Non-seroconversion in an HIV-tuberculosis co-infected patient-case report

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ABSTRACT

Background: Most cases of negative serology in proven human immunodeficiency virus (HIV) patients occur due to testing during the window period. However, true non-seroconversion is a phenomenon that should always be considered.

Case Presentation: A 13-year-old female with a family history of multi-drug resistant tuberculosis (MDR-TB) presented with cough without fever for 1 month. She was vitally stable and the physical examination was unremarkable. Chest X-ray was suggestive of active TB, sputum positive for acid-fast bacilli, and GeneXpert positive for Mycobacterium tuberculosis. Her HIV RNA polymerase chain reaction (PCR) was positive at 28,866 IU/ml. She was registered as presumed MDR-TB and started on anti-tuberculosis treatment. Serum specimen sent inadvertently 6 weeks later was positive for HIV- p24 antigen while negative for anti-HIV 1/2 on Determine HIV Early Detect fourth generation lateral flow assay. A fresh specimen 2 weeks later showed similar results and was also negative by Alinity HIV Ag/antibody Combo (Abbott Diagnostics) and Bio-Rad Geenius HIV-1/2 Supplemental Assay. HIV antibodies were still negative at 20 weeks (5 months) after positive PCR.

Conclusion: This is a case of a seronegative HIV/TB co-infected patient. Non-seroconversion should always be considered in patients with clinical suspicion and discordant results.

Keywords: Seroconversion, tuberculosis, acquired immunodeficiency syndrome, human immunodeficiency virus, case report.

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Background

The worldwide prevalence of co-infection with human immunodeficiency virus (HIV) and tuberculosis (TB) is 8.6%. The presence of HIV infection increases the chances of active TB. Immunosuppression in HIV infection is a significant factor in the re-activation of latent TB infection, and it also predisposes the patient to a new concurrent infection [1-3]. Co-infection with TB compounds the immunosuppression caused by HIV [2,4].

HIV infection is followed by a distinct clinical course. During the acute phase, there is rapid viral replication and release of viral antigen, p24, in the blood. This is followed by a chronic phase in which the patient may be asymptomatic, yet the replication continues at a low rate. The patient starts to produce antibodies during this stage, which can cause a reduction in the viral load. Seroconversion usually occurs in 4-6 weeks following infection. The mean time for seroconversion has been reported as 43.9 days (95% confidence interval 37.3-50.5) [5]. The time period from infection to when the antibodies become detectable is known as the window period. This is followed by the third stage of active acquired immunodeficiency syndrome (AIDS) in which there is continued viral replication and reduction in CD4 cells.

The usual cause of negative HIV antibodies in a patient with HIV infection is testing too soon during the disease course when the levels of the produced antibodies are below the detection limit of the assay or during the window period. Here, we report a rare occurrence of true seronegative HIV infection in a child who failed to develop antibodies to HIV due to exaggerated immunosuppression from co-infection with TB.

Case presentation

A 13-year-old Pakistani female was seen in the outpatient clinic in October 2021 with a history of cough for 1 month without accompanying fever. She was vitally stable and her physical examination was unremarkable. There was a strong family history of TB, her sister had just completed treatment for TB while her brother was on anti-tuberculous treatment (ATT). Chest X-ray was suggestive of pulmonary TB, showing non-homogenous opacities in the middle and lower zones, more marked on the right, and a few

thin-walled cavities in the right lung middle zone (Figure 1). Sputum acid-fast bacilli (AFB) smear showed 18 AFB/100 fields. Molecular TB diagnosis using Xpert MTB/RIF (GeneXpert) was positive with no Rifampicin resistance detected. The patient was presumed multi-drug-resistant (MDR) TB as her brother was on MDR treatment and her sister was on a second-line tuberculous treatment regime. The treatment was initiated with Bedaquiline 200 mg, Cycloserine 500 mg, Clofazimine 100 mg, and Linezolid 600 mg four times a day (QID) and the patient was counseled about the disease, its spread, and treatment.

A polymerase chain reaction (PCR) test for HIV-1 RNA was also requested which was strongly positive at 28,866 IU/ml (cut-off for positive 72 IU/ml). Blood specimen was inadvertently sent for HIV serology on a subsequent follow-up visit after more than 6 weeks. Testing performed by the clinical laboratory on Determine HIV Early Detect lateral flow test gave a positive result for p24 antigen while it was negative for HIV IgG and IgM antibodies. Repeat testing on a fresh specimen received 2 weeks later also showed the same results. Analyses by fourth-generation Alinity HIV Antigen/antibody Combo on chemiluminescent microparticle immunoassay and Bio-Rad Geenius HIV-1/2 Supplemental Assay (Figure 2) were both negative.

Patient follow-up

Sputum AFB smear was performed subsequently 6 weeks after the initial presentation which was negative for AFB while sputum culture was positive for *Mycobacterium tuberculosis* and showed resistance to Isoniazid while it was sensitive to Rifampicin, Ethambutol, Streptomycin, and Pyrazinamide. Repeat sputum AFB smear and culture a month later were negative. The patient was kept on regular follow-ups.

Serology was still negative for HIV1/2 antibodies on the serum specimen received nearly 5 months after the initial PCR result. In view of the clinical presentation of cough with a diagnosis of MDR-TB and positive HIV-1 RNA PCR, it was decided to start the patient on anti-retroviral therapy (ART) for HIV infection.

Discussion

Here we report a case of HIV-TB co-infection in a 13-year-old female child. This case is a unique presentation in which the patient failed to develop detectable antibodies to proven HIV infection. HIV PCR was requested for this patient with a presumptive diagnosis and family history of MDR TB.

The different modalities of laboratory investigations help in diagnosing HIV at various disease stages. HIV serological assays have evolved over time. First-generation HIV assays detected only antibodies. These were gradually improved upon to increase their sensitivity by including detection of both p24 antigen and antibodies to HIV1 and 2. These combo (antigen plus antibodies) tests are available on lateral flow as well as automated chemiluminescent assays. The fourth- and fifth-generation

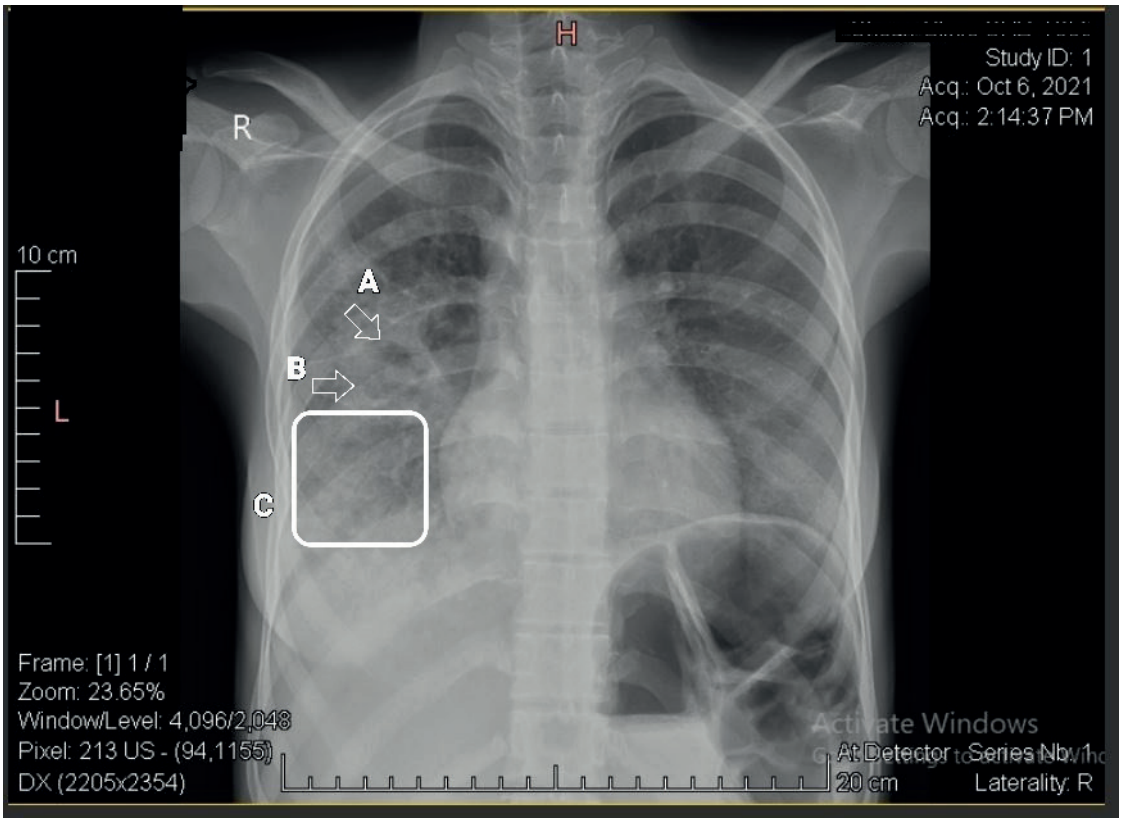

Figure 1. *Chest X-ray suggestive of active TB. A, B: Thin-walled cavities, C: Non-homogenous opacities.*

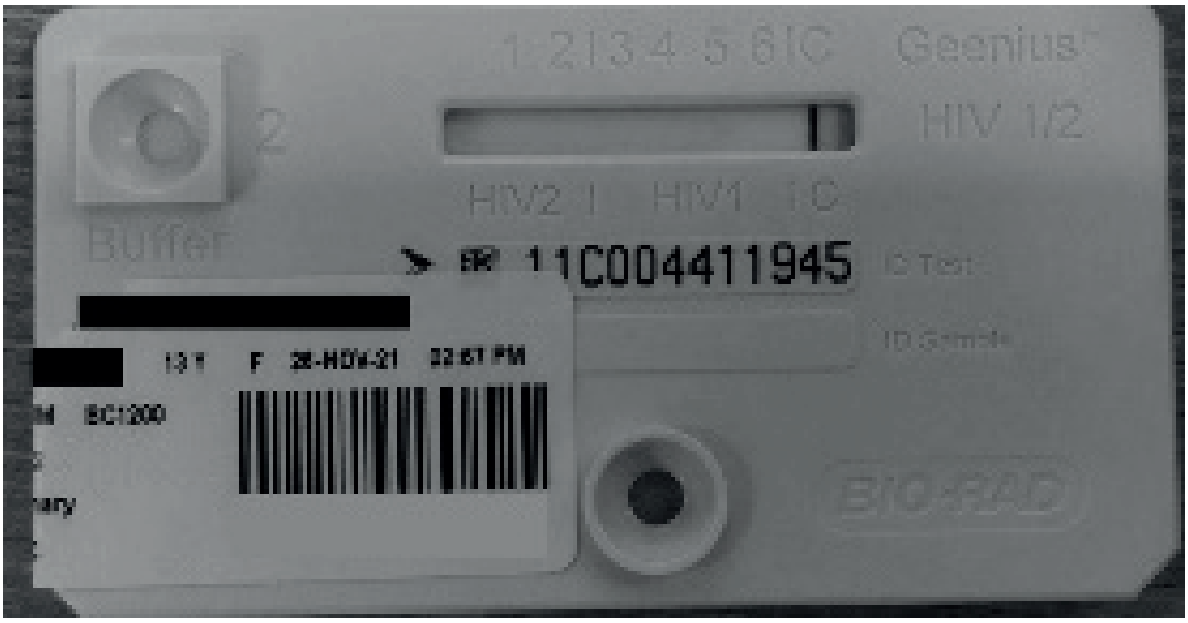

Figure 2. *Bio-Rad Geenius HIV1/2 Supplementary Assay.*

combo assays, especially those based on chemiluminescence or electrochemiluminescence, have substantially reduced window period because of their enhanced analytical sensitivities and earlier detection of the p24 antigen.

WHO recommends offering HIV testing in all cases of diagnosed and presumed TB settings [6]. However, both CDC [7] and WHO [8] have proposed algorithms for HIV diagnosis in which an initial screening test is followed up by confirmatory testing. The testing algorithm starts with the most sensitive tests and is followed by more specific ones. A fourth-generation assay, which detects p24 antigen as well as antibodies against HIV-1 and HIV-2 is the first screening test. If the antibodies are positive, this should be followed by a confirmatory immunoassay differentiating HIV-1 and HIV-2 or a molecular-based diagnostic test [7, 9]. The previous recommendation of Western Blot is now being superseded by other confirmatory tests which are more convenient and less time- and labor-intensive. Bio-Rad Geenius HIV-1/2 Supplemental Assay is one such assay that has gained FDA approval [7].

In our patient, the first test was mistakenly ordered as HIV PCR followed by serology. The serological assay initially conducted on determine HIV Early Detect lateral flow gave a positive result for the p24 antigen while it was negative for the antibodies. This was surprising considering the highly positive PCR results 6 weeks earlier. Therefore, a fresh specimen was collected from the patient 2 weeks later which yielded the same result. The fourth-generation Alinity i HIV Ag/Ab Combo and confirmatory testing of antibodies on Geenius HIV1/2 Supplemental Assay (Bio-Rad Laboratories) were both negative.

HIV PCR was performed using artus HI Virus-1 RG RT Kit (Qiagen, GmbH) with a detection limit of 72 copies per milliliter. Geenius HIV1/2 Bio-Rad is an FDA-approved multi-target lateral flow confirmatory test against various HIV-1 antigens (p41, gp160, p24, and p31) and HIV-2 antigens (gp36 and gp140). Interpretation can be made manually or with a reader. The Abbott Alinity i HIV Ag/Ab Combo assay (Abbott Combo; Abbott Laboratories, Abbott Park, IL, USA) is a chemiluminescent microparticle immunoassay that simultaneously detects HIV-1 p24 antigen, HIV-1 gp41 antibody, and HIV-2 gp36 antibody. The signal intensity is reported as a signal-to-cutoff (S/CO) ratio.

Seronegative HIV is generally more aggressive clinically [10]. Whether this is the result of the lack of adequate humoral response is less clear. A fall in the levels of CD4 T cells has been postulated to be the major cause of co-infection or reactivation of latent TB in HIV-infected patients. In the absence of anti-retroviral treatment, patients progress to AIDS in a few years [10]. In a case series from London, there were eight out of eighteen children with TB and HIV co-infection who were not diagnosed with HIV until after their clinical presentation with TB [4]. HIV testing should be considered in all children developing TB. TB in a child with HIV infection is considered to be an indication to initiate ART. The WHO recommends starting anti-retroviral treatment within 2-8 months of ATT in such cases [6].

Our case highlights the limitations of using the standard serological tests to screen for HIV in unrecognized non-seroconverts. The case was only brought to the surface by the erroneously requested test for HIV serology

in an HIV-proven patient and could have been missed if the antibody testing was performed initially. A similar case was reported recently in which a woman with AIDS lost the ability to produce HIV antibodies due to severe immune suppression. HIV PCR and p24 antigen were positive, confirming HIV infection [11]. There is another reported case of a patient infected with HIV who remained seronegative for 4 years, where the humoral response deficit was specific to HIV [9]. There have been rare reports of seronegative HIV patients who were not on anti-retroviral treatment. These patients were more likely to suffer from rapid clinical deterioration [10]. This, therefore, calls for having a high index of suspicion in patients with a suggestive history or discordant results, and consideration for timely ART. There is a need to monitor these patients for possible disease progression.

What is new?

Testing for HIV antibodies during the window period is a well-established cause for false negative serology results in a proven HIV-infected patient. Here, the authors present a case of an HIV/TB co-infected child with undetectable antibodies even 5 months after a positive HIV-1 RNA PCR.

Conclusion

Seronegative HIV is an entity that should not be ignored while screening potentially infected individuals or patients with ambiguous results.

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Anti-retroviral therapy
ATT	Anti-tuberculous treatment
HIV	Human immunodeficiency virus
MDR-TB	Multi-drug resistant tuberculosis
TB	Tuberculosis
CDC	Centers for Disease Prevention and Control
FDA	Food and Drug Administration
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MTB	Mycobacterium Tuberculosis
QID	Four times a day (Latin: quarter in die)
RIF	Rifampicin

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Consent for publication

Written and informed consent was taken from the family of the patient to publish this case report.

Ethics approval

Ethical approval is not required at our institution to publish an anonymous case report.

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Summary of the case

1	**Patient (age/gender)**	13-year-old female
2	**Diagnosis**	TB/HIV co-infection
3	**Symptoms**	Cough for 1 month
4	**Specialty**	Clinical Chemistry/Immunology
5	**Background**	Absence of HIV antibodies in a patient with highly positive HIV PCR
6	**Conclusion**	Non-seroconversion can result in the missing diagnosis of HIV infection in immunosuppressed patients