Clinical characteristics that portend a positive Xpert Ultra test result in patients with pleural tuberculosis

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Background. The performance of Xpert-MTB/RIF, including the newer Xpert Ultra test, for the diagnosis of pleural TB is poor (~28 - 38%). There are no data on patient characteristics that portend a positive Xpert-Ultra test in pleural fluid. These characteristics could be useful for selecting patients for Xpert-Ultra testing, thus maximising benefits of a positive test while minimising cost.

Objective. To determine the clinical, radiological, microbiological and biochemical characteristics associated with Xpert-Ultra positivity in patients with suspected pleural tuberculosis.

Methods. We performed a subgroup analysis of a prospective observational cohort (N=165 patients with suspected pleural TB) evaluating same-day diagnostic tools for pleural tuberculosis. Forty-nine patients with confirmed pleural tuberculosis (culture and/or histology) were included in the final analysis.

Results. Of the 49 participants, 17 (35%) were female and 9 (18.4%) were HIV-infected. In the multivariate analysis including demographic, radiological and pleural fluid test characteristics, there were no independent predictors of Xpert-Ultra positivity. However, when pleural fluid test results were excluded, and when only rapidly ascertainable pre-test factors (demographic and radiologic variables) were considered, the multivariable analysis showed that only a chest X-ray (CXR) suggestive of active TB (cavities, consolidation and hilar lymphadenopathy) was associated with Xpert-Ultra positivity (p=0.021). Notably, only 22% (n=11/49) of the participants had a CXR suggestive of active TB and of these, 73% (n=8/11) had a positive Xpert-Ultra result.

Conclusion. CXR features suggestive of active TB are significantly associated with a positive Xpert-Ultra test result on pleural fluid. These data inform clinical practice in resource-poor settings.

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as at least one positive *M. tuberculosis* culture (pleural fluid and/or biopsy) and/or caseating granulomatous inflammation suggestive of TB on histological examination of pleural biopsy tissue, and with improvement on anti-TB treatment (all patients with definite TB received anti-TB treatment). There were 49 patients with definite TB and all of them were included in this subgroup analysis.

**Demographic characteristics**
Clinical information (age, sex, HIV status and CD4 count) was extracted from the research database and patient source documents. Previous TB was reported by the patient and in most cases corroborated by medical records.

**Pleural fluid tests**
Microbiological (Z-N stain, pleural fluid culture and time to positive culture (TTP)), biochemical (pleural fluid protein and adenosine deaminase (ADA)) and Xpert Ultra tests were performed.

**Radiological characteristics**
For the purpose of this study, effusions occupying less than one-third, one-third to two-thirds and greater than two-thirds of hemithorax were characterised as small, moderate and large, respectively, on PA films. Presence of cavities, consolidation and hilar lymphadenopathy on CXR were defined as features suggestive of active TB. CXR records between 2012 and 2018 were retrieved from the iSite Enterprise electronic system while the plain CXRs done between 2009 and 2012 were retrieved from medical records.

**Statistical analysis**
As the objective was to ascertain the predictive value of Xpert Ultra in those patients with pleural TB, the analysis was restricted to 49 definite TB cases. Medians and interquartile ranges (IQRs) were calculated for continuous variables and frequency (percent) for categorical variables. Comparisons of continuous variables were performed using Wilcoxon rank sum tests while $\chi^2$ tests were used for categorical variables. Logistic regression analysis included variables that were significantly associated with the Xpert Ultra result. A significance value of $p < 0.05$ was used. Statistical analyses were done in Stata version 13 (StataCorp., USA).

**Results**

**Univariate and multivariate analysis using all variables** Overall, 35% of our study population was composed of females, while 18.4% of the participants had HIV co-infection. Individual patients’ characteristics and their association with the Xpert ultra test have been summarised in Table 1. Pleural fluid tests such as pleural fluid protein ($p=0.01$) and ADA ($p=0.001$) showed individual predictive powers for a positive Xpert Ultra result. Furthermore, pleural effusion size

| Variables | Ultra-negative (N=30), n (%) | Ultra-positive (N=19), n (%) | $p$-value |
|-----------|-----------------------------|-----------------------------|-----------|
| Gender    |                             |                             |           |
| Male      | 18 (60)                     | 14 (73.7)                   | 0.33      |
| Female    | 12 (40)                     | 5 (26.3)                    |           |
| Age (mean), years | 45.6                     | 38.6                       | 0.14      |
| Previous TB |                             |                             |           |
| Yes       | 5 (16.7)                    | 4 (21)                      | 0.70      |
| No        | 25 (83.3)                   | 15 (79)                     |           |
| HIV status |                             |                             | 0.25      |
| Positive  | 4 (13.3)                    | 5 (26.3)                    |           |
| Negative  | 26 (86.7)                   | 14 (73.7)                   |           |
| CD4 count (mean), cells/µL | 115.5                    | 182.2                      | 0.19      |
| CXR suggestive of TB |                             |                             | 0.01      |
| Yes       | 3 (10)                      | 8 (42)                      |           |
| No        | 27 (90)                     | 11 (58)                     |           |
| Pleural effusion size |                             |                             | 0.06      |
| Small     | 12 (40)                     | 9 (47.4)                    |           |
| Moderate  | 15 (50)                     | 4 (21.5)                    |           |
| Large     | 3 (10)                      | 6 (31.6)                    |           |
| TTP (days) |                             |                             | 0.09      |
| Mean pleural fluid protein, g/L | 54.9                      | 63.5                       |           |
| Mean adenosine deaminase, U/L | 48.5                      | 88.5                       | 0.001     |
| Pleural fluid TB culture |                             |                             | 0.71      |
| Positive  | 22 (73.3)                   | 13 (68.4)                   |           |
| Negative  | 8 (26.7)                    | 6 (31.6)                    |           |
| Pleural fluid lymphocytes, $\times 10^9$ | 2.5                        | 7.4                        | 0.17      |

CXR = chest X-ray; TB= tuberculosis; TTP = time to positive culture.

*Unless otherwise specified.*
(0.06) and time to positive culture (p=0.09) showed a trend towards significance. In a model including all variables with p<0.1, individual characteristics lost significance on multivariate analysis as shown in Table 2. ADA was the only variable that showed a trend towards significance (p=0.08).

**Univariate and multivariate analysis of immediately ascertainable pre-test variables (demographic and radiological variables)**

In the univariate analysis, CXR suggestive of active TB was the only rapidly ascertainable variable that was predictive of a positive Xpert Ultra result (p=0.01). More importantly, in the multivariate analysis, CXR suggestive of active TB (p=0.021) was associated with a positive Xpert Ultra result (Table 3).

**Xpert Ultra results in association with CXR findings**

More than a quarter of the patients (27.3%; n=11/49) had a CXR suggestive of active TB. Of these, 72.7% (n=8/11) had a positive Xpert Ultra result (Table 4). Similarly, 66.7% (n=6/9) patients with a large pleural effusion size had a positive Xpert Ultra result (Table 1).

**Discussion**

We evaluated clinical characteristics that predict a positive Xpert Ultra test result in pleural fluid. The key finding of this study was that CXR was an independent predictor of Xpert Ultra positivity when taking into account immediately ascertainable pre-test variables. This has obvious implications for selection of tests and costs.

In the multivariate analysis using immediately ascertainable factors, CXR was the only independent predictor of Xpert positivity. On reflection, this finding is perhaps not surprising, given that patients with radiological features of active TB are likely to have parenchymal lung involvement. Indeed, 72.7% of patients in our study with features suggestive of active TB had a positive Xpert Ultra result. It is important to note that only 27.3% of patients had such CXR characteristics, thus limiting the utility of this approach. However, looking at this from a different angle would suggest that in a quarter of patients, this strategy may still be useful by allowing cost-effective usage of Xpert Ultra in TB-endemic countries. Indeed, in many countries such as Malawi and Mozambique, Xpert Ultra is not readily available and is used selectively. This approach will be very useful in such settings. To the best of our knowledge, this is the first time that a readily available investigation such as a CXR, often available prior to pleural fluid aspiration and testing, has been shown to predict Xpert ultra positivity on pleural fluid.

| OR | p-value | 95% CI |
|----|---------|--------|
| CXR suggestive of TB | 12.33 | 0.22 | 0.22 - 681.31 |
| Pleural effusion size | 0.34 | 0.16 | 0.07 - 1.54 |
| TTP | 1.00 | 0.98 | 0.88 - 1.13 |
| Pleural fluid protein | 1.12 | 0.19 | 0.94 - 1.33 |
| Adenosine deaminase | 1.06 | 0.08 | 0.99 - 1.13 |

**Table 2. Multivariate analysis incorporating demographic, radiological and pleural fluid test characteristics (biochemical and microbiological tests): Model 1**

**Table 3. Multivariate analysis incorporating only immediately ascertainable (demographic and radiological) characteristics: Model 2**

In the univariate model using all the characteristics (including immediately ascertainable factors and pleural fluid test results), pleural effusion size (p=0.06) and time to positive culture (p=0.09) showed a trend towards significance. Patients with a large pleural effusion were more likely to test positive for Xpert Ultra, suggesting a larger burden of disease compared with those patients with small or moderate effusion size. Similarly, time to positive culture (TTP), which is a surrogate of mycobacterial load, was shorter in those with positive Xpert Ultra compared to those who had a negative test (18.8 days vs. 23 days, respectively). Therefore, patients with a higher disease burden (borne out of a lower TTP and large pleural effusion size) were more likely to test positive for Xpert Ultra.

When all characteristics with p<0.1 were included in a multivariate model, individual characteristics lost their significance. This was surprising given that pleural fluid ADA and pleural fluid protein are well recognised for their role in facilitating a TB diagnosis. However, in our model, ADA only showed a trend towards significance (p=0.08). This might be an effect of our small sample size. In any event, waiting for an ADA result (not rapidly ascertainable prior to any pleural fluid testing) will result in unnecessary delay and will require aspiration and diagnostic testing in its own right, which is also resource-consuming.

**Table 4. Xpert Ultra result in participants with a CXR suggestive of active TB**

In the univariate analysis, CXR suggestive of active TB was the only rapidly ascertainable variable that was predictive of a positive Xpert Ultra result (p=0.01). More importantly, in the multivariate analysis, CXR suggestive of active TB (p=0.021) was associated with a positive Xpert Ultra result (Table 3).

**Discussion**

We evaluated clinical characteristics that predict a positive Xpert Ultra test result in pleural fluid. The key finding of this study was that CXR was an independent predictor of Xpert Ultra positivity when taking into account immediately ascertainable pre-test variables. This has obvious implications for selection of tests and costs.

In the multivariate analysis using immediately ascertainable factors, CXR was the only independent predictor of Xpert positivity. On reflection, this finding is perhaps not surprising, given that patients with radiological features of active TB are likely to have parenchymal lung involvement. Indeed, 72.7% of patients in our study with features suggestive of active TB had a positive Xpert Ultra result. It is important to note that only 27.3% of patients had such CXR characteristics, thus limiting the utility of this approach. However, looking at this from a different angle would suggest that in a quarter of patients, this strategy may still be useful by allowing cost-effective usage of Xpert Ultra in TB-endemic countries. Indeed, in many countries such as Malawi and Mozambique, Xpert Ultra is not readily available and is used selectively. This approach will be very useful in such settings. To the best of our knowledge, this is the first time that a readily available investigation such as a CXR, often available prior to pleural fluid aspiration and testing, has been shown to predict Xpert ultra positivity on pleural fluid.

| OR | p-value | 95% CI |
|----|---------|--------|
| CXR suggestive of TB | 4.48 | 0.021 | 1.25 - 16.04 |
| Pleural effusion size | 1.64 | 0.20 | 0.77 - 3.49 |

**Table 3. Multivariate analysis incorporating only immediately ascertainable (demographic and radiological) characteristics: Model 2**

Thus, when all characteristics with p<0.1 were included in a multivariate model, individual characteristics lost their significance. This was surprising given that pleural fluid ADA and pleural fluid protein are well recognised for their role in facilitating a TB diagnosis. However, in our model, ADA only showed a trend towards significance (p=0.08). This might be an effect of our small sample size. In any event, waiting for an ADA result (not rapidly ascertainable prior to any pleural fluid testing) will result in unnecessary delay and will require aspiration and diagnostic testing in its own right, which is also resource-consuming.

HIV status was not significantly associated with a positive Xpert Ultra result. Given the impaired cellular immunity in HIV-infected participants, one would expect a higher disease burden and hence a higher likelihood of a positive test. This was not the case in
our study. In a study on extrapulmonary TB in Durban, South Africa, Gounden et al.\(^3\) showed that HIV co-infection was a common risk factor for extrapulmonary TB, suggesting impaired cellular immunity as an explanation for the higher rate in HIV-infected individuals. One of the reasons why HIV was not associated with a positive Xpert Ultra result could have been the low proportion of the HIV-infected participants (18.4%) in our study compared with the Gounden cohort (88%).

**Study limitations**

The present study had a number of limitations. Firstly, the sample size was small. However, recruiting 165 patients for pleural biopsy is one of the largest studies evaluating same-day diagnostic tools for the diagnosis of pleural TB. It is challenging to carry out a large study but clearly this will need to be undertaken. Secondly, clinical variables studied were limited. However, this was a retrospective analysis and a future prospective study could collect variables such as presence of systemic lymphadenopathy, fever and history of positive TB contact. These pre-test variables might be important in determining selection of patients for Xpert Ultra testing.

**Conclusion**

CXR features suggestive of active TB are significantly associated with a positive Xpert Ultra test result on pleural fluid. These data inform clinical practice in resource-poor settings.

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**Author contributions.** EM and HM prepared the first draft and final manuscript, MF and MD analysed data. RM prepared the dataset used for this sub-study. AE edited the first draft and final manuscript. KD conceptualised the sub-study and approved the final manuscript for submission.

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