

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We present a case of a 20-year-old male who had ambiguous HIV test results after entering new provider care and whose status was later complicated by undetectable viral RNA off antiretroviral therapy (ART). Verifying HIV infection status may occasionally require sensitive DNA testing that might need to be considered in diagnostic guidelines to resolve diagnosis and ensure appropriate ART management.

Keywords. antiretroviral therapy; diagnostic testing; DNA; HIV; rapid start.

In 2017, an estimated 85.8% of persons with HIV in the United States were diagnosed, and in 42 jurisdictions with data available, 62.7% of persons with diagnosed HIV were virally suppressed [1]. The national Ending the HIV Epidemic plan (EHE) [2] aims to raise awareness of infection status among persons with HIV to 95% and to achieve virologic suppression in 95% of persons with diagnosed HIV [1].

Despite highly advanced HIV testing capabilities, there are still substantial problems with providing and interpreting HIV test results, delivering results of testing, linkage to HIV care providers, retention on ART, and achieving and maintaining HIV viral suppression.

The introduction of antiretroviral therapy (ART) has markedly reduced morbidity and mortality, and viral suppression greatly reduces HIV transmission to the uninfected [3]. When taken as prescribed, ART can suppress viral load (VL), maintain CD4 cell counts, prevent AIDS, and prolong survival. The current recommendation is to initiate ART as soon as possible after diagnosis [4]. Early initiation of ART has been reported to improve prognosis but also presents challenges for future HIV testing and diagnosis when re-verification of HIV status may be required [5].

We present a case of a 20-year-old male military veteran who underwent rapid-start ART initiation after an HIV-1 Ab–positive test result, but before receiving HIV-1 viral load results. Follow-up testing 1 year later at the Veterans Affairs Connecticut Healthcare System (VACHS) led to a series of ambiguous HIV test results, in particular with HIV screening and supplemental tests. In this brief report, we review HIV test results in the context of verifying infection status, the recommended HIV diagnostic algorithm, and when and what type of additional HIV testing could be performed to resolve unusual test results.

METHODS

HIV diagnostic testing performed when a 20-year-old black male engaged in new provider care at a VA Infectious Disease (ID) Clinic in August of 2019 yielded ambiguous results that prompted a series of additional laboratory testing to verify HIV-1 infection (Table 1). Importantly, the resolution of HIV status required coordination with the laboratory testing done with the VACHS, commercial laboratories, and the CDC.

RESULTS

The individual reported at the visit that he had been diagnosed with HIV-1 during routine military academy admission testing in July 2018 and referred to a nearby academic ID clinic 1 month later. Of note, he was discharged from the military academy based on the test result. One month after this initial HIV-1 antibody diagnosis and 3 days before his follow-up ID visit, HIV-1 RNA viral load (VL) testing was performed, but the VL results were not yet available at the time of the ID visit. At the ID visit, the individual reported to the provider that he had recently become sexually active and had engaged in receptive condomless anal intercourse with male partners of unknown HIV status that year, including ~1 month before the test result on his admission to the military academy. He reported that he received an HIV-negative test result the year before entrance into the military academy at a local walk-in clinic; he also reported that he had not been taking pre-exposure prophylaxis (PrEP) at that time. His first ID provider prescribed dolutegravir + tenofovir/emtricitabine based on the positive HIV-1 Ab test result he received at the military academy; the VL results were not available at the time of the visit. After the

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Table 1. Summary of HIV Test Results Performed in 2018–2019 in the Investigation of Infection Status of a 20-Year-Old Male

Sample Date	Procedure	Method	Result
Before VA Presentation: July 2018–March 2019			
July 31, 2018	HIV Ab serum test	Unknown at military academy	HIV-1 Ab positive
September 4	HIV supplemental assay	Geenius	HIV-1 Ab positive
	HIV-1 RNA quantitative	Roche Cobas at ID clinic	28 copies/mL (test done 9/4 but results reported after 9/7/18)
September 7	Treatment initiation	Dolutegravir + tenofovir/emtricitabine	
October 8	HIV-1 RNA quantitative	Roche Cobas at ID clinic	Not detected
December 8	Treatment change	Bictegravir/TAF/emtricitabine	
January 2019	HIV-1 RNA quantitative	Roche Cobas at ID clinic	Not detected
March 2019	HIV-1 RNA quantitative	Roche Cobas at ID clinic	Not detected
Start of VA Presentation: August 2019			
August 19	HIV screening assay	Architect at VACHS	Repeatedly reactive S/CO 2.63
	HIV supplemental assay	Geenius at VACHS	HIV-1 indeterminate
	HIV-1 RNA quantitative	Roche Cobas at VACHS	Not detected
	HIV screening assay	HIV combo Ag/Ab EIA at CT DPH laboratory	Repeatedly reactive
	HIV supplemental assay	HIV Ab differentiation assay at CT DPH laboratory	HIV-1 indeterminate
	HIV-1 RNA quantitative	Roche Cobas at CT DPH	Not detected
August 30	HIV screening assay	Architect at VACHS	Reactive S/CO 2.27
	HIV supplemental assay	Geenius at VACHS	HIV-1 positive
	HIV-1 DNA qualitative	Lab-developed test at Quest	Not detected
	HIV-1 RNA quantitative	Roche Cobas at VACHS	Not detected
	HIV-1 coreceptor tropism, ultradeep sequencing	Lab-developed test at Quest	Test not performed, unable to amplify viral nucleic acid for sequence analysis due to low viral load or viral sequence heterogeneity
	T-cell subsets	Flow cytometry	CD3 + 4+# 811
			CD3 + 4+% 50
			CD4/CD8 ratio 2.20
September 2	HIV-RNA quantitative	Roche Cobas at VACHS	Not detected
	HIV-1 DNA qualitative	Lab-developed test at Quest	Not detected
September 6	Treatment stopped		
October 2	HIV screening assay	Architect at VACHS	Reactive S/CO 57.77
	HIV supplemental assay	Geenius at VACHS	HIV-1 positive
	HIV-RNA quantitative	Roche Cobas at VACHS	Not detected
October 25	Treatment restarted	Bictegravir/TAF/emtricitabine	
November 15	HIV screening assay	Architect at VACHS	Reactive S/CO 51.63
	HIV supplemental assay	Geenius at VACHS	HIV-1 positive
	HIV-RNA quantitative	Roche Cobas at VACHS	Not detected
	T-cell subsets	Flow cytometry (VACHS)	CD3 + 4+# 774
			CD3 + 4+% 51
			CD4/CD8 ratio 0.61
	HIV-1 drug resistance proviral DNA, deep sequencing	Lab-developed test at Quest	HIV proviral DNA: Detected HIV subtype: B (no ART-related mutations detected)
November 22	HIV screening assay	Architect	Reactive S/CO 44.15
	HIV-RNA quantitative	Roche Cobas	Not detected
	HIV screening assay	Bio-Rad GS HIV combo Ag/Ab EIA at CDC	Repeated reactive
	HIV supplemental assay	Geenius at CDC	HIV-1 positive
	HIV-1 RNA qualitative	Aptima HIV-1 RNA qualitative assay at CDC	Not detected

Geenius: Bio-Rad Geenius HIV-1/2 supplemental assay; a positive Geenius result is any 2 bands of the 4 HIV-1 test lines with at least 1 ENV gp160 (Band 4) or gp41 (Band 6); indeterminate is 1ENV (Band 4 or 6), or 1GAG (Band 5), or 1POL (Band 3), or 1GAG and 1POL (Bands 5 and 3). Architect: Abbott Architect HIV Ag/Ab combo; S/CO is an average of 3 values. The lower limit of detection of the Roche Cobas HIV RNA test is 22.0 IU/mL, and the lower limit of quantitation is 33 IU/mL.

Abbreviations: ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; CT DPH, Connecticut State Department of Public Health; EIA, enzyme immunoassay; ID, infectious diseases; S/CO, sample/cutoff ratio; VA, Veterans Administration; VACHS, Veterans Administration Connecticut Healthcare System.

initial ID visit, the pre-ART VL result was reported as 28 RNA copies/mL, the Geenius HIV supplemental test was HIV-1 Ab positive, and the CD4 count was 1114 cells/mL. The client was then contacted to confirm HIV-positive infection status and instructed to continue the prescribed ART regimen. One month post–ART initiation, the VL was reported as target not

detected (TND), and his CD4 count was 650 cell/mL. He was continued on the prescribed ART regimen until the provider decided to change his ART regimen in December 2018 to once-daily bictegravir/TAF/emtricitabine for ease of dosing before his move to Connecticut (CT) to attend college. Subsequently, he had 2 HIV VL TND test results in January and March of 2019, and in March 2019 he had a CD4 of 755 cells/mL. In July 2019, the individual moved to CT and sought to initiate care with the VACHS.

The individual then presented to the ID clinic visit at the VACHS in August 2019 to establish new HIV care. He reported no interruptions in ART, and his VACHS HIV Ab/Ag screen was repeatedly reactive with a low signal-to-cutoff (S/CO) ratio, a Geenius HIV-1/2 supplemental assay (Geenius) HIV-1 indeterminate, and HIV-1 RNA reported as TND. The CT state lab confirmed the HIV combo Ag/Ab enzyme immunoassay (EIA) as repeatedly reactive, and Geenius HIV-1 indeterminate and Western blot (WB) results corroborated the Geenius, which was TND. A qualitative HIV-1 DNA test was performed 2 weeks later in an attempt to detect proviral DNA that might be detectable despite suppression of viral replication, but HIV DNA was not detected. From these results, the VA ID provider now had concerns that the individual could have been misdiagnosed with HIV because the original HIV RNA was only 28 copies/mL and could have been a spurious result. The individual communicated regularly with the VACHS ID provider, and at one point the patient asked if he was truly HIV-positive. The ID provider stated that she was uncertain but would work with experts to determine his status. Further, the ID provider was very concerned that if he did not currently have HIV infection and treatment were discontinued, he would be at risk of acquiring HIV via condomless anal intercourse. After consultation with numerous HIV providers, the CT state lab, and a hotline, ART was discontinued on September 6, and 1 month after ART cessation on October 2 the patient's HIV-1 RNA and DNA remained TND. However, also at this time his Ab/Ag S/CO ratio increased to 25 times the previous result, and a Geenius differentiation test was now HIV-1-positive, an unexpected result given that HIV nucleic acid was undetectable. The individual's prior ART regimen was restarted on October 25 as a cautionary prophylaxis due to the individual reporting continued condomless sexual intercourse. The plan was to repeat blood testing 1 month later, and specimens were obtained on November 15 at the VACHS. Blood samples were also sent to the CDC on November 22, 2019, for testing. The CDC reported HIV combo Ag/Ab EIA repeatedly reactive and Geenius HIV-1-positive; continued absence of detectible RNA after reinitiating ART was verified by a nonreactive Aptima HIV-1 RNA qualitative assay. While the progression of serological testing strongly suggested HIV infection and the patient met serological criteria for diagnosis, our prior inability to detect HIV RNA or DNA was troubling. After consultation with the CDC HIV Reference Laboratory, a specimen from the same collection date on November 22 was also sent out for commercial HIV-1 proviral DNA resistance testing by deep sequencing. This DNA test was reported as HIV-1 subtype B detected. The individual was informed of the DNA test results and was recommended to continue ART.

DISCUSSION

This case report highlights the limitations in health care providers' ability to verify HIV infection status after initiation of therapy, when needed, where a hampering of immunologic responses that lead to ambiguous diagnostic results could be an issue, particularly if ART was initiated early in infection. The use of VL for confirming HIV infection may not be useful in cases of an atypical lack of RNA expression, and dialogue with HIV clinicians on appropriate interpretation is warranted. The reasons behind these discordant diagnostic findings are speculative, as duration of infection before ART initiation and the possibility of undocumented PrEP use are unknown. Given the low initial VL value reported, the person described here may not have had a reproducible detectable VL result with repeat testing. Hence, with very low initial VL results and the absence of a definitive second serologic test, consideration should be given as to whether it is appropriate to immediately begin treatment. A subsequent laboratory-based serologic test may be recommended to rule out potentially spurious reactivity in either the initial serologic and/or VL assay. The case reported here highlights the complexity of considering VL (or a qualitative nucleic acid test) the second step in a diagnostic algorithm, and dialogue with HIV care clinicians on its limitations is warranted.

As illustrated here, persistently undetectable HIV VL even after cessation of ART led to a great deal of uncertainty about infection status, whereas laboratory-based antibody testing pointed to the presence of HIV. To resolve rare cases of conflicting or discordant HIV test results, sensitive assays for DNA detection may be needed. The clinical performance characteristics of these tests are not well described in complex patients similar to the patient described here, and we benefited from discussion with the CDC and local experts in HIV diagnostics. In this case, direct (standard polymerase chain reaction) testing for HIV DNA was surprisingly negative; only our very high clinical suspicion led us to pursue a sensitive DNA assay involving deep sequencing. We are unaware of any comparison of the sensitivity of these modalities for clinical detection of proviral DNA. However, the availability of an approved sensitive DNA diagnostic early in the course of testing would resolve cases like this much sooner. Information gained from additional research into cellular testing might need to be considered for testing guidelines. Diagnostic advances in non-plasma-based testing could

prove to be advantageous in resolving difficult diagnoses and ensuring appropriate ART management.

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