LETTERS

Bronchoalveolar lavage immunodiagnosis for tuberculosis suspects in Europe and Africa

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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 (IFNγ) 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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