



Case report

High positive HIV serology results can still be false positive

Joanna Reid^{a,*}, Gert Van Zyl^a, Michael Linström^a, Stephen Korsman^b, Gert Marais^b, Wolfgang Preiser^a

^a Division of Medical Virology, Department of Pathology, Stellenbosch University and National Health Laboratory Service Tygerberg, South Africa

^b Division of Medical Virology, University of Cape Town and Groote Schuur National Health Laboratory Service, South Africa

ARTICLE INFO

Article history:

Received 26 November 2019

Received in revised form 23 May 2020

Accepted 23 May 2020

Keywords:

Human immunodeficiency virus

HIV serology

False positive HIV serology

HIV diagnosis

HIV Elisa

ABSTRACT

The consequences of falsely reactive HIV test results can be significant, for patients and healthcare providers. This case describes a diagnostic investigation of a patient with pronounced discordant HIV serological results, to determine HIV status. The fourth generation serological screening assay (Roche COBAS Elecsys HIV combiPT) had high positive results but confirmatory testing was negative (Abbott HIV Ag/Ab Combo). Five separate samples over 13 days were tested using multiple fourth generation HIV immunoassays and molecular tests for HIV-1 and HIV-2. Potential causes of falsely reactive serological results were investigated. Samples were sent to the manufacturer for analysis.

The screening assay was positive on all samples with a very high signal to cut-off ratio (S/CO) of greater than 400. However, multiple serological and molecular assays did not detect HIV-1 or HIV-2 specific antibodies, antigen or nucleic acid. A recombinant immunochromatographic assay had faint reactivity to gp41 peptide and the manufacturer investigation reported cross-reactivity to one of the screening assay's synthetic peptides. Possible causes of the false positive result include cross reactivity to other antigens, including prior schistosomiasis infection, or the patient's previously excised ameloblastoma (a rare germ cell tumor of the jaw). This is a rare case of false high positive results on fourth-generation HIV serology testing due to high level non-specific reactivity to an isolated synthetic peptide component of the assay. It highlights the need for confirmatory testing even in settings with HIV high prevalence and awareness that false-positive serological results may have a high S/CO.

© 2020 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

A falsely reactive HIV test result has serious consequences for patients, both emotionally and socially. It may also result in unnecessary initiation of antiretroviral treatment, with potential for side effects and other complications. If later proven to have been false-positive, this can have a negative impact on the perception of, and trust in the health care services by the public. Most falsely reactive HIV serological result are low positive, and can be excluded on the basis of a low positive or indeterminate range, which is appropriate for the local population. In South Africa, HIV testing guidelines for adults and infants over the age of 18 months recommend the use of a series of rapid

immuno-chromatographic assays supplemented by enzyme linked immunosorbent assays (ELISA) in case of discrepant rapid results [1].

Case presentation

The patient was a 35 year old Nigerian man, living in South Africa, who had an unremarkable medical history until 2012, and described himself as previously fit and healthy. Regular screening for HIV at the local community health care clinic had been negative, including three weeks prior to his hospital admission. He denied recent flu-like symptoms or treatment for sexually transmitted infections. He did not use regular medications, traditional medicine, vitamins or dietary supplements. He had no history of HIV vaccine exposure or antiretroviral drug use for pre- or post-exposure prophylaxis. In June 2012, he had a subtotal hemi-mandibulectomy for an ameloblastoma, a benign odontogenic tumor of the mandible [2]. This was done in Nigeria, according to the patient's self-reported history, but there is neither a histology report nor access to the specimen to confirm the diagnosis. After moving to South Africa, he experienced surgical

* Corresponding author at: Department of Medicine, University of the Witwatersrand, Johannesburg, South Africa.

E-mail addresses: reidjoanna@gmail.com (J. Reid), guvz@sun.ac.za (G. Van Zyl), michael.linstrom@nhls.ac.za (M. Linström), stephen.korsman@nhls.ac.za (S. Korsman), Kruger.Marais@nhls.ac.za (G. Marais), preiser@sun.ac.za (W. Preiser).

site complications, and was admitted for replacement of the mandibular reconstruction plate. In 2019, as part of his pre-surgical assessment and workup, he had blood taken for HIV screening. During the operation, one of the surgeons sustained a needle-stick injury, and the virology laboratory was contacted to enquire about the results of the HIV test requested on admission.

His regular partner was then pregnant and had tested HIV negative multiple times during antenatal care visits, including a week before the patient's admission to hospital. He reported concurrent, consistently protected casual intercourse with multiple other female partners up until two to three weeks prior to the current admission but did not report any intravenous drug use or intercourse with men.

Serological screening for HIV-1 and HIV-2 infection was done according to the routine virology testing algorithm at the National Health Laboratory Service (NHLS) Tygerberg laboratory. The 4th generation Elecsys HIV combi PT assay on the Roche COBAS[®] e 601 analyser (Roche Diagnostics, Mannheim, Germany) showed a reactive result with a high signal to cut-off ratio of 478. However, confirmatory serology using the HIV Ag/Ab Combo assay on the Abbott Architect i2000SR analyser (Abbott Laboratories, Wiesbaden, Germany) was non-reactive with a signal to cut-off ratio of 0.71. Both assays were repeated on four independently obtained samples, obtained over a period of five days, as well as 12 days after the first sample, with similar values obtained (Table 1). The Roche Elecsys HIV combi PT assay is a sandwich electrochemiluminescence (ELICA) serological assay [3]. It captures HIV-1 and HIV-2 antibodies and p24 antigens, using biotinylated and ruthenylated conjugates (anti-p24 antibodies, HIV-specific recombinant antigens/ HIV-specific peptides) to form sandwich complexes, thereafter streptavidin-coated microparticles are added to capture the complexes. A signal is generated from the electrochemiluminescent reaction and the assay readout is light, emitted by the ruthenium complexes when a voltage is passed through the reaction. This signal is compared to a cut off value obtained by

calibration of the instrument, giving the signal to cut off ratio (S/CO) [3]. The Abbott HIV Ag/Ab Combo assay (Abbott Laboratories) employs a similar chemiluminescent principle.

Diagnoses considered at this stage included acute HIV infection/seroconversion, a second window period, HIV-2 infection (with variable sensitivity for HIV-1 and 2 on the different testing platforms accounting for the discrepant results), or false positive results. A second diagnostic window period in HIV serological testing describes a time period where a fourth-generation assay will be nonreactive because the antigen (p24) component becomes undetectable, but before the antibody component is high enough to be detected [4]. False positive results may be caused by non-specific binding of other immunoglobulins in the sample to the target antigens or antibodies or to one of the other components of the assay. Cross-reacting immunoglobulins may represent antibodies to a different antigen, rheumatoid factor or be due to polyclonal activation.

Acute HIV infection was excluded via molecular testing, using the COBAS[®] AmpliPrep/ COBAS[®] TaqMan HIV-1 Quantitative Test V2.0 (Roche Diagnostics), showing a viral load of lower than detectable. Qualitative total nucleic acid testing for HIV-1 with the HIV-1 Qualitative Test V2.0 (Roche Diagnostics) also yielded a negative result. (See Table 2 for details). Considering the patient's country of origin, a diagnosis of HIV-2 was considered, and an EDTA whole blood sample was tested for HIV-2 via polymerase chain reaction (PCR), using the Alere[™] Q analyser HIV 1 /2 Detect test (Roche Diagnostics), which gave negative results for both HIV 1 group M/N, group O and HIV-2.

In order to exclude established HIV infection with low or variable antibody levels, as a result of undisclosed treatment with antiretrovirals, or as a possible elite controller of HIV, additional serological testing was performed. A variety of rapid immunochromatographic tests were performed on the patient's samples, all with negative results (see Table 1 for details). An alternative 4th generation serological assay, the GS Bio-Rad HIV Combo Ag/Ab

Table 1
Serological results.

Instrument /platform	Date: April 2019 Sample ID Assay/ test	5/4 1	9/4 2	9/4 3	10/4 4	10/4 5	17/4 6
COBAS e 601	Elecsys [®] HIV combi PT S/CO:	R 478	R 488	R 493	R 415		R 471
Abbott Architect i2000SR	HIV Ag/Ab Combo S/CO:	NR 0.71	NR 0.79	NR 0.77	NR 0.66		NR 0.7
Bio-Rad	Bio-Rad Genscreen ULTRA HIV Ag/Ab S/CO:					N	
Rapid test	Visitect HIV1/2 Rapid					NR	
Rapid test	Alere [™] HIV Combo			N			
Rapid test	Abon HIV 1/2/O Tri-line					NR	
Rapid test	ADVANCED QUALITY [™] Rapid Anti-HIV(1&2)					NR	

Table 2
Molecular results.

Instrument /platform	Date: April 2019 Sample ID Assay/ test	5/4 1	9/4 2	9/4 3	10/4 4	10/4 5	17/4 6
COBAS [®] AmpliPrep/ COBAS [®] TaqMan	HIV-1 Quantitative Test V2.0			LDL		LDL	
COBAS [®] AmpliPrep/ COBAS [®]	HIV-1 Qualitative					ND	ND
TaqMan Alere q	Test V2.0 HIV-1/2 Detect (quant PCR)					ND	

Assay (Bio-Rad Diagnostics, Marnes-la-Coquette, France) was used as a tiebreaker which too produced a negative result. CD4 count testing was performed with a result of 1 454 cells/microliter.

To investigate a non-specific cross reaction related to the Roche Elecsys® HIV combi PT assay principle, such as non-specific cross-linking of conjugate, tests based on the same principle but for other serological markers for which the patient might be negative, were investigated on the same Roche COBAS® e 601 analyser. These included hepatitis A IgM, hepatitis B surface, and core IgM and hepatitis C antibodies as well as hepatitis B surface antigen. The sample tested negative with all these assays, suggesting that the false cross-reactivity was likely restricted to a component of the HIV combi PT assay. A series of dilutions of one of the patient samples were made using the Roche 'Dil Uni' reagent and tested on the Roche screening HIV assay. Results reflected a linear dilution of the reactive component as follows: Undiluted sample: reactive (S/CO 492.6); sample diluted 1:10: reactive (S/CO 48.8), sample diluted 1:100: reactive (S/CO 4.22), sample diluted 1:1000: reactive (S/CO 1.01). Serial dilution has been used as a method of distinguishing false positive results due to cross reacting antibodies from true positive results. In incidence surveys, dilution is used to alter the sensitivity of antibody tests to detect recent HIV infection [5]. In principle, during early HIV infection, lower antibody titers means that reactivity can be diluted out earlier than for samples with high titers. Low avidity, broadly reactive antibodies produced to infectious antigens other than HIV can also cause a false positive result in serological testing for HIV and may theoretically become negative on dilution, as this makes a functionally less sensitive assay [5]. It is significant in this case that the sample tested positive on repeated dilutions, indicating that this false positive result was most likely not due to this type of low avidity antibody response. This finding is supported by repeatedly positive results, with a similar S/CO result on repeat testing 12 days after the initial sample was taken, making a transient response to another antigen less likely.

Cross reacting Schistosoma antibodies remains a possible cause, as the patient tested positive on an immunofluorescent assay for anti-cercarial IgM and IgG antibodies. This has been described as a possible cause of falsely reactive fourth generation immunoassays in other African populations [6]. Other common causes of cross reactivity include autoimmune disease and rheumatoid factor; screening tests for these two conditions were negative. To further investigate specific cross reactive antibodies, the patient's sample was tested with the Geenius HIV 1/ 2 confirmatory assay (Bio-Rad Diagnostics, Marnes-la-Coquette, France) which is a 'rapid' recombinant immunochromatographic test used for the confirmation and differentiation of individual antibody responses to HIV-1 and 2 [7]. Criteria for interpretation of a positive test result for HIV -1 include the presence of any two bands of the HIV-1 test lines with at least one envelope band positive (gp160 or gp41), while a positive test for HIV-2 must include both HIV-2 bands (gp36 and gp140). Testing of this patient's sample using this assay produced a faint band in the gp41 test line (HIV-1 group M and O envelope peptides) but no other bands were produced, indicating an indeterminate result. This correlates with findings from a report of a more than five million samples collected over four years from low risk blood donors, showing that the most common profile of false positive HIV-1 western blot tests were antibodies to envelope only [8].

Patient samples were sent to the screening assay manufacturer for analysis and were found to be falsely reactive to one of the seven HIV specific antigens the test employs. It was determined that the COI (cut off index) of these samples was around 400, and highly reactive against a particular synthetic peptide but non-reactive against the recombinant antigens. The clinicians involved were advised that it was safe to stop post exposure prophylaxis.

The patient was informed about these false positive results and advised to relate this history to future health care providers.

Discussion

Reasons for false reactive serological tests can be classified as pre-analytical, which include sample swaps or sample contamination [9]; analytical, such as false reactive results due to cross reacting antibodies; or post-analytical, when non-reactive results are incorrectly reported as reactive. When assays are performed in settings that are very different from the population in which they were validated, specificity may be lower than expected. For instance, poor specificity has been reported for HIV and hepatitis C virus serological assays in tropical countries [10].

Most false reactive results, however, are low positive and could be excluded based on a low positive/indeterminate range, appropriate for the local population. This high-positive false positive result (more than 400 times the cut-off for positivity) on the screening assay is therefore very unusual. It should be emphasized that all laboratory-based HIV diagnoses require confirmatory testing on a separate independent serological platform and require an independently collected sample. In this case, independently collected samples all showed high positive values on the screening assay, but negative values on the confirmatory assay.

Multiple biological reasons for false reactive serology have been reported, which include polyclonal activation during systemic infections, molecular mimicry with other infectious agents, pregnancy, vaccination and various malignancies [11–13]. No prior case reports regarding ameloblastoma and false positive HIV serological results have been published, although a variety of other malignancies have been implicated in generating false positive HIV results [12]. The reason for the falsely reactive screening assay result remains unknown at present, although the possibility of cross reactivity with schistosomiasis antibodies or antibodies produced towards his previously excised ameloblastoma, or another antigen remains.

Conclusion

HIV serological diagnosis in adults and children older than 18 months necessitates confirmatory testing to exclude assay-specific false reactivity and the testing of an independently collected sample to avert sample-associated causes of false positivity such as mislabelling, transient cross-reactivity or on-instrument contamination. Although the cause for this high positive assay-specific false reactive result could not be elucidated fully, this case highlights the indispensable need for confirmatory HIV serological testing and the use of independent serological and molecular assays as tiebreakers in investigating discordant results.

Funding

No funding sources involved in this work.

Consent and ethics approval

This case report was discussed with the patient who gave written and verbal consent for the report. Ethics approval for the case study was obtained from Stellenbosch University Health Research Ethics Committee (Project ID 10106, Ref: C19/05/014).

The Stellenbosch University Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the World Medical Association (2013), Declaration of Helsinki: Ethical

Principles for Medical Research Involving Human Subjects; the South African Department of Health (2006), Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015) Ethics in Health Research: Principles, Processes and Structures (2nd edition).

CRedit authorship contribution statement

Joanna Reid: Conceptualization, Investigation, Writing - original draft. **Gert Van Zyl:** Conceptualization, Writing - review & editing, Supervision. **Michael Linström:** Investigation. **Stephen Korsman:** Investigation, Writing - review & editing. **Gert Marais:** Investigation. **Wolfgang Preiser:** Writing - review & editing, Supervision.

Declaration of Competing Interest

No conflicts of interest to declare.

Acknowledgements

The authors would like to thank Louise Wilsdorf for assistance in performing the assays.

References

- [1] National Department of Health (South Africa). National HIV counselling and testing policy Guidelines. 2015 May, p17. Available at <http://www.nicd.ac.za/assets/files/HCT-Guidelines-2015.pdf>. Accessed 09/09/2019.
- [2] Masthan KM, Anitha N, Krupaa J, Manikkam S. Ameloblastoma. *J Pharm Bioallied Sci* 2015;(April (Suppl 1)):S167–70, doi:<http://dx.doi.org/10.4103/0975-7406.155891>.
- [3] Roche Diagnostics. HIV combi PT package insert. 2016-07(V8.0), Mannheim, Germany.
- [4] George CR, Robertson PW, Lusk MJ, Whybin R, Rawlinson W. Prolonged second diagnostic window for human immunodeficiency virus type 1 in a fourth-generation immunoassay: are alternative testing strategies required? *J Clin Microbiol* 2014;52(November (11)):4105–8, doi:<http://dx.doi.org/10.1128/JCM.01573-14>.
- [5] Shanks L, Siddiqui MR, Abebe A, Piriou E, Pearce N, Ariti C, et al. Dilution testing using rapid diagnostic tests in a HIV diagnostic algorithm: a novel alternative for confirmation testing in resource limited settings. *Virol J* 2015;12(May (75)), doi:<http://dx.doi.org/10.1186/s12985-015-0306-4>. https://www.ncbi.nlm.nih.gov/pubmed/?term=Pearce%20N%5bAuthor%5d&cauthor=true&cauthor_uid=25972188%22.
- [6] Everett DB, Baisely KJ, McNerney R, Hambleton I, Chirwa T, Ross DD. Association of schistosomiasis with false-positive HIV test results in an African adolescent population. *J Clin Microbiol* 2010;48(February (5)):1570–7, doi: <http://dx.doi.org/10.1128/JCM.02264-09>.
- [7] Bio-Rad diagnostics Geenius HIV 1/ 2 Confirmatory Assay package insert 883601. 2013/0. Marnes-la-Coquette, France.
- [8] Kleinman S, Busch MP, Hall L, Thomson R, Glynn S, Gallahan D, et al. False positive HIV-1 test results in a low-risk screening setting of voluntary blood donation. *JAMA* 1988;80(September (12)):1080–5, doi:<http://dx.doi.org/10.1001/jama.280.12.1080>.
- [9] Hardie DR, Korsman SN, Hsiao NY, Morobadi MD, Vawda S, Goedhals D. Contamination with HIV antibody may be responsible for false positive results in specimens tested on automated platforms running HIV 4th generation assays in a region of high HIV prevalence. *PLoS One* 2017;12(July (7)): e0182167, doi:<http://dx.doi.org/10.1371/journal.pone.0182167>.
- [10] Van Zyl G, Maritz J, Newman H, Preiser W. Lessons in diagnostic virology: expected and unexpected sources of error. *Rev Med Virol* 2019;29(May (4)): e2052, doi:<http://dx.doi.org/10.1002/rmv.2052>.
- [11] Zacharias NM, Athanassaki JD, Sangi-Haghpeykar H, Gardner MO. High false-positive rate of Human Immunodeficiency Virus rapid serum screening in a predominantly Hispanic prenatal population. *J Perinatol* 2004;(August):743–7, doi:<http://dx.doi.org/10.1038/sj.jp.7211184>.
- [12] Liu P, Jackson P, Shaw N, Heysell S. Spectrum of false positivity for the fourth generation human immunodeficiency virus diagnostic tests. *AIDS Res Ther* 2016;13(January (1)):1742–6405, doi:<http://dx.doi.org/10.1186/s12981-015-0086-3>.
- [13] Shida S, Takahashi N, Fujishima N, Kameoka Y, Nara M, Fujishima M, et al. False-positive human immunodeficiency virus antibody test and autoimmune hemolytic anemia in a patient with angioimmunoblastic T-cell lymphoma. *Jpn J Med* 2011;50(October (20)):2383–7, doi:<http://dx.doi.org/10.2169/internalmedicine.50.5764>.