



Hyporexia and cellular/biochemical characteristics of pleural fluid as predictive variables on a model for pleural tuberculosis diagnosis

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ABSTRACT

Objectives: Pleural tuberculosis (PITB) diagnosis is a challenge due to its paucibacillary nature and to the need of invasive procedures. This study aimed to identify easily available variables and build a predictive model for PITB diagnosis which may allow earlier and affordable alternative strategy to be used in basic health care units.

Methods: An observational cross-sectional study compared PITB and non-TB patients followed at a tertiary Brazilian hospital between 2010 and 2018. Unconditional logistic regression analysis was performed and a Decision Tree Classifier (DTC) model was validated and applied in additional PITB patients with empiric diagnosis. The accuracy (Acc), sensitivity (Se), specificity (Sp), positive and negative predictive values were calculated. **Results:** From 1,135 TB patients, 160 were considered for analysis (111 confirmed PITB and 49 unconfirmed PITB). Indeed, 58 non-TB patients were enrolled as controls. Hyporexia [adjusted *odds ratio* (aOR) 27.39 (95% CI 6.26 – 119.89)] and cellular/biochemical characteristics on pleural fluid (PF) (polimorphonuclear in two categories: 3-14% aOR 26.22, 95% CI 7.11 – 96.68 and < 3% aOR 28.67, 95% CI 5.51 – 149.25; and protein \geq 5g/dL aOR 7.24, 95% CI 3.07 – 17.11) were associated with higher risk for TB. The DTC constructed using these variables showed Acc=87.6%, Se=89.2%, Sp=84.5% for PITB diagnosis and was successfully applied in unconfirmed PITB patients.

Conclusion: The DTC model showed an excellent performance for PITB diagnosis and can be considered as an alternative diagnostic strategy by using clinical patterns in association with PF cellular/biochemical characteristics, which were affordable and easily performed in basic health care units.

Keywords: Pleural tuberculosis; Diagnosis; Decision tree classifier.

INTRODUCTION

Tuberculosis (TB) is a major public health problem worldwide.⁽¹⁾ Pleural tuberculosis (PITB) is the commonest extrapulmonary TB presentation, representing 42% of all extrapulmonary cases.^(2,3) In addition to its frequency, PITB diagnosis can be a challenge due to the paucibacillary nature of patients' biological samples and to the need for invasive procedures.⁽⁴⁾

The Ziehl Neelsen (ZN) stain and the mycobacterial culture of pleural fluid (PF) and/or pleural tissue are the gold standard methods for tuberculous pleural effusion diagnosis, but their success rates are relatively poor.⁽⁵⁾ The identification of granuloma in histopathological examination of the pleural tissue is also considered as diagnostic criteria.⁽⁶⁾ However, besides being invasive, the pleural biopsy is operator-dependent, relatively expensive, may be limited by clinical contraindications and

is associated with complications.⁽⁷⁻⁹⁾ Adenosine deaminase (ADA) measurement in pleural fluid can provide a putative diagnosis of PITB in high prevalence settings, considering that high ADA levels can also be observed in other infectious, inflammatory or malignant diseases.^(6,10,11) In addition, the evaluation of biomarkers on pleural effusion configures an alternative for TB diagnosis.⁽¹²⁻¹⁴⁾ Recently, our research group have shown that interferon-gamma (IFN- γ) was an excellent rule-in and rule-out test compared to other two biomarkers (IFN- γ inducible protein 10 kD and ADA) and that the combination of IFN- γ and ADA, in a reviewed cut-off point, showed to be particularly useful to PITB confirmation.⁽¹⁵⁾ However, these methods can be available only at reference centers, whereas basic health care units cannot count on them.

In order to surpass the limitations stated forementioned, which compels the practice of a not uncommon empiric diagnosis based on clinical and radiological criteria, as

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previously published,⁽¹⁶⁻¹⁹⁾ our research group proposed the application of conventional biochemical and cellular parameters of pleural fluid, which lacks specificity when isolated considered,⁽⁴⁾ but when in combination with each-other or with clinical features could discriminate between tuberculous pleural effusion or not. For this purpose, a predictive model for PITB diagnosis was validated by us based on hyporexia, polymorphonuclear cells (PMN cells) and protein levels on pleural fluid, which was applied on empiric PITB cases to perform an internal validation.

METHODS

Study design

A cross-sectional study was conducted based on medical records of patients with pleural effusion under investigation attended between January 2010 and January 2018 at Pedro Ernesto University Hospital from Rio de Janeiro State University (*Hospital Universitário Pedro Ernesto/Universidade do Estado do Rio de Janeiro* (HUPE/UERJ)), in the city of Rio de Janeiro (RJ), Brazil. Patients at least 18 years and who attended the Outpatient Clinic of Tuberculosis of HUPE with PITB diagnosis were included. Those patients whose TB treatment outcomes were unknown (loss of follow-up) were excluded. Patients with non-TB pleural effusion were considered controls and were drawn from the Outpatient Clinic of Pleural Diseases of the same hospital.

The ethics committee approved the study of the institution under protocol #2612/2010.

Diagnostic criteria

PITB was stratified as follows: i) *Confirmed PITB (C-PITB)*: ZN stain positive or isolation of *Mycobacterium tuberculosis* on the respiratory specimen, pleural fluid or pleural tissue, or identification of granuloma on histopathological analysis, or patients with clinical manifestations of PITB and a lymphocytic and exudative pleural effusion with ADA dosage above 40 IU/L that fully recover after at least six months of antituberculosis treatment; ii) *Unconfirmed PITB (UC-PITB)*: cases with clinical manifestations of PITB that did not fulfill the C-PITB and fully recovered after at least six months of antituberculosis treatment.

Non-tuberculosis (Non-TB): patients were defined as those with pleural or pleuropulmonary diseases other than TB in which the diagnose was concluded based on clinical, laboratory, radiological, microbiological, or cytopathological/histopathological features.

Data collection

The medical records of all patients were reviewed in order to evaluate physical, clinical, and demographic information, medical history, and laboratory data. According to subjective reported presence and duration, signs and symptoms such as cough, fever, chest pain, dyspnea, night sweats, hyporexia, and weight

loss were included. The Human Immunodeficiency Virus (HIV) status and other comorbidities were also recorded. Data on PF routine diagnostic tests, including a chemistry panel, total and differential cell count, ADA measurement, cytopathology, and microbiological analysis (ZN and solid media culture) were registered. In those cases where pleural biopsy with Cope's needle was performed, histopathology analysis, ZN stain, and mycobacterial culture results were registered.

Statistical analysis

For statistical analysis, categorical and continuous data were presented in frequency (percentage) and median and interquartile range (IQR), respectively. Fisher Exact Test and T-test were used for group comparisons. Simple and multiple unconditional logistic regression analyses were performed, and *odds ratios* (OR) and adjusted OR (aOR) with its 95% Confidence Interval (95% CI) were calculated. The level of significance used was 0.05.

Decision Trees Classifier (DTC) was selected as a predictive model because it allows a straightforward and easy interpretation of the rules, and it was built with an implementation of the Quinlan's C4.5 algorithm available in the packages 'rpart' version 4.1-10 for the open-source software R version 3.3.1.⁽²⁰⁾ The DTC was built with cases of C-PITB and non-TB in a subset of nine [viral hepatitis, fever, dyspnea, hyporexia, weight loss, percentage of PMN cells, percentage of mononuclear cells (MN cells), protein and albumin on pleural fluid] of 22 variables pre-selected (age, diabetes mellitus, renal failure, cardiac failure, cancer, previous transplant, viral hepatitis, autoimmune disease, immunosuppressive treatment, fever, cough, chest pain, dyspnea, weight loss, hyporexia, night sweating, chest X-ray, percentage of PMN cells, percentage of MN cells, protein and albumin on pleural fluid) by successively eliminating at least 20% important variables (with importance as returned from random forest) using the out-of-bag error as minimization criterion from random forest (number of trees equal to 1,000) classifiers implemented in the R package 'varSelRF' version 0.7-5. The fitted tree was pruned to minimize the expected 10-fold cross-validation prediction accuracy. Pruning included a complexity parameter of 0.25, informing the algorithm that any split that does not improve the fit by 25% will likely be pruned off by 10-fold cross-validation. Hence, the algorithm needs not to pursue it. In addition, the performance of the built DTC was estimated by its leave-one-out cross-validation accuracy (Acc), sensitivity (Se), specificity (Sp), Positive Predictive Value (PPV) and Negative Predictive Value (NPV), and false-positive and negative ratios with 95% CI and area under the Receiver Operating Characteristics (ROC) and the Area Under the Curve (AUC).

RESULTS

From January 2010 to January 2018, 1,135 patients were diagnosed with active TB at HUPE/UERJ. Of these, 397 had an extrapulmonary presentation of TB, and

212 (53%) had PITB, being 160 patients considered for analysis. The other 52 patients were excluded according to the exclusion criteria. The 58 non-TB cases included 34 malignancies (16 adenocarcinomas, four lymphomas, two carcinomas, one spindle epithelial, and 11 non-specified cell types), 11 renal/cardiac failures, four empyema, two systemic lupus erythematosus, one hepatic disease, one chylothorax and five cases of undefined pleural effusion (Figure 1).

The sociodemographic and clinical characteristics from all patients are shown in Table 1. PITB patients were younger than non-TB and were less likely to present other comorbidities such as cancer, cardiac failure, viral hepatitis, and previous transplant. In addition, fever, chest pain, hyporexia, weight loss, and night sweats were more frequently observed in PITB cases and higher levels of ADA measurement, MN cells frequency, total protein, and albumin levels, and reduced PMN cells frequency. Confirmed and unconfirmed PITB were homogeneous unless for age (Table 1). Compared to non-TB, younger patients presented a higher risk for TB diagnosis (30 – 44 years: OR 4.6, 95% CI 1.63 – 12.99; 18 – 29 years: OR 6.16, 95% CI 1.79 – 21.16). This association persisted in multiple unconditional logistic analyses adjusting for gender, comorbidities, signs/symptoms, radiological appearance and cytological, and biochemical pleural fluid analyses (Table 2).

Fever, chest pain, weight loss, and hyporexia were the signs/symptoms considered risk factors for confirmed PITB. Of these, hyporexia showed the highest aOR [27.39 (95% CI 6.26 – 119.89)]. Cytological

characteristics on PF showed a reverse behavior once a reduced frequency of PMN cells has exhibited a greater chance of being diagnosed with PITB (PMN cells 3 – 14%: aOR 8.78, 95% CI 3.35-22.97 and PMN cells < 3%: aOR 28.67, 95% CI 5.51-149.25) and the higher the percentage range of MN cells the greater the chance of PITB diagnosis (MN cells 85 – 97.5%: aOR 9.04, 95% CI 3.34 – 24.42 and MN cells \geq 97.5%: aOR 33.67, 95% CI 6.26 – 181.01). Protein levels above 5g/dL were also identified as a risk factor for the infectious disease (aOR 7.24, 95% CI 3.07 – 17.11) (Table 2).

From twenty-two pre-selected variables, our model chose nine to DTC building, which has included viral hepatitis, fever, dyspnea, hyporexia, weight loss, percentage of PMN cells, percentage of MN cells, protein, and albumin on PF. By the end of the analysis, the pruned DTC used only three predictive variables for PITB discrimination: PMN cells and protein in PF and hyporexia.

Figure 2 depicts the DTC analysis built to discriminate confirmed PITB from non-TB patients. Based on three variables' results (presence of hyporexia, PMN cells levels, and protein levels, both on PF), in 21 cases (12 PITB and nine non-TB), the diagnostic classification of the DTC was mistaken. In these cases, the results of microbiological tests, pleural tissue biopsy, and ADA dosage were of extreme importance for differential diagnosis.

With an area under the ROC curve of 88.7%, the DTC proved to be 90.23% accurate with a Se of 92.47% (95% CI 88.99-95.95%) and a Sp of 87.6% (95% CI

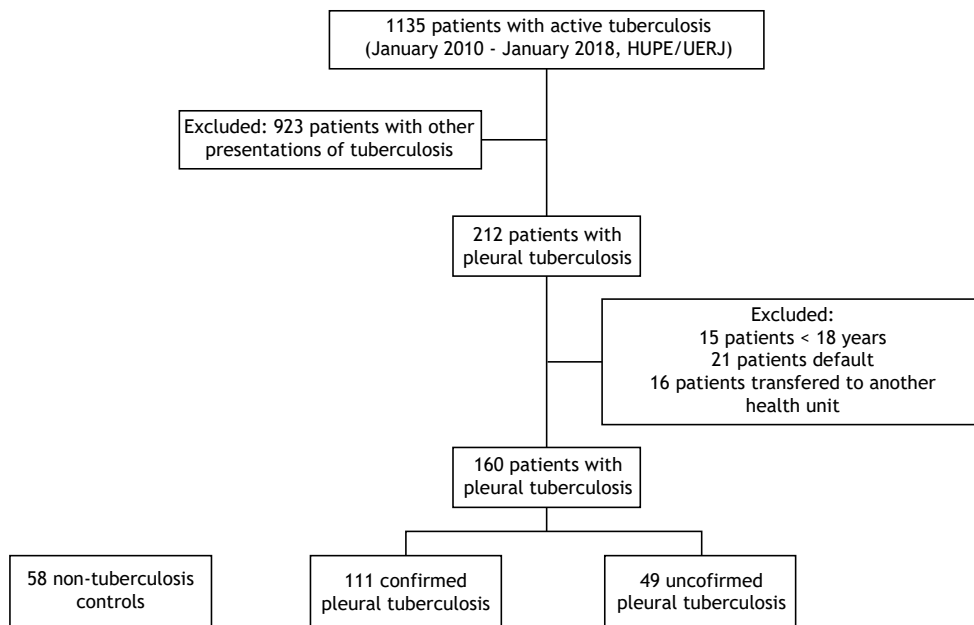


Figure 1. Flowchart of the study design. Pleural tuberculosis according to the eligibility criteria and non-TB cases considered as controls.

Table 1. Baseline characteristics of the study population. Univariate analysis was performed comparing PITB and non-TB groups. Also confirmed and unconfirmed PITB groups were compared.

	PITB			<i>p</i>	
	Non-TB	Confirmed	Unconfirmed	NTB vs TB	C-PITB vs NC-PITB
	(58) N (%)	(111) N (%)	(49) N (%)		
Gender, %					
Male	32 (55.2)	64 (57.7)	30 (61.2)		
Female	26 (44.8)	47 (42.3)	19 (38.8)	0.64	0.73
Age, years					
Median (IQR)	62 (49-73)	43 (30-53)	32 (25-48)	< 0.0001	0.004
HIV status, %					
Positive	2 (3.4)	1 (0.9)	3 (6.1)		
Negative	37 (63.8)	66 (59.5)	29 (59.2)		
Refuse testing	-	2 (1.8)	1 (2.0)	0.68	0.26
Unknown	19 (32.8)	42 (37.8)	16 (32.7)		
Previous comorbidities, %					
Arterial Hypertension	17 (29.3)	25 (22.5)	6 (12.2)	0.27	0.28
Diabetes mellitus	8 (13.8)	9 (8.1)	2 (4.1)	0.29	0.27
Chronic Renal Failure	4 (6.9)	4 (3.6)	2 (4.1)	0.59	0.83
Cancer	10 (17.2)	2 (1.8)	1 (2.0)	< 0.0001	0.83
Cardiac Failure	7 (12.1)	-	1 (2.0)	< 0.0001	0.26
COPD / Asthma	2 (3.4)	1 (0.9)	-	0.27	0.67
Previous transplant	4 (6.9)	-	1 (2.0)	0.02	0.26
Autoimmune disease	2 (3.4)	6 (5.4)	3 (6.1)	0.79	0.82
Viral hepatitis	5 (8.6)	-	1 (2.0)	0.006	0.26
Immunosuppressive therapy	5 (8.6)	5 (4.5)	6 (12.2)	0.91	0.07
Signs/symptoms, %					
Fever	13 (22.4)	83 (74.8)	36 (73.5)	< 0.0001	0.63
Cough	29 (50)	65 (58.6)	30 (61.2)	0.41	0.54
Chest pain	25 (43.1)	71 (64.0)	26 (53.1)	0.04	0.41
Dyspnea	43 (74.1)	66 (59.5)	28 (57.1)	0.09	0.47
Hyporexia	2 (3.4)	57 (51.4)	22 (44.9)	< 0.0001	0.56
Weight loss	20 (34.5)	69 (62.2)	35 (71.4)	< 0.0001	0.13
Night sweats	10 (17.2)	58 (52.3)	22 (44.9)	< 0.0001	0.53
Duration of signs/symptoms, Days					
Median (IQR)	75 (30-180)	30 (20-60)	30 (17-90)	0.001	0.94
PF Characteristics					
Median (IQR)					
Total cell count, mm ³	1135 (500-2600)	2305 (795-3875)	2435 (1300-3800)	0.72	0.65
Mononuclear (MN), %	78 (56-90)	95 (90-98)	85 (70-95)	< 0.0001	0.44
Polimorphonuclear (PMN), %	20 (10-41)	5 (2-10)	15 (5-30)	< 0.0001	0.43
Total protein, g/dL	4.2 (3.6-5.3)	5.5 (5.0-5.9)	5.5 (5.1-6.3)	< 0.0001	0.44
Albumin, g/dL	2.6 (2.0-3.1)	2.9 (2.6-3.2)	2.9 (2.9-3.3)	0.006	0.19
LDH, UI/L	210 (137-697)	411 (256-780)	513 (324-727)	0.25	0.73
ADA, IU/L					
Median (IQR)	39 (24.5-74.5)	71.5 (46.5-93.2)	-	0.008	-
Chest Xray					
UPE	41 (70.7)	73 (65.8)	32 (65.3)		
BPE	9 (15.5)	3 (2.7)	1 (2.0)		
UPE + Pulmonary infiltrates	6 (10.3)	28 (25.2)	15 (30.6)	0.001	0.78
BPE + Pulmonary infiltrates	1 (1.7)	1 (0.9)	-		
Missing data	1 (1.7)	6 (5.4)	1 (2.0)		

Table 2. Analysis of clinical, radiographic and laboratorial features in multiple unconditional logistic regression models for pleural tuberculosis.

Features	Odds Ratio (95%CI)	Adjusted Odds Ratio (95% CI)	p
Gender			
Male	1	1	
Female	0.9 (0.48-1.71)	1.09 (0.54-2.21)	0.80
Age			
60 - 74 years	1	1	
≥ 75 years	0.29 (0.08-1.06)	0.2 (0.05-0.82)	0.02
45 - 59 years	1.66 (0.68-4.04)	1.25 (0.47-3.34)	0.65
30 - 44 years	4.6 (1.63-12.99)	5.02 (1.53-16.54)	0.008
18 - 29 years	6.16 (1.79-21.16)	6.31 (1.54-25.93)	0.01
Comorbidities			
Diabetes Mellitus	0.55 (0.2-1.5)	0.53 (0.18-1.56)	0.25
Cancer	0.09 (0.02-0.41)	0.07 (0.01-0.36)	0.001
Autoimmune disease	1.59 (0.31-8.12)	1.39 (0.27-7.23)	0.70
Renal failure	0.5 (0.12-2.08)	0.52 (0.11-2.5)	0.41
Immunosuppressive therapy	0.5 (0.14-1.79)	0.4 (0.11-1.47)	0.16
Fever	11.66 (5.37-25.3)	10.43 (4.62-23.58)	< 0.0001
Cough	0.7 (0.36-1.36)	0.67 (0.32-1.37)	0.27
Chest pain	2.51 (1.28-4.9)	3.32 (1.57-6.99)	0.002
Dyspnea	2.23 (1.05-4.71)	2.03 (0.89-4.61)	0.09
Weight loss	3.18 (1.61-6.25)	3.13 (1.51-6.49)	0.002
Hyporexia	30.83 (7.14-133.07)	27.39 (6.26-119.89)	< 0.0001
Night sweats	5.44 (2.48-11.92)	7.07 (2.87-17.43)	< 0.0001
Polimorphonuclear (PMN) % in PF			
≥ 15%	1	1	
3 - 14%	7.31 (3.1-17.2)	8.78 (3.35-22.97)	< 0.0001
< 3%	29.23 (6-142.5)	28.67 (5.51-149.25)	0.0001
Missing data	55.54 (11.73-262.99)	58.45 (11.34-301.32)	< 0.0001
Mononuclear (MN) % in PF			
< 85%	1	1	
85 - 97.4%	7.31 (3.02-17.67)	9.04 (3.34-24.42)	< 0.0001
≥ 97.5%	34 (6.76-171.04)	33.67 (6.26-181.01)	< 0.0001
Missing data	64.6 (13.21-315.85)	66.61 (12.65-350.84)	< 0.0001
Total protein in PF (g/dL)			
< 5.0	1	1	
≥ 5.0	6.32 (2.91-13.7)	7.24 (3.07-17.11)	< 0.0001
Missing data	86.32 (11.03-675.53)	69.69 (8.76-554.25)	0.0001

Table 3. Performance characteristics of the decision tree analysis for confirmed and unconfirmed PITB cases.

	Confirmed Pleural Tuberculosis (95% CI)	Unconfirmed Pleural Tuberculosis (95% CI)
Accuracy	87.6 (84.4 - 90.7)	88.8 (84.8 - 92.8)
Sensitivity	89.2 (85.5 - 92.8)	93.9 (89.4 - 98.4)
Specificity	84.5 (78.5 - 90.4)	84.5 (78.2 - 90.7)
Positive Predictive Value (PPV)	91.7 (88.4 - 95)	83.6 (77.1 - 90.2)
Negative Predictive Value (NPV)	80.3 (74 - 86.7)	94.2 (90 - 98.5)
Area under ROC curve	88.7	90.1

CI: Confidence Interval; ROC: Receiver Operating Characteristic.

84.4-90.7) (Table 3). The performance of the DTC applied for unconfirmed PITB classification to perform an internal validation was 88.8% accurate, 93.9% sensitive, and 84.5% specific (Table 3). Noteworthy,

almost 90% of the cases would be correctly treated by applying the DTC as a diagnostic tool and using laboratory and clinical variables, which can be afforded and performed in a basic care health system.

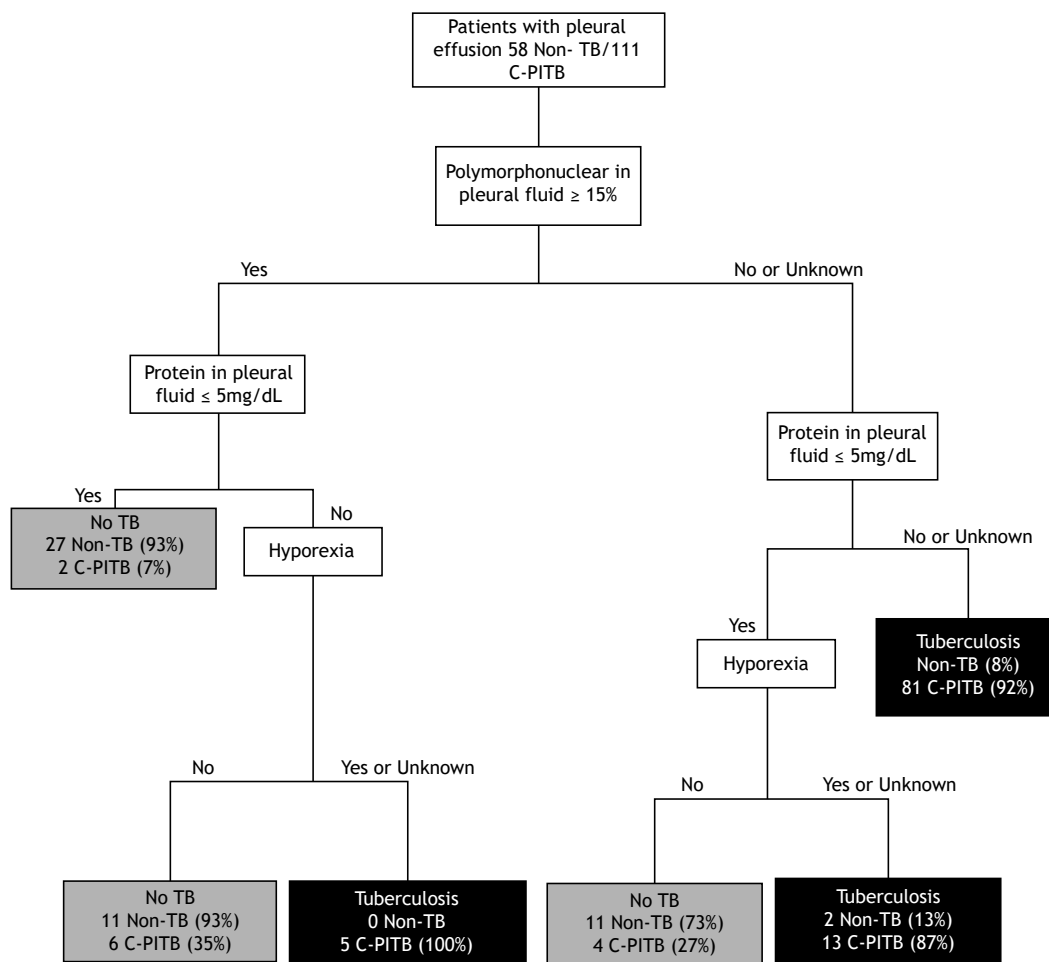


Figure 2. Discrimination between confirmed pleural tuberculosis and non-TB cases according to the decision tree classifier. Graphical representation of a decision tree classifier where the terminal branches filled gray were classified as non-TB and filled black terminal branches were classified as TB. The numbers inside the branches are the original diagnosis and their false positive results.

DISCUSSION

The challenge of differential diagnosis of tuberculous pleural effusion among a range of etiologies encouraged a great number of studies that employed efforts to develop tools for differentiating PITB from other entities using scoring systems, DTC, and artificial intelligence neural networks.⁽²¹⁻²⁷⁾ Our DTC behaved as well as them, with excellent accuracy. Besides that, we consider our model, with only three predictive variables, and applied to a broad spectrum of patients, easier to use in clinical practice. Moreover, the DTC presented here was validated using an independent sample of patients, leading to almost 90% of cases being correctly classified.

In this study, a DTC model was built with excellent accuracy for discriminating PITB in an area with a high incidence of TB. The performance of the DTC was similar when either applied on C-PITB or unconfirmed PITB cases, with sensitivity higher than 89%, for both

groups. Noteworthy, the sensitivity of the proposed DTC model is much higher than either microbiological or histopathological analyses of pleural specimens for TB diagnosis.^(5,28) Moreover, the decision tree presented here can also be considered an alternative to ADA dosage, given the various performances of ADA for PITB diagnosis^(11,29,30) and the lack of availability of the test in some scenarios. In addition, physicians would be more confident to initiate anti TB therapy in cases empirically diagnosed using this DTC in scenarios without other diagnostic methods available.

To the best of our knowledge, there are only two studies published that have proposed DTCs that could be used in clinical practice.^(26,27) In 2008, a similar model was used to discriminate between tuberculous and malignant pleural effusions based on four parameters: age > 35 years, ADA > 38 IU/L, temperature, and DHL on pleural fluid.⁽²⁶⁾ This model was 92.2% sensitive and 98.3% specific, and the validation using an

independent sample showed a sensitivity of 85% and a specificity of 97%. Even though TB and cancer are the two most frequent causes of exudative pleural effusions,⁽³¹⁾ other etiologies must be discarded during pleural diseases diagnosis. In our study, among the 58 non-TB patients, 41.4% had other etiologies than cancer. Valdés *et al.*⁽²⁷⁾ proposed a DTC to classify PF as tuberculous or non-tuberculous. The first proposed model included pleural fluid lymphocyte count > 31.5% and ADA > 35 IU/L with a mean accuracy of 99%. Then, to be applied in health care settings without the availability of ADA, a second model, including PF lymphocyte count > 31.5%, fever, and cough, showed to be less accurate than the first one. Only patients under 40 years old were included in that study, whereas our study population had patients from 18 to 89 years and more than 50% were older than 45.

Our statistical model identified PMN cells percentage instead of MN cells included in the DTC, although lymphocytic pleural effusions are most typical among tuberculous pleural fluid analyses.⁽⁹⁾ However, in many cases, PMN rather than MN are the predominant cell type in the pleural effusion of TB cases, especially during the earlier phases of the pleural inflammatory process, as shown by Jeon *et al.*⁽⁹⁾ According to Lyadova,⁽³²⁾ neutrophils are probably the least understood among immune cell populations, playing a dual role during TB pathophysiology. It is already known that these cells participate in the acquired immunity and granuloma formation and may kill *Mtb*. Notwithstanding this characteristic, at the same time, neutrophils could support mycobacterial growth and have been implicated in the transition from infection to active TB, mediate tissue destruction, disease severity, and progression.⁽³²⁾ However, recently published studies⁽³³⁻³⁶⁾ showed that TB disease alters the neutrophil population, leading to the accumulation of heterogeneous subsets of immature and activated dysfunctional cells with a decline in true neutrophils.

Based on the observations in the present study, it would be relevant not to reduce the importance of lymphocytes but to bring out the role of neutrophils in diagnosing TB. In our study, 13 confirmed PITB cases (13/111; 12%) had a frequency of neutrophils higher than 15%. Lin *et al.*⁽³⁷⁾ showed that among 354 tuberculous pleural effusion, 39 (11%) presented a PMN predominance in the pleural fluid. Interestingly, these patients presented a high mortality rate and a high risk for transmission.

Moreover, in our study, the addition of total protein levels of pleural fluid on the DTC contributed to correct discrimination of PITB, considering that according to Choi *et al.*,⁽³⁸⁾ PITB with predominant PMN in PF showed a more intense inflammatory response with higher levels of total protein and albumin. As already indicated, since Light's criteria, an exudative pleural effusion is characteristic of inflammatory pleural diseases, including TB.⁽³⁹⁾ Our findings can be compared to those of Samanta *et al.*⁽³⁰⁾ that showed higher levels

of total protein and albumin on the pleural effusion of TB patients than in lung cancer patients.

All of the most typical signs and symptoms of PITB and other prevalent pleural diseases, namely fever, cough, chest pain, dyspnea, hyporexia, weight loss, and night sweat, were considered in the training of the DTC here proposed. Among them, just hyporexia was preserved in the final model. This symptom also showed the highest aOR for PITB in the multiple unconditional logistic regression and was included in the DTC model (Figure 2). However, a literature search did not find any other publications that identified hyporexia as an essential clinical presentation for PITB diagnosis and other classical signs/symptoms.

There were some limitations in our study, most of which were attributable to the use of the information from a retrospective cohort. We were dependent on documentation and interpretation of data in the medical records. However, data were reported in medical records according to standard questionnaires by the attending physicians of a university institution, where physicians, nurses, and students were trained to document data that could be used for research. In addition, many missing data regarding biochemical and cellular features of PF (40 cases) were identified. Besides that, the absence of data, which can happen in routine, was included in the DTC. Maybe, with more results available, our model could present a higher precision. Also, most non-TB cases had cancer (58.6%), with a minority of cases composed of less frequent pleural conditions such as empyema and autoimmune diseases. Our University Hospital is not a reference center for HIV patients, and this could explain the few cases of co-infected patients. Also, although some readers understand that hyporexia and protein levels in the DTC can be considered an incorporation bias, we comprehend that these isolated variables were not used to confirm or exclude TB diagnosis. Even if this bias could be found, it would not invalidate the results and only overestimate the DTC accuracy. Finally, but not a limitation of our study, but of the DTC model proposed itself, TB prevalence can affect the performance of this model in different scenarios and should be used with caution in low-prevalence settings.

Recently, clinical pathways have been used to optimize patient care for specific clinical conditions. Mummadi and Hahn⁽⁴⁰⁾ published their experience applying what they called the "pleural pathway" in an institution in the United States of America (USA). They concluded that this "pleural pathway" and a centralized pleural service are associated with reducing case charges, inpatient admissions, and length of stay for pleural conditions. We believe that the DTC proposed in this article could be included as a screening test to organize steps of diagnosis without neglecting the possibility of less frequent presentations of tuberculous pleural effusion.

The DTC based on pleural fluid cellular and biochemical characteristics does not replace microbiological tests for TB. Its disadvantage is associated with the failure to provide microbiological confirmation and antituberculosis sensitivity tests. Thus, we still advocate that pleural

fluid and the respiratory specimen should also be obtained whenever possible. Additionally, pathological analysis of pleural fluid and fragment may be indicated for cases with high suspicion of cancer.⁽²⁶⁾ However, the variables selected by this model can be readily available in primary health care units, which cannot count on ADA's dosage for PITB diagnosis on a routine basis.

In conclusion, the DTC model proposed in this study could identify PITB cases based on only three simple predictive variables readily available and collected in basic health care units, with a mean accuracy of almost 90%. ADA, microbiological and pathological analysis of pleural fluid should also be obtained whenever possible. Invasive diagnostic procedures, such as pleural biopsy may be reserved for specific cases considering their risks *versus* benefits.

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AUTHOR CONTRIBUTIONS

APS: idealization, investigation, data collection, writing of the paper. MRA: methodology, statistical analysis, writing of the paper. RC, IL, MAS e JL: data collection, writing of the paper. TTM: investigation, data collection, writing of the paper. LSR and RR: idealization, methodology, statistical analysis, final revision of the paper.

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