LETTERS

Bronchoalveolar lavage immunodiagnosis for tuberculosis suspects in Europe and Africa

We read with interest the article by Dheda *et al*¹ who followed our approach for a rapid diagnosis of smear-negative tuberculosis by bronchoalveolar lavage (BAL) enzyme-linked immunospot (ELISpot)² in a country of high tuberculosis incidence, including individuals with HIV-1 infection.

The authors report a sensitivity of 88.9% and specificity of 94.7% of the BAL ELISpot (T-SPOT.TB test) for the diagnosis of tuberculosis in suspects with scarce or negative acid-fast bacilli (AFB) sputum smears. This observation is important, as it confirms the findings of other recent studies performed in low tuberculosis incidence countries where flow cytometric assays were performed with BAL cells in order to obtain a rapid diagnosis of tuberculosis.^{3–4} However, flow cytometry is technically more demanding and time-consuming than ELISpot.

Results from the largest study performed on this topic to date, a recent prospective multicentre TBNET study, showed that the BAL ELISpot is superior to blood ELISpot, tuberculin skin test and *Mycobacterium tuberculosis*-specific nucleic acid amplification to diagnose sputum smear-negative tuberculosis.⁵

However, an important difference between this study and that of Dheda *et al* is the high frequency of indeterminate BAL ELISpot test results (9.2% vs 33.7%) that could be related to different cell processing procedures. Fifty-four percent of indeterminate results in the cohort from South Africa were due to lack of sufficient numbers of cells or failure of the positive control, interestingly unrelated to the patients' HIV serostatus. In 46.4% of the South African cohort and 82.1% of the European cohort the reason for indeterminate results was a high number of cells already producing interferon γ (IFN γ) without stimulation in the negative control. These are probably prestimulated terminally differentiated, cytokine-secreting effector T cells.

Different definitions of indeterminate test results are another important explanation for the observed variability between the two studies. When we reanalysed the data set of the TBNET study with the cut-offs used by Dheda *et al*, the sensitivity and specificity of the BAL ELISpot for the detection of sputum AFB smear-negative tuberculosis changed from 90.9% and 79.9% to 87.2% and 88.1%, and the frequency of indeterminate test results increased to 30.5%. Therefore, it would be interesting to know whether application of the cut-offs used in the TBNET study will substantially reduce the proportion of indeterminate test results in the study by Dheda *et al*.

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Authors' response

We thank Lange and colleagues for their insightful comments about our data.¹ In our study, one-third of the bronchoalveolar lavage (BAL) enzyme-linked immunospot (ELISpot) test results were indeterminate.¹ Lange *et al* pose the question of whether the number of indeterminate results could be reduced by redefining the cut-off point used for the analysis.

There were 28/83 indeterminate results (33.7%), of which less than half (13/28 or 46.4%) were due to high spot counts in the negative control well. When we reanalysed

the data with the cut-off point used by Lange and colleagues,² four additional subjects had valid results. On reanalysis the sensitivity remained unchanged and the specificity was marginally reduced from 93.75% (95% CI 79.85 to 98.27) to 91.67% (95% CI 78.17 to 97.13). Many of the high spot counts in the negative control well were not close to the cut-off point. In our original analysis we were not able to reduce the number of indeterminate results without significantly compromising the sensitivity when changing the cut-off point of the negative control. Furthermore, in most cases there was little difference between the counts in the negative control and antigen-specific wells, suggesting an effect of terminally differentiated effector cells rather than one attributed to antigen-specific cells.

Nevertheless, we found that 53.6% (15/28) of our indeterminate results were due to failure of the positive control. We showed that using staphylococcal enterotoxin B (SEB), in addition to phytohaemagglutinin (PHA), substantially reduced failure of the positive control (25–3%; $p=0.02$). We estimate that if SEB was used as a positive control throughout the study then the proportion of inconclusive RD-1 ELISpot results would have dropped from 34% to 25%. We therefore recommend that SEB and PHA be used as positive controls in the BAL ELISpot assay.

In addition to the selection of cut-off points, the variable performance (sensitivity and specificity) of these assays are to be expected given the differences in methodological and technical aspects (skills of the bronchoscopist, lavage technique and the BAL processing protocol), tuberculosis case definitions (culture confirmation alone vs a clinical definition for tuberculosis) and the populations studied.^{3–4} What both studies indicate, however, is that a BAL ELISpot would approximately double the yield of a rapid positive diagnosis over a smear alone. This additive value makes the test clinically promising. Further studies refining the assay and validating the cut-off points used in different settings are now required.

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Exercise-induced bronchoconstriction and exercise testing in an international rugby union team

Exercise-induced bronchoconstriction (EIB) is an acute, transient airway narrowing that occurs during or after exercise, defined as a $\geq 10\%$ decline in forced expiratory volume in 1 s (FEV₁) after exercise.¹ Exercise-induced fatigue or dyspnoea due to EIB are often incorrectly attributed to deconditioning.² In elite athletes, EIB has a prevalence of 7–50%.³

The prevalence of EIB in rugby union players has not been reported despite the sport's popularity, with >2 million players worldwide. We developed a rugby-specific exercise protocol and questionnaire to measure the prevalence of asthma/EIB in all players in the Irish Senior Rugby squad who attended preseason training.

The exercise protocol differed from regular field or laboratory-based testing, reflecting the type of exertion experienced by elite rugby players where whole-body musculature is recruited.⁴ The combination of sport-specific manoeuvres and sprinting with a 4 kg exercise ball was designed to provoke 8 min of hyperpnoea (as per field testing guidelines). Pre-exercise and postexercise spirometric data were measured using a calibrated, computerised, pneumotachograph spirometer. Exertion was quantified using rates of perceived exertion, in-test heart rate and serum lactate levels (for additional methodology see supplementary material online). Players were grouped into two cohorts; an airflow obstruction group (AOG) included players with a previous diagnosis of asthma/EIB or spirometric airflow obstruction, and a non-airflow obstruction group (NAOG) included players with no history of asthma/EIB and normal spirometry.

Forty-two players were assessed with comparable levels of exertion in both groups (table 1). Twelve players (29%) demonstrated baseline airflow obstruction. The group consisted of seven players previously diagnosed with asthma/EIB who used their regular inhaled treatment at the time of testing (salbutamol (n=7), salmeterol/fluticasone combination (n=3) and salbutamol,

Table 1 Player anthropometry, levels of exertion (heart rate and levels of perceived exertion) and spirometry

Characteristics	All (n=42)	NAOG (n=30)	AOG (n=12)	
Mean (range) (SD)				
Age (years)	26.5 (20–33) (± 2.8)	26.1 (20–33) (± 2.7)	27.6 (23–30) (± 3.1)	
Height (m)	1.86 (1.72–1.98) (± 0.06)	1.87 (1.77–1.96) (± 0.06)	1.83 (1.72–1.98) (± 0.06)	
Weight (kg)	99 (73–116) (± 10.9)	101 (83–116) (± 11.2)	95 (73–116) (± 9.5)	
Heart rate (bpm)	175 (156–195) (± 9)	174 (156–195) (± 9)	176 (162–192) (± 9.1)	
Perceived exertion	15.6 (14–18) (± 0.9)	15.5 (14–17) (± 0.9)	15.8 (15–18) (± 0.9)	
Lactate	11.3 (5.7–16.2) (± 2.3)	11.3 (8.1–16.2) (± 2.1)	11.2 (5.7–15.8) (± 2.3)	
Symptoms				p Value*
Wheezing	7 (17%)	2 (7%)	5 (42%)	0.006
Woken by dyspnoea	4 (10%)	0	4 (33%)	0.001
Attack of dyspnoea	5 (12%)	1 (3%)	4 (33%)	0.001
Atopy	6 (10%)	4 (13%)	2 (17%)	0.78
Pneumonia	4 (10%)	2 (7%)	2 (17%)	0.318
Dyspnoea postexercise	8 (19%)	3 (10%)	5 (42%)	0.015
Cough postexercise	13 (31%)	6 (20%)	7 (58%)	0.047
Spirometry; litres $\pm$ SD (% predicted $\pm$ SD)				
FEV ₁ pre-exercise	4.73 $\pm$ 0.73 (100 $\pm$ 12.63)	4.8 $\pm$ 0.62 (100 $\pm$ 11.8)	4.53 $\pm$ 0.96 (98 $\pm$ 14.9)	
FEV ₁ postexercise	4.66 $\pm$ 0.79 (98 $\pm$ 14.1)	4.86 $\pm$ 0.62 (101 $\pm$ 12.3)	4.14 $\pm$ 0.93 (90 $\pm$ 15.7)	
FVC pre-exercise	5.88 $\pm$ 0.79 (104 $\pm$ 10.5)	5.79 $\pm$ 1.04 (105 $\pm$ 11.2)	5.92 $\pm$ 0.68 (102 $\pm$ 10.4)	
FVC postexercise	5.81 $\pm$ 0.82 (102 $\pm$ 10.8)	5.52 $\pm$ 1.06 (100 $\pm$ 11.7)	5.93 $\pm$ 0.68 (103 $\pm$ 10.6)	

*Pearson χ^2 test.

AOG, airflow obstruction group; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; NAOG, non-airflow obstruction group.

salmeterol/fluticasone and montelukast (n=1). In this group, four (57%) had a >10% drop in FEV₁ after exercise challenge, despite regular therapy. Three additional players who had a positive exercise challenge test had a previous diagnosis of asthma but no longer took regular inhaled treatment. One of these had spirometric airflow obstruction before testing and a second had a strongly positive response to exercise challenge (FEV₁ decreased 18%). Two further athletes with no previous history of asthma/EIB were positive after exercise challenge.

Wheeze was reported by 42% (n=5) of the AOG and 7% (n=2) of the NAOG (p=0.006). Exercise-increased dyspnoea (42% vs 10%; p=0.015) and cough (58% vs 20%; p=0.047) were reported in the AOG versus the NAOG (table 1).

Asthma/EIB is common in professional rugby players (29% vs 12–15% of the general population),⁵ often occurring despite standard treatments. Exercise performance poorly reflects airflow obstruction. Wheeze, being woken from sleep by dyspnoea and cough postexercise are important symptoms in rugby players which, if present, warrant further investigation. The high prevalence of asthma/EIB in this study supports routine testing in professional rugby union players. We propose a sport-specific screening challenge that is acceptable to players/medical staff and compliant with World Anti-Doping Authority testing criteria. Spirometry with reversibility and/or inhalation challenge may prove useful where exercise challenge testing is non-diagnostic but players' symptoms suggest asthma/EIB.

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