

Table 1 Blood gas and ECG parameters at baseline and while breathing the 15% hypoxic mixture

Parameter	Mean	N	SD	SE mean	95% CI lower	95% CI upper	Significance
H ⁺ (0.21%)	36.58 nmol/l	65	2.35	0.29			
H ⁺ (0.15%)	36.06 nmol/l	65	2.41	0.30			
ΔH ⁺ (21–15%)	0.52 nmol/l	65	2.60	0.32	-0.1282	1.1590	0.12
Paco ₂ (0.21%)	5.11 kPa	65	0.45	0.06			
Paco ₂ (0.15%)	4.87 kPa	65	0.47	0.06			
ΔPaco ₂ (21–15%)	0.25 kPa	65	0.40	0.05	0.14904	0.34942	<0.001
PaO ₂ (0.21%)	10.56 kPa	65	1.17	0.14			
PaO ₂ (0.15%)	6.82 kPa	65	0.77	0.09			
ΔPaO ₂ (21–15%)	3.75 kPa	65	1.06	0.13	3.48188	4.00920	<0.001
HCO ₃ (0.21%)	25.62 mmol/l	65	4.88	0.61			
HCO ₃ (0.15%)	24.46 mmol/l	65	2.33	0.29			
ΔHCO ₃ (21–15%)	1.16 mmol/l	65	4.15	0.51	0.1310	2.1860	0.03
BE (0.21%)	1.09 mmol	65	2.04	0.25			
BE (0.15%)	0.74 mmol	65	2.18	0.27			
ΔBE (21–15%)	0.35 mmol	65	1.7378	0.22	-0.0814	0.7798	0.11
SaO ₂ (0.21%)	95.82%	65	1.19	0.15			
SaO ₂ (0.15%)	87.15%	65	3.61	0.45			
ΔSaO ₂ (21–15%)	8.67%	65	3.38	0.42	7.8326	9.5090	<0.001
PtccO ₂ (0.21%)	5.12 kPa	39	0.69	0.11			
PtccO ₂ (0.15%)	4.84 kPa	39	0.74	0.12			
ΔPtccO ₂ (21–15%)	0.28 kPa	39	0.28	0.05	0.1874	0.3715	<0.001
HR (21%)	83.22 bpm	101	14.97			1.49	
HR (15%)	86.89 bpm	101	15.09			1.50	
ΔHR (21–15%)	3.67 bpm	101	0.58	-4.809	-2.537	0.57	<0.001
PR (21%)	161.23 ms	96	16.09			1.64	
PR (15%)	158.01 ms	96	20.31			2.07	
ΔPR (21–15%)	3.22 ms	96	12.63	0.660	5.778	1.29	0.01
QRSd (21%)	91.93 ms	101	15.97			1.59	
QRSd (15%)	90.27 ms	101	15.92			1.58	
ΔQRSd (21–15%)	1.66 ms	101	9.13	-0.138	3.465	0.91	0.07
QT (21%)	357.75 ms	101	40.97			4.08	
QT (15%)	348.83 ms	101	35.03			3.49	
ΔQT (21–15%)	8.92 ms	101	24.05	4.173	13.669	2.39	<0.001
QTc (21%)	415.16 ms	101	25.86			2.57	
QTc (15%)	416.95 ms	101	24.02			2.39	
ΔQTc (21–15%)	1.79 ms	101	26.70	-7.062	3.478	2.66	0.50

21%, baseline measurement while breathing room air; 15%, test measurement after breathing 15% O₂ hypoxic mixture for 15 min; BE, base excess; ΔBE, change in base excess between 21% and 15% O₂; H⁺, hydrogen ion concentration; ΔH⁺, change in hydrogen ion concentration between 21% and 15% O₂; HCO₃, bicarbonate ion concentration; ΔHCO₃⁺, change in bicarbonate ion concentration between 21% and 15% O₂; HR, electrocardiographic heart rate; ΔHR, change in heart rate between 21% and 15% O₂; Paco₂, partial pressure of CO₂; ΔPaco₂, change in partial pressure of CO₂ between 21% and 15% O₂; PaO₂, partial pressure of O₂; ΔPaO₂, change in partial pressure of O₂ between 21% and 15% O₂; PR, electrocardiographic PR interval; ΔPR, change in PR interval between 21% and 15% O₂; PtccO₂, transcutaneous CO₂; ΔPtccO₂, change in transcutaneous CO₂ between 21% and 15% O₂; QRSd, electrocardiographic QRSd interval; ΔQRSd, change in QRSd interval between 21% and 15% O₂; QT, electrocardiographic QT interval; ΔQT, change in QT interval between 21% and 15% O₂; QT_c, electrocardiographic QT_c interval; ΔQT_c, change in QT_c interval between 21% and 15% O₂; SaO₂, oxygen saturation; ΔSaO₂, change in oxygen saturations between 21% and 15% O₂.

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A new potential biomarker for childhood tuberculosis

One of the major research areas for tuberculosis (TB) focuses not only on diagnostics but also on biomarkers that can provide prognostic data about the disease course and response to treatment. Although progress has been made, improved tests for paediatric TB are especially needed. Young children are at increased risk of progressing to TB after exposure, and may suffer from disseminated forms of the disease. Due to the paucibacillary nature of paediatric disease, the current armamentarium and future pipeline of TB diagnostics that largely rely on microbial growth and/or molecular detection are unlikely to demonstrate performance equivalent to that in adults. Thus, an accurate surrogate marker of disease may be crucial to improving the diagnosis of paediatric TB. We have tested and evaluated a novel B-cell assay called the antibodies in lymphocyte supernatant, or ALS, which has performed very well in diagnosing TB disease both in Asia^{1,2} and Africa (manuscript in preparation). Here, we report the performance of ALS as a biomarker in children with culture-confirmed TB.

The ALS assay is based on a principle similar to that of the enzyme-linked immunosorbent spot assay, measuring antibody-secreting cells in cultures of peripheral blood mononuclear cells (PBMCs). The ALS assay detects antibody secretion from in vivo activated plasma B cells that migrate throughout the peripheral circulation in response to TB antigens that are present during active disease but not latent TB infection.³ The ALS methodology for children includes phlebotomy of 3.5 ml of blood in order to isolate 5 million PBMCs; these cells are incubated in tissue culture plates without stimulation for 48–72 h. The supernatant is collected, placed into BCG-coated microtitre plates and IgG responses to

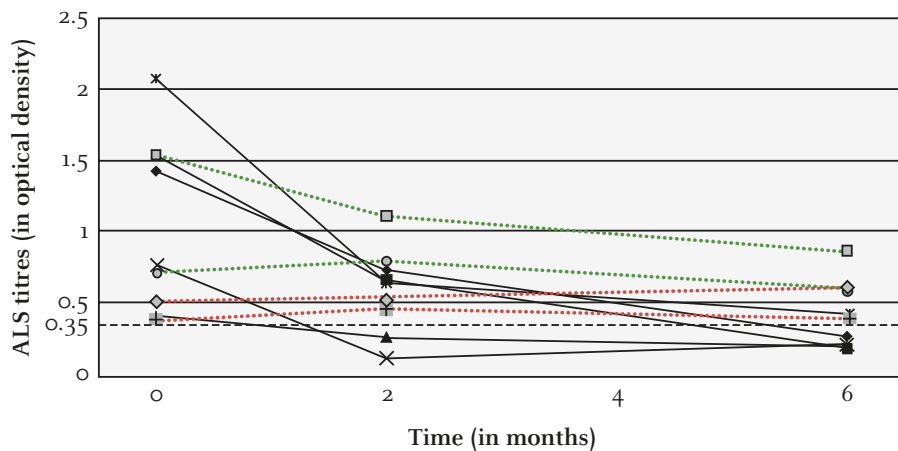


Figure 1 Antibodies in lymphocyte supernatant (ALS) titres during the course of anti-tuberculosis (TB) therapy. The black dashed line represents the threshold value for a positive test, 0.35 optical density. Solid data lines depict changes in ALS titres for children found to have culture-confirmed drug-susceptible TB. Dotted data lines depict changes in ALS titres for children found to have drug-resistant TB; red dotted lines represent children with multidrug-resistant TB (isoniazid and rifampin resistant) and green dotted lines represent children with drug-resistance to isoniazid or rifampin (but not both). ALS titres from the five children with drug-susceptible TB significantly declined after 2 months of first-line anti-TB treatment ($p=0.016$). However, ALS titres failed to decline significantly after 2 months of first-line anti-TB treatment among the four children who were later found to have drug-resistant TB ($p=0.62$). After these four children initiated second-line anti-TB treatment, the ALS titres gradually declined, mirroring their clinical improvement.

BCG are measured by ELISA.¹⁻³ A positive titre is defined as ≥ 0.350 optical density units, calculated from data on healthy children.³ In its present format, the method requires skilled personnel and equipment for PBMC separation from blood samples, sterile cell cultures and ELISA.

A prospective study of children from a low-HIV prevalence area of Bangladesh assessed ALS responses among 58 hospitalised children with clinically diagnosed TB (ages 1–14 years, 15% confirmed by culture), 16 hospitalised children ruled out for TB and 58 age-matched controls. The ALS assay was 91% sensitive and 87% specific when

compared with the clinical diagnostic algorithm for diagnosing TB disease and performed equally well in TB cases < 5 years old compared with those ≥ 5 years.³ Notably, the BCG-specific IgG titres declined after starting appropriate anti-TB treatment. Figure 1 illustrates the trends in ALS titres for nine paediatric patients with culture-confirmed TB. Five of these patients had drug-susceptible TB and four patients had drug-resistant TB, including one patient with TB resistant to isoniazid and streptomycin, another with TB resistant to rifampin, streptomycin and ethambutol, and two patients with TB resistant to isoniazid and

rifampin (multidrug-resistant (MDR) TB). First-line anti-TB therapy (isoniazid, rifampin, pyrazinamide) in patients with drug-susceptible TB resulted in a significant reduction in ALS titres after 2 months ($p=0.016$), whereas the four patients with drug-resistant TB demonstrated only a marginal decline in ALS titres after 2 months of first-line anti-TB therapy ($p=0.62$), reflecting their clinical status (table 1). However, modification of the antibiotic regimen led to clinical improvement and gradual reduction of the ALS titres in three of these four patients. The fourth patient—with MDR TB—took longer time to recover clinically, despite appropriate second-line anti-TB therapy, which paralleled the delayed reduction in ALS titres. Previously, we have illustrated a similar profile of the ALS response in adults with MDR TB.²

The ALS assay is different from traditional serological tests that quantify TB-specific IgM, IgG or IgA levels in serum. Since the serum containing pre-existing antibodies *in vivo* is removed on isolation of PBMCs, the ALS assay focuses on assessing active antibody secretion from TB-specific plasma cells circulating during TB disease. Traditional serological tests have greatly varied sensitivity and poor specificity for TB disease, and the results cannot discriminate active or subclinical disease from latent TB infection.⁴ The sensitivity is greatly reduced in sputum-smear negative and/or HIV-infected individuals and may also be affected by prior BCG vaccination, or exposure to non-tuberculous mycobacteria.⁵ By contrast, the performance of the ALS assay is not adversely affected by these clinical conditions.

The ALS assay is a novel method that is feasible and has high accuracy in detecting TB disease in children³ and adults^{1,2}; however, it warrants further assessment as a biomarker for response to therapy. Interestingly, evaluation of the ALS method for TB diagnosis in Ethiopian adults demonstrated significant positive results for both TB-infected and TB–HIV co-infected patients suggesting that this test, in contrast to presently available immunological assays, can perform well in HIV-infected populations (manuscript in preparation).

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Table 1 Demographic and clinical characteristics of patients (N=9)

	Drug-susceptible TB (n=5)	Drug-resistant TB (n=4)
Median age in years (range)	2.5 (1.6 to 5)	10.5 (5 to 13)
Female gender (%)	2 (40%)	4 (100%)
Known TB contact	4 (80%)	4 (100%)
Chest x-ray findings on presentation	Hilar LAD only: 3 (60%) LAD + infiltrates: 2 (40%)	Hilar LAD only: 1 (25%) LAD + infiltrates: 3 (75%)
Median baseline ALS titre, in optical density units (range)	1.421 (0.412 to 2.070)	0.615 (0.380 to 1.532)
Resolution of fever by 2 months	3 (75%)*	0 (0%)
Resolution of cough by 2 months	4 (80%)	1 (25%)
Median change in BMI for age/gender over 2 months (range)	1.18 (–1.06 to 4.15)	0.48 (0.01 to 0.57)
Median change in BMI for age/gender over 6 months (range)	2.1 (1.01 to 4.95)	0.6 (–0.5 to 0.7)
Median change in ALS titre over 2 months, in optical density units (range)	–0.690 (–1.452 to –0.152)	0.041 (–0.421 to 0.077)
Median change in ALS titre over 6 months, in optical density units (range)	–1.151 (–1.636 to –0.214)	–0.057 (–0.664 to 0.100)

*Of the four children with drug-susceptible TB who presented with fevers. ALS, antibodies in lymphocyte supernatant; BMI, body mass index; LAD, lymphadenopathy; TB, tuberculosis.

Bangladesh (ICDDR,B)—had no role in the study design, data interpretation or writing of this manuscript.

Competing interests RR and DS are the inventors of the antibodies in lymphocyte supernatant assay which is patented; however, the ICDDR,B is the patent holder. Any potential financial gain will be for the Centre if/when this assay is commercialised. There are no other potential conflicts of interest related to the work presented.

Ethics approval This study was conducted with the approval of the Ethics Review Committee of the ICDDR,B.

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A comparison between interferon gamma release assays and the tuberculin skin test in the contact tracing of patients with chronic kidney disease

We welcome the recent guidelines from the British Thoracic Society on the management of *Mycobacterium tuberculosis* infection and disease in patients with chronic kidney disease (CKD).¹ We note the paucity of evidence (particularly from the UK) in this population regarding the use of interferon gamma release assays (IGRA) in screening patients for latent tuberculosis infection (LTBI).

We present data to show the first UK-based cohort comparing the tuberculin skin test (TST) and the two commercially available IGRA—T-SPOT.TB (Oxford Immunotec, Abingdon, UK) and Quantiferon-Gold-in-Tube (Cellestis, Carnegie, Australia)—in a population of inpatients with CKD. It involves the follow-up of 61 patients from a renal inpatient ward who were screened for LTBI following exposure to a staff member with smear-negative, culture-positive pulmonary tuberculosis in 2008.

Overall Results

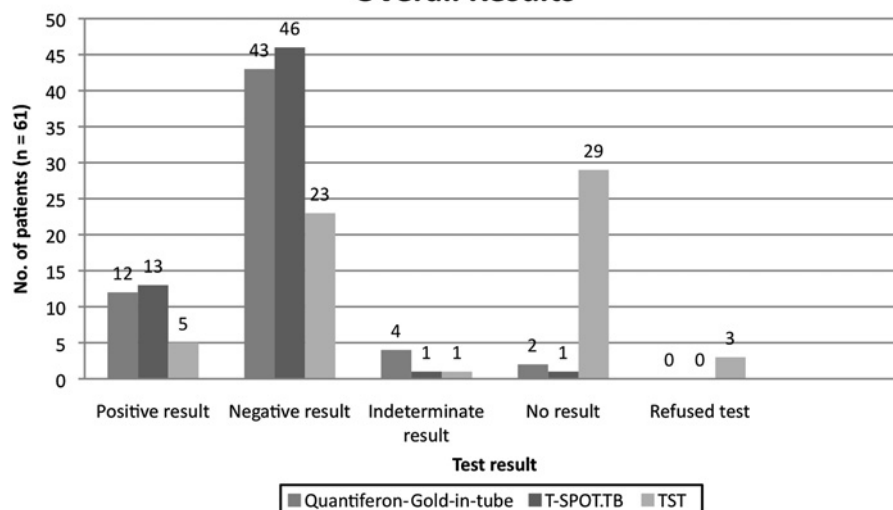


Figure 1 Overall results comparing TST, T.SPOT.TB and Quantiferon-Gold-in-tube across the whole cohort.

The mean age of the cohort was 62 years (range 28–88). Thirty patients were male. Eight patients were of Afro-Caribbean ethnicity, three middle eastern, one south-east Asian, 31 Caucasian and 18 from the Indian subcontinent. Forty-five patients were receiving haemodialysis; three patients had CKD stages II–IV; one patient had acute kidney injury; 12 patients were post-renal transplant; 29.5% were positive for at least one of the three tests; none of these had evidence of active disease. The results of each test are shown diagrammatically in figure 1. Of note, 48% of patients did not receive a completed TST despite standard follow-up by experienced tuberculosis nurses; 19.6% of the cohort had a positive Quantiferon-Gold-in-Tube test, 21.3% of the cohort had a positive T-SPOT.TB test and 8.1% of the cohort had a positive TST (≥ 15 mm if *Bacillus Calmette–Guérin* vaccinated; ≥ 5 mm if not). There were no significant associations between age, gender, diagnosis, or ethnicity and the likelihood of TST completion.

Twenty-five patients had all three tests performed. Of this group, four patients had a positive TST, five patients had a positive Quantiferon-Gold-In-Tube and eight patients had a positive T-SPOT.TB (see supplementary table 1 and supplementary figure 2, available online only). χ^2 tests (using Fisher's exact methods) were used to calculate associations between test modalities in this group: (1) T-SPOT.TB/Quantiferon-Gold-in-Tube: $\kappa=0.694$; $p<0.002$; (2) T-SPOT.TB/TST: $\kappa=0.364$; $p=0.08$; (3) Quantiferon-Gold-in-Tube/TST: $\kappa=0.324$; $p=0.17$.

When repeating the above analysis in the whole cohort ($n=61$), using positive test versus non-positive test as comparators, similarly significant associations were observed (see supplementary table 2, available online only).

On multivariate analysis, there were no significant associations with any of the three tests and gender, age, ethnic background or mode of renal replacement therapy. Length of exposure to the index case had no effect on the test results. (Mann–Whitney U test, $p>0.1$ for all three tests, comparing the median number of 'exposed' shifts for those with a positive test and those with a negative test).

In conclusion, these data support the growing body of evidence that IGRA appear more sensitive and accurate than the TST in detecting LTBI in this immunosuppressed group of patients.^{2–4} Importantly, they also provide evidence of the clinical utility of IGRA in contact tracing, and the difficulty of performing the TST, in large numbers of patients undergoing dialysis.

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