Development of a clinical prediction rule for the diagnosis of pleural tuberculosis in Peru

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\begin{abstract}
Objectives: To develop a clinical prediction rule (CPR) for the diagnosis of pleural tuberculosis (PT) in patients with pleural exudates in Peru.

Methods: Clinical and laboratory information was collected from patients with exudative pleural effusion attending two reference hospitals in Lima, Peru. Predictive findings associated with PT in a multiple logistic regression model were used to develop the CPR. A definite diagnosis of PT was based on a composite reference standard including bacteriological and/or histological analysis of pleural fluid and pleural biopsy specimens.

Results: A total of 238 patients were included in the analysis, of whom 176 had PT. Age, sex, previous contact with a TB patient, presence of lymphadenopathy, and pleural adenosine deaminase (ADA) levels were found to be independently associated with PT. These predictive findings were used to construct a CPR, for which the area under the receiver operating characteristics curve (AUC) was 0.92. The single best cut-off point was a score of \( \geq 60 \) points, which had a sensitivity of 88\%, specificity of 92\%, a positive likelihood ratio of 10.9, and a negative likelihood ratio of 0.13.

Conclusions: The CPR is accurate for the diagnosis of PT and could be useful for treatment initiation while avoiding pleural biopsy. A prospective evaluation is needed before its implementation in different settings.

\end{abstract}

Introduction

Pleural tuberculosis (PT) is one of the most common forms of extrapulmonary TB and a common cause of exudative pleural effusion in developing countries (Sharma and Mohan, 2004). The differential diagnosis of exudative pleural effusion includes several conditions, the most important one being neoplasms with pleural involvement (Porcel and Vives, 2003). Bacteriological studies of pleural fluid to confirm the presence of \textit{Mycobacterium tuberculosis} have low sensitivity (Gopi et al., 2007), and even molecular tests are only helpful in a small group of patients (Denkinger et al., 2014). Consequently, reaching a definite diagnosis in many cases requires performing an invasive procedure such as pleural biopsy or thoracoscopy for histopathological examination. However, access to these procedures is frequently restricted, particularly in settings with constrained resources and a high incidence of TB. Moreover, these procedures are not exempt from complications such as bleeding or pneumothorax, which, even if infrequent, can have serious consequences (Cao et al., 2015).

In some high incidence settings, clinical features and pleural fluid analyses are the only available tools for decision-making. In alignment with this, guidelines have been issued to standardize the use of laboratory tests. The World Health Organization (WHO) proposes ‘coagulation ability of the pleural fluid’ and ‘presence of mononuclear predominance in pleural fluid’ as parameters suggesting a diagnosis of PT when assessing a pleural exudate (WHO, 2007). Locally, Peruvian guidelines do not propose a structured approach, but instead only consider microbiological procedures as criteria for the diagnosis of PT (MINSA, 2013). Although currently not endorsed by the WHO, the use of adenosine deaminase (ADA) for the diagnosis of extrapulmonary TB and
specifically for PT is supported by several meta-analyses (Morisson and Neves, 2008; Liang et al., 2008; Greco et al., 2003; Goto et al., 2003).

Several clinical prediction rules (CPRs) or scores (tools that use simple clinical and/or laboratory findings to assess the likelihood of a condition or to inform a clinical decision) (Laupacis et al., 1997) have been proposed to guide the diagnosis of PT in different settings and populations, but most of all in low-incidence countries (Porcel and Vives, 2003; Antonangelo et al., 2007; Burgess et al., 1996; De Oliveira et al., 1994; Demirer et al., 2012; Ghanie et al., 2004; Neves et al., 2007; Porcel et al., 2008). In Latin America, the only country in which CPRs have been developed is Brazil (De Oliveira et al., 1994; Neves et al., 2007). Considering the importance of the setting for the strength of particular predictive findings, it would be useful to develop additional CPRs in other high-incidence Latin American countries, in order to compare the predictive findings included and their diagnostic performance. These local CPRs could constitute an adequate tool to support clinical decision-making. The objective of this study was to derive a CPR for the diagnosis of PT in patients with pleural exudates in Lima, Peru.

Methods

Patients

Clinical information was obtained from patients attending Hospital Nacional Cayetano Heredia and Hospital Nacional Hipolito Unanue, two reference hospitals in Lima, Peru, a country with a TB incidence of 119/100,000 (http://www.who.int/tb/publications) (WHO, 2016) and a concentrated HIV epidemic. All adult patients with pleural effusion diagnosed through chest X-ray or ultrasound, attending the two hospitals between October 2009 and February 2012, were screened using Light’s criteria for pleural exudates (Light et al., 1972). Those meeting the criteria for pleural exudates were referred to the pulmonology units for inclusion in the study until the required sample size was attained.

After giving informed consent, demographic and clinical information was collected through direct interviews and physical examination by certified physicians. Standard blood tests were performed. Trained pulmonologists performed a thoracentesis and a blind closed pleural biopsy with an Abrams needle according to recommended procedures (McLeod et al., 1989). Cell count and differential, glucose, lactate dehydrogenase (LDH), protein, and ADA levels (using the Giusti method) in the pleural fluid were determined.

In addition, Ziehl–Neelsen acid-fast bacillus (AFB) staining, Gram staining, and cultures for mycobacteria in Ogawa medium and Mycobacterial Growth Indicator Tube (MGIT) medium, as well as PCR for M. tuberculosis and cytology for malignant cells were performed. The pleural biopsy was sent for histopathological examination and read by certified pathologists.

The required sample size was 259 participants based on an estimated accuracy of 80% for the clinical prediction rule, a precision of 5%, and a confidence level of 95%, and allowing for up to 5% of missing data.

Reference standard

The diagnosis of PT was based on a composite reference standard consisting of the following elements: positive AFB or culture for M. tuberculosis in any of the culture media or positive PCR for M. tuberculosis (all of these in pleural fluid) or presence of caseating granulomas in the histopathology of the pleural biopsy, or histopathological findings compatible with TB (fibrosis, lymphocytic infiltration, unspecific granulomas) followed by complete resolution of the effusion and all of the clinical symptoms within the 3 months after initiating anti-TB therapy.

Malignant pleuritis was defined by the presence of malignant cells in the pleural fluid or biopsy. Other causes of pleural exudate diagnosed through immunohistochemistry or cultures included connective tissue diseases and bacterial and fungal infections. Patients for whom neither the pleural fluid analysis, nor the pleural biopsy, nor specific complementary tests allowed a diagnosis to be reached within 3 months of follow-up, were considered to have ‘pleural exudates of unknown cause’ and were excluded from the analysis. Diagnostic thoracoscopy could not be performed.

Development of the prediction rule

The aim was to differentiate patients with PT from those with other pathologies. Predictive findings that were identified as relevant in previously published clinical prediction rules for the diagnosis of PT, or that had a strong association with TB in general, were used (Porcel and Vives, 2003; Antonangelo et al., 2007; Burgess et al., 1996; De Oliveira et al., 1994; Demirer et al., 2012; Ghanie et al., 2004; Neves et al., 2007; Porcel et al., 2008). Those following were selected as relevant predictive anamnestic and clinical findings for evaluation: age (in years), sex, having had previous contact with a patient with TB (defined as living in the same dwelling or working in the same area as a person with a diagnosis of TB during the last 2 years), disease duration (duration of symptoms in days), and presence of fever (as reported by the patient, not necessarily confirmed at the examination), night sweats, haemoptysis, and lymphadenopathy (presence of lymph nodes at any site, observed by the patient or detected during physical examination). The laboratory predictive findings that were withheld were the white blood cell count, pleural protein, pleura/serum protein ratio, pleural LDH, percentage of lymphocytes in pleural cells, and pleural ADA.

Univariate logistic regression was performed to assess the association of the predictor variables with PT. Those predictors found to be associated with an odds ratio (OR) > 2 or < 0.5 and/or a p-value of < 0.10 were retained and used to develop a multiple logistic regression model. Variables with the highest p-values were dropped from the full model in a backward fashion until only variables with a p-value of < 0.05 were kept. A bootstrapped linear regression of the expected individual PT probabilities (based on the final logistic regression model) on the withheld variables was then performed. The beta coefficients for the predictors in this linear regression model were multiplied by 100 and rounded to the nearest integer to assign the specific number of points for each predictor in the final score.

The objective for defining the cut-off point of the score was to reach a positive likelihood ratio (LR) of ≥ 10 or a negative LR of ≤ 0.1. These values are considered adequate for diagnostic purposes (Deeks, 2004). If no single cut-off point fulfilled this objective, three categories were to be defined: low likelihood of PT for those scores with a negative LR of ≤ 0.1, high likelihood of PT for those scores with a LR of ≥ 10, and intermediate likelihood for the scores in between.

The sensitivity, specificity, predictive values, and LRs of the score were calculated for the selected cut-off point(s). Additionally a receiver operating characteristics (ROC) curve analysis was used, and the area under the ROC curve (AUC) for the score was compared to that for pleural ADA alone. All calculations were performed using STATA version 11 for Windows (StataCorp., College Station, TX, USA).

Written informed consent was obtained from all participants. The study was approved by the ethics committee of Universidad Peruana Cayetano Heredia, Hospital Nacional Cayetano Heredia,
Table 1
Clinical and laboratory predictive findings and their association with pleural tuberculosis in the univariate and multiple logistic regression models; Lima, Peru.

<table>
<thead>
<tr>
<th>Predictive findings</th>
<th>Patients (n = 176)</th>
<th>Other conditions (n = 62)</th>
<th>Univariate logistic regression OR (95% CI) p-Value</th>
<th>Final multiple logistic regression model OR (95% CI) p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
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<tr>
<td>Mean age, years (95% CI)</td>
<td>37.1 (34.4–39.8)</td>
<td>61.9 (56.6–64.6)</td>
<td>0.94 (0.92–0.96) &lt;0.001</td>
<td>0.96 (0.94–0.98) &lt;0.001</td>
</tr>
<tr>
<td>Mean disease duration, days (95% CI)</td>
<td>35 (33–37)</td>
<td>77 (74–82)</td>
<td>0.90 (0.85–0.95) &lt;0.001</td>
<td>Not withheld in the final model</td>
</tr>
<tr>
<td>Fever % (95% CI)</td>
<td>81 (74–85)</td>
<td>45 (32–58)</td>
<td>5.07 (2.71–9.47) &lt;0.001</td>
<td>Not withheld in the final model</td>
</tr>
<tr>
<td>Male % (95% CI)</td>
<td>70 (66–80)</td>
<td>45 (32–58)</td>
<td>2.50 (1.60–5.25) &lt;0.001</td>
<td>2.77 (1.16–6.67) 0.02</td>
</tr>
<tr>
<td>Contact with a patient with TB % (95% CI)</td>
<td>50 (42–58)</td>
<td>23 (13–32)</td>
<td>3.43 (1.76–6.66) &lt;0.001</td>
<td>3.25 (1.30–8.07) 0.01</td>
</tr>
<tr>
<td>Night sweats % (95% CI)</td>
<td>60 (53–68)</td>
<td>34 (22–47)</td>
<td>2.69 (1.61–5.42) &lt;0.001</td>
<td>Not withheld in the final model</td>
</tr>
<tr>
<td>Haemoptysis % (95% CI)</td>
<td>7 (4–12)</td>
<td>15 (7–26)</td>
<td>0.43 (0.17–1.08) &lt;0.07</td>
<td>Not withheld in the final model</td>
</tr>
<tr>
<td>Lymphadenopathy % (95% CI)</td>
<td>16 (11–22)</td>
<td>32 (21–45)</td>
<td>0.40 (0.20–0.78) &lt;0.01</td>
<td>0.18 (0.06–0.56) 0.003</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean WBC count in blood, × 10^9/l (95% CI)</td>
<td>7.750 (7.267–8.232)</td>
<td>8.525 (7.642–9.409)</td>
<td>0.99 (0.99–1.00) 0.11</td>
<td>Not considered for inclusion in the model</td>
</tr>
<tr>
<td>Mean proteins in pleural fluid, g/dl (95% CI)</td>
<td>5.16 (5.03–5.29)</td>
<td>4.16 (3.83–4.49)</td>
<td>2.53 (1.79–3.37) &lt;0.001</td>
<td>Not considered for inclusion in the model</td>
</tr>
<tr>
<td>Pleural/serum protein ratio (95% CI)</td>
<td>0.58 (0.53–0.62)</td>
<td>0.61 (0.54–0.68)</td>
<td>0.67 (0.24–1.89) 0.49</td>
<td>Not considered for inclusion in the model</td>
</tr>
<tr>
<td>Mean LDH in pleural fluid, g/dl (95% CI)</td>
<td>880.2 (809.1–951.4)</td>
<td>866.9 (553.2–1180.6)</td>
<td>1.00 (1.00–1.00) 0.90</td>
<td>Not considered for inclusion in the model</td>
</tr>
<tr>
<td>Mean lymphocyte proportion in pleural fluid (95% CI)</td>
<td>63 (58–68)</td>
<td>67 (61–74)</td>
<td>0.66 (0.25–1.72) 0.40</td>
<td>Not considered for inclusion in the model</td>
</tr>
<tr>
<td>Mean ADA in pleural fluid, IU/l (95% CI)</td>
<td>79.2 (73.7–84.6)</td>
<td>28.0 (19.9–36.1)</td>
<td>1.06 (1.04–1.07) &lt;0.001</td>
<td>1.05 (1.03–1.06) &lt;0.001</td>
</tr>
</tbody>
</table>

TB, tuberculosis; OR, odds ratio; CI, confidence interval; WBC, white blood cell; LDH, lactate dehydrogenase; ADA, adenosine deaminase.

Hospital Nacional Hipolito Unanue, and the Institute of Tropical Medicine, Antwerp.

Results

Three hundred and eighty-three patients were eligible for inclusion in the study. Of these, 101 did not consent to participate and a further 41 were excluded: 21 because a definite diagnosis could not be reached, 12 had empyema, and eight had incomplete data for analysis. Three had concomitant evidence of TB and malignancy. Of the 238 patients eventually included in the analysis, 176 had a diagnosis of PT. One hundred and forty were confirmed through microbiology or the presence of caseating granulomas on histopathology and 36 were diagnosed through histopathological findings compatible with TB followed by complete resolution of the symptoms after treatment. Sixty-two had other causes of pleural exudates, out of which 50 were neoplastic, seven were associated with connective tissue diseases, three had a bacterial aetiology, and two had a fungal origin.

The mean age of the 238 included patients was 43.6 years (95% confidence interval (CI) 40.9–46.3 years). One hundred and fifty-two (64%) were male, 28 (12%) had a previous history of TB, and 102 (43%) reported having had contact with a patient with TB. The mean duration of symptoms was 46.3 days (95% CI 39.0–53.6 days). Fourteen (6%) of the tested patients were infected with HIV (12 patients refused testing).

The univariate logistic regression analysis found that age, sex, contact with a patient with TB, duration of illness, fever, night sweats, hemoptysis, lymphadenopathy, ADA, and proteins in the pleural fluid were associated with PT at a p-value of <0.10 (Table 1). After developing the multiple logistic regression model, only age, sex, contact with a TB patient, lymphadenopathy, and pleural ADA remained significantly and independently associated with PT at a p-value of <0.05. The corresponding ORs and individual p-values are shown in Table 1.

Table 2 shows the final CPR and the number of points assigned to each predictive finding.

Figure 1 shows the scores that patients with PT and those with other conditions obtained with the application of this CPR.

Based on the ROC curve analysis, the best single cut-off point for the score was ≥60 points and for ADA was ≥30 IU/l (Figure 2). This cut-off point for the score had an overall accuracy (patients correctly classified) of 89.1%, a sensitivity of 88.1% (95% CI 82.3–92.5%), a specificity of 91.9% (95% CI 82.2–97.3%), a positive

![Figure 1](image-url)
Among the variables included, male sex and contact with a TB patient were positively associated with PT. Age had a negative association with PT, as found in many other studies (Porcel and Vives, 2003; Antonangelo et al., 2007; Demirer et al., 2012): PT was more likely to be present in younger patients and conversely malignancy was more frequent in older patients. Lymphadenopathy was also found to be negatively associated with PT, as it was found more often in cases of pleural effusion associated with neoplastic metastatic disease with lung and lymphatic involvement.

Of note, ADA activity was the only pleural fluid analysis, and even laboratory finding, associated with PT. Pleural protein and in particular the percentage of lymphocytes in pleural cells, which is a predictive finding included in several CPRs (Sales et al., 2009), was not independently associated with PT in the present study patients. Since M. tuberculosis-induced lymphocytic activation is the main mechanism for ADA generation, the predictive ability of lymphocytes may be limited when ADA levels have already been taken into consideration. With respect to the protein levels, this study population consisted of patients with pleural exudates, so its discriminatory power was not expected to be high. Currently, one of the few available recommendations on tuberculous pleuritis suggests strongly taking into account the results of pleural fluid analyses when determining the diagnosis (Light, 2010).

It is also of note that this CPR performed better than ADA alone (7% more sensitive and 5% more specific). This is in contrast with earlier findings (Solari et al., 2017), possibly because the present study made comparisons using a locally derived CPR instead of an external one. The former perform better than those developed in different epidemiological settings, as the strength of association between the predictive findings and PT varies.

A limitation of this study is the quite high proportion of patients who could not be included due to refusal of the pleural biopsy. In addition, the possible contribution of molecular tools as potential predictive findings, in particular of GeneXpert and interferon gamma release assays (IGRAs), was not evaluated. In principle, CPRs should only include results from anamnesis, physical examination, and simple tests, and both of these tests are considered high complexity ones by the food and drug administration clinical laboratory improvement amendments FDA-CLIA (FDA, 2018). Their availability in hospitals in resource-constrained settings such as ours is very limited. Furthermore, evidence on their utility for PT is still being debated. Recent papers have failed to provide conclusive evidence concerning the diagnostic accuracy of GeneXpert for PT (Denkinger et al., 2014), and a systematic review showed suboptimal performance of IGRAs in the diagnosis of PT (Aggarwal et al., 2015). More recently, the measurement of serum and pleural interleukins such as IL-31 (Gao et al., 2016) has shown sensitivities and specificities over 90% for the diagnosis of pleural TB, but once more these are high complexity tests that need further evaluation.

In conclusion, the CPR derived in this study is a useful tool for the diagnosis of PT. As is the case for every CPR, it requires further validation before being implemented, particularly in different settings. In Peru, it could be used immediately as a tool for decision-making in patients with pleural exudates and would allow the management of such patients to be standardized, avoiding invasive procedures, preventing their complications, and saving resources.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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