


SHORT COMMUNICATION

Diagnostic accuracy of Abbott Architect Assay as a screening tool for human T-cell leukaemia virus type-1 and type-2 infection in a London teaching hospital with a large solid organ transplant centre

Nathaniel Lee¹  | Jamie Murphy¹ | Rasheed Al-Khudairi¹ | Ann Sturdy¹ |
Tabitha Mahungu¹ | Tanzina Haque^{1,2} | Paul Griffiths¹ | Jennifer Tosswill³ |
Dianne Irish¹

¹Royal Free Hospital NHS Foundation Trust, London, UK

²Centre for Virology, Division of Infection and Immunity, UCL, London, UK

³Department of Virology, UK Health Security Agency, London, UK

Correspondence

Nathaniel Lee, Royal Free Hospital NHS Foundation Trust, London, UK.
Email: nathaniel.lee@doctors.org.uk

Abstract

Aim: In the United Kingdom, organ donors/recipients are screened for evidence of human T-cell leukaemia virus type-1 and type-2 (HTLV-1/2) infections. Since the United Kingdom is a low prevalence country for HTLV infections, a screening assay with high sensitivity and specificity is required. Samples with repeat reactivity on antibody testing are sent to a reference lab for confirmatory serological and molecular testing. In the case of donor screen, this leads to delays in the release of organs and can result in wastage. We aim to assess whether a signal/cut-off (S/CO) ratio higher than the manufacturer's recommendation of 1.0 in the Abbott Architect antibody assay is a reliable measure of HTLV-1/2 infection.

Methods: We conducted a 5 year retrospective analysis of 7245 patients from which 11 766 samples were tested on the Abbott Architect rHTLV I/II assay. Reactive samples (S/CO >1) were referred for confirmatory serological and molecular detection (Western Blot and proviral DNA) at UK Health Security Agency, (formerly PHE, Colindale), the national reference laboratory. Electronic, protected laboratory and hospital patient databases were employed to collate data.

Results: A total of 45 patients had initially reactive samples. 42.2% ($n = 19/45$) had an S/CO ratio > 20, with HTLV infection confirmed in $n = 18/19$ and indeterminate confirmatory results in $n = 1/19$. No samples with an S/CO ratio <4 (48.9%, $n = 22/45$) or 4–20 (8.9%, $n = 4/45$) had positive confirmatory results on subsequent confirmatory testing.

Conclusion: Samples with an S/CO >20 likely represent a true HTLV-1/2 infection. Reactive samples with an S/CO <4 were unlikely to confirm for HTLV infections. Interpretation of these ratios can assist clinicians in the assessment of low reactive samples and reiterates the need for faster access to confirmatory testing.

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KEYWORDS

HTLV-1/2, screening, transplant

1 | INTRODUCTION

Human T-cell leukaemia virus type-1 and type-2 (HTLV-1 and HTLV-2) were first isolated in 1979 and 1981 respectively, the former the first retrovirus to be discovered.¹ They are enveloped, single-stranded RNA viruses members of the genus Deltaretrovirus of the Orthoretrovirinae subfamily of the family of Retroviridae.² HTLV-1 has been found to cause lifelong infection of T-lymphocytes, and is associated with the development of haematological malignancies and neurological sequelae.³⁻⁵ Although myelopathy is a recognised association with HTLV-2, other disease associations with HTLV-2 are less well established.

It has only been since 2011 that pre-screening for HTLV infection in solid organ transplant recipients and donors was introduced in the United Kingdom because of growing concern about the infection risk and progression to disease by this route.⁶⁻⁸ Timely and accurate diagnostics are vital given the occasionally short assessment window for serological evaluation at or around the time of organ transplantation.²

The aim of this study was to assess whether a signal/cut-off (S/CO) ratio higher than the manufacturer's (Abbott Architect) recommendation of 1.0 is a reliable measure of HTLV-1/2 infection using data from a large UK solid organ transplant centre. Secondary objectives were included which evaluated the diagnostic accuracy of various S/CO ratio categories to improve screening outcomes.

2 | METHODS

A retrospective analysis was performed on data from 11 766 blood serum samples from 7254 patients submitted for HTLV-1 and HTLV-2 testing over a 5 year period between 2013 and 2017 at the Royal Free Hospital (RFH) in London, United Kingdom. Samples were drawn from screening patients from the renal, hepatology and haematology/oncology departments, most of them screened as potential transplant recipients but also included samples from organ donors. Samples were also included from patients screened from immunology and neurology departments. Basic demographic information was recorded from all patients, including co-presenting viral infections such as Hepatitis B, C, D and HIV. Retrospective request for intravenous immunoglobulin (IVIG) therapy was confirmed from the Immunoglobulin Database (<https://igd.mdsas.com/>) and cross-checked with RFH's pharmacy dispensing records. Ethics approval was not sought as all data were collected routinely for clinical purposes.

2.1 | Primary analysis

Samples were tested using the Abbott Architect rHTLV I/II assay (Abbott Laboratories Weisbaden, Germany) at our laboratory. All

TABLE 1 Patient demographics of this study population

Patient demographics	
Patients (%)	Male: 64.4% (29/45) Female: 25.6% (16/45)
Mean age (range)	49.8 (19–85)
Reason for testing (%)	
Pre-transplant screening	80% (36/45)
Clinical indication	15.7% (7/45)
Donor screening	2.2% (1/45)
Not documented	2.2% (1/45)
HIV positive (%)	2.2% (1/45)
Hepatitis B positive (%)	6.7% (3/45)
Hepatitis C positive (%)	6.7% (3/45)

samples with values S/CO ≥ 1.0 were sent to the UK National Reference Department (VRD; Virus Reference Department, UK Health Security Agency) for confirmatory serological testing, where the initial clotted samples were again tested on the Abbott Architect rHTLV I/II, and reactive samples were then tested by Western blot. Where unseparated whole blood on ethylenediaminetetraacetic (EDTA) samples were available from patients who had HTLV I/II reactive serology, the EDTA samples were tested by a nested HTLV DNA polymerase chain reaction (PCR). A final status was determined by the reference lab using the Western blot (WB) and PCR results, and was reported as the following: HTLV-1 positive, HTLV-2 positive, HTLV untyped, HTLV indeterminate or HTLV negative.

The sensitivity, specificity, positive predictive values and negative predictive values were calculated for S/CO ratios in the ranges 1–4, 4.01–20 and >20 . The standard was a composite of tests run by the VRD; defined as a positive confirmatory WB or proviral PCR.

2.2 | Secondary analysis

S/CO values were stratified into groups first proposed in the paper by Tosswill & Taylor to aid in the clinical interpretation of results.² Values were stratified into the following groups: S/CO 1–4, 4.01–20, and >20 . Tosswill & Taylor suggested that an S/CO cut-off of <4 could be considered a negative result as no samples with values in this range subsequently confirmed positive for HTLV-1/2 infection. All samples that had an S/CO value initially >20 were subsequently found to have HTLV infection. Although the samples with S/CO values between 4.01 and 20 were found to have an indeterminate status on additional testing, subsequent repeat sampling found most of these to be false positive results.

	In-house Abbott Architect S/CO values		
	S/CO 1–4	S/CO 4.01–20	S/CO > 20
Western blot testing			
Seropositive	—	—	94.7% (18/19)
Seronegative	90.9% (20/22)	100% (4/4)	—
Indeterminate	9.1% (2/22)	—	5.3% (1/19)
HTLV-1 DNA PCR			
Positive	—	—	63.2% (12/19)
Negative	45.4% (10/22)	50% (2/4)	36.8% (7/19)
Not tested	54.5% (12/22)	50% (2/4)	—

TABLE 2 Western blot and viral PCR testing outcomes across the samples stratified by in-house Abbott Architect S/CO groups

3 | RESULTS

Of the 11 766 samples tested there were 114 samples (1%) from 45 patients that were initially reactive that formed this cohort, with patient demographics described in Table 1. Each patient had an initial serology sample with an Abbott Architect S/CO value ≥ 1.0 . When repeated at the reference lab, all the initial first positive samples had an S/CO value ≥ 1.0 .

A 26.7% (12/45) of patients were bone marrow or stem cell transplant recipients, 15.6% (7/45) were renal transplant recipients, 8.9% (4/45) were liver transplant recipients, 28.9% (13/45) were end-stage renal failure on or being considered for dialysis, 8.9% (4/45) were undergoing plasmapheresis, and 6.7% (3/45) had other medical comorbidities. In addition, one patient (2.2%) was under investigation for spastic paraparesis, and one (2.2%) was a transplant donor.

The Western blot and viral PCR testing outcomes are given in Table 2. Samples tested in WB were seropositive in 40% ($n = 18/45$) of patients, seronegative in 53.3% (24/45) and indeterminate in 6.7% (3/45) of patients. Of the in-house S/CO 1–4 group, 90.9% (20/22) were WB seronegative and 9.1% (2/22) were indeterminate. Of the in-house S/CO 4.01–20 group, 100% (4/4) of patients were WB negative. 94.7% (18/19) of patients in the in-house S/CO >20 group were WB positive, and 1 patient was WB indeterminate on the initial sample but negative by PCR on a subsequent sample.

HTLV-1 DNA was detected by PCR in 26.7% (12/45) of patients, all of whom had S/CO >20 on initial HTLV serology. None of the samples from the 45 patients were positive for HTLV-2 DNA by PCR. In the in-house S/CO 1–4 and 4.01–20 group there were no positive HTLV-1 DNA PCR results, although 53.8% (14/26) of these patients did not undergo PCR testing at the reference lab as an EDTA sample was never sent. In the in-house S/CO 4.01–20 group, 50% (2/4) of the patients had samples which had undergone PCR testing.

Follow-up samples were received for 62.2% (28/45) of patients, totalling 65 samples, of which 50% (14/28) were in the initial in-house S/CO 1–4 group, 7.1% (2/28) in the S/CO 4.01–20 group, and 42.9% (12/28) were in the S/CO > 20. A total of 53.3% (24/45) patients had confirmatory proviral DNA sent, including 71% (10/14) of those in the initial in-house S/CO 1–4 who were followed up, and all the patients followed up in the remaining two groups. It is advised by our

laboratory, as well as the reference laboratory, that repeat samples should be sent at least 2 weeks after the initial reactive samples. In our cohort, there were a proportion of patients who had samples sent <2 weeks as well as >2 weeks as recommended. Of all patients who had follow-up samples, 46.4% (13/28) had repeat samples sent <2 weeks after the first sample, of which the majority (76.9%; 10/13) were EDTA samples to confirm proviral DNA. Repeat samples sent >2 weeks were received from 85.7% of follow-up patients (24/28), with 50% (12/24) in the initial in-house S/CO 1–4 group, 4% (1/24) for the S/CO 4.01–20 group, and 45.8% (11/24) in the in-house S/CO >20 group.

Eight patients had IVIG administered before submitting at least one of their samples for HTLV serological testing, equating to a 10.5% (12/114) of samples. In the in-house testing of these samples, 58.3% (7/12) had an S/CO 1–4, 33.3% (4/12) were S/CO 4.01–20, and 8.3% (1/12) was S/CO >20. The one patient with a positive S/CO >20 subsequently had HTLV-1 PCR detected on confirmatory sampling. None of the S/CO 4.01–20 had confirmed HTLV-1 from the reference lab testing.

The diagnostic accuracy against reference lab positive from the initial S/CO ratio of >20, the sensitivity and the PPV of the initial Abbott Architect is 100% (95% CI 79.4%–100%) and 96.3% (95% CI 81%–99.9%) respectively. HTLV infection was not confirmed in any individual with S/CO ratio 1–4, with a negative predictive value of 21.7% (95% CI 7.5%–43.7%). In the S/CO group 4.01–20 there were also no confirmed infections, and this had a negative predictive value of 56.1% (95% CI 39.7%–71.7%).

4 | DISCUSSION

The aim of this study was to describe the experience of HTLV-1/2 screening in a tertiary solid organ transplant centre in the United Kingdom. From the large cohort of samples tested, there were a significant number of confirmed HTLV-1 cases compared with the experience of other research groups both historically and internationally.^{5,9}

The goal of screening is to ensure that blood, tissues and organs are safe for donation. Identification of recipients with HTLV is

important, as transplantation and in particular associated immunosuppressive therapy, may be important in altering immunological control of HTLV infection.⁶ The majority of local hospital labs that embark on HTLV-1/2 testing only have a screening test such as Abbott Architect rHTLV I/II assay at their disposal. Therefore when samples are tested and reactive results obtained, these patient samples must undergo additional testing before the recipient or donor organs are deemed safe for transplantation. Delays in testing and laboratory processing ultimately lead to delays in donation and in most cases wastage. The ability to draw conclusions about the HTLV status of the patient based on the S/CO value could allow for appropriate risk-stratification and reduced time lost.

Similar to datasets previously described, categorization of S/CO values can aid in rapid identification of cases suggestive of true HTLV-1/2 infection.² It is significant that none of the patients with S/CO values ≤ 4 had detectable virus on subsequent PCR testing. This has potential significant implications on transplantation risk assessments, particularly pertaining to the clinical interpretation of low-titre serological positivity suggestive of low risk of true HTLV infection for a transplant recipient or from donor tissue for an organ recipient facing an imminent transplant. This has ramifications for cost-effectiveness considerations in transplant delay and the avoidance of organ wastage.

Intravenous immunoglobulin therapy (IVIG) is a therapy used in a variety of conditions involving the infusion of donor-derived IgG.¹⁰ False-positive serological testing due to non-specific reactivity of donor IgG is a widely known consequence following IVIG use.^{11,12} In our cohort, of those who had received IVIG prior to serological testing only eight had HTLV-1/2 reactive serology. Of the 12 samples with reactive serology, the majority (92%; 11/12) were within the low (1–4) and indeterminate (4.01–20) S/CO value range. The one case with an S/CO value >20 was subsequently demonstrated to be PCR positive for HTLV-1. As HTLV has been routinely screened for in the United Kingdom in blood products since 2002 and donor transmission of HTLV is very low risk from leucodepleted blood components, this was likely to be un-related to IVIG administration.¹³ This further supports the stratification of S/CO > 20 as likely representative of true infection despite the use of IVIG, however our data size precludes any concluding statement on contribution of IVIG to HTLV-1 reactivity.

There were some limitations in the analysis of our cohort. The stratification of S/CO is dependent on the use of the Abbott Architect rHTLV I/II assay. However, this is one of the more widely used assays in the United Kingdom and worldwide for HTLV screening. Less than half of the samples sent to the reference lab received HTLV PCR testing because whole blood on EDTA blood was not available, though of the samples tested none were positive in the S/CO 1–4 and S/CO 4.01–20 groups, which supports the findings of Tosswill et al. There was limited demographic information including ethnicity recorded on patients records, therefore it was impossible to assess impact. It should also be noted that our analysis is conducted in a low-prevalence country setting for primary HTLV disease – our proposed cut-off may not be applicable in high prevalence settings but would be worthy of further investigation.

Though our data indicates that low level reactive samples are likely to represent false positives the ideal situation would be a commercially available HTLV PCR test, which would enable laboratories which perform initial HTLV testing and require rapid confirmatory HTLV investigation to more confidently exclude HTLV infection in the event of reactive samples. It is acknowledged that stratification of S/CO values into groups reduced sample sizes resulting in wider confidence intervals in the diagnostic accuracy analysis. However, the significant positive predictive value of the S/CO >20 group aids the role of serological testing, which is known to have limitation in low seroprevalence populations.¹⁴

5 | CONCLUSION

Samples with an S/CO >20 are likely to represent a true HTLV-1/2 infection. Reactive samples with an S/CO ≤ 4 are unlikely to confirm for HTLV infections. Interpretation of these ratios can assist clinicians in the assessment of low reactive samples. A commercially available HTLV PCR would be a valuable tool in certain hospital settings such as solid organ transplantation where rapid confirmation is desirable.

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CONFLICT OF INTEREST

The authors have no competing interests.

ORCID

Nathaniel Lee  <https://orcid.org/0000-0002-4393-6891>

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