Optimising the utility of pleural fluid adenosine deaminase for the diagnosis of adult tuberculous pleural effusion in Hong Kong

KC Chang *, MC Chan, WM Leung, FY Kong, Chloe M Mak, Sammy PL Chen, WC Yu

ABSTRACT

Introduction: Pleural fluid adenosine deaminase level can be applied to rapidly detect tuberculous pleural effusion. We aimed to establish a local diagnostic cut-off value for pleural fluid adenosine deaminase to identify patients with tuberculous pleural effusion, and optimise its utility.

Methods: We retrospectively reviewed the medical records of consecutive adults with pleural fluid adenosine deaminase level measured by the Diazyme commercial kit (Diazyme Laboratories, San Diego [CA], United States) during 1 January to 31 December 2011 in a cluster of public hospitals in Hong Kong. We considered its level alongside early (within 2 weeks) findings in pleural fluid and pleural biopsy, with and without applying Light's criteria in multiple scenarios. For each scenario, we used the receiver operating characteristic curve to identify a diagnostic cut-off value for pleural fluid adenosine deaminase, and estimated its positive and negative predictive values.

Results: A total of 860 medical records were reviewed. Pleural effusion was caused by congestive heart failure, chronic renal failure, or hypoalbuminaemia caused by liver or kidney diseases in 246 (28.6%) patients, malignancy in 198 (23.0%), non-tuberculous infection in 168 (19.5%), tuberculous pleural effusion in 157 (18.3%), and miscellaneous causes in 91 (10.6%). All those with tuberculous pleural effusion had a pleural fluid adenosine deaminase level of ≤100 U/L. When analysis was restricted to 689 patients with pleural fluid adenosine deaminase level of ≤100 U/L and early negative findings for malignancy and non-tuberculous infection in pleural fluid, the positive predictive value was significantly increased and the negative predictive value non-significantly reduced. Using this approach, neither additionally restricting analysis to exudates by Light's criteria nor adding closed pleural biopsy would further enhance predictive values. As such, the diagnostic cut-off value for pleural fluid adenosine deaminase is 26.5 U/L, with a sensitivity of 87.3%, specificity of 93.2%, positive predictive value of 79.2%, negative predictive value of 96.1%, and accuracy of 91.9%. Sex, age, and co-morbidity did not significantly affect prediction of tuberculous pleural effusion using the cut-off value.

Conclusion: We have established a diagnostic cut-off level for pleural fluid adenosine deaminase in the diagnosis of tuberculous pleural effusion by restricting analysis to a level of ≤100 U/L, and considering early pleural fluid findings for malignancy and non-tuberculous infection, but not Light's criteria.

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New knowledge added by this study

- There are limitations to the use of pleural fluid adenosine deaminase (pfADA) level as a surrogate marker for tuberculous pleural effusion (TBPE); thus, it must be interpreted alongside other findings that help exclude non-tuberculous diseases, thereby increasing the pre-test probability of TBPE and the positive predictive value (PPV).
- We demonstrated that TBPE was unlikely when pfADA level was >100 U/L.
- Restricting analysis to patients with pfADA level of ≤100 U/L and early (within 2 weeks) negative findings for malignancy and non-tuberculous infection in pleural fluid significantly increased PPV and non-significantly reduced the negative predictive value (NPV). Using this approach, neither additionally restricting analysis to exudates by Light's criteria nor adding closed pleural biopsy would further enhance predictive values of pfADA for TBPE. As such, the local pfADA diagnostic cut-off value is set at 26.5 U/L, with a sensitivity of 87.3%, specificity of 93.2%, PPV of 79.2%, NPV of 96.1%, and accuracy of 91.9%.

Implications for clinical practice or policy

- Among patients with pfADA level of ≤100 U/L, when pfADA level is ≥26.5 U/L with early negative findings in pleural fluid for malignancy and non-tuberculous infection, it is probably appropriate to manage the patient as a case of TBPE, without additionally performing pleural biopsy (also a surrogate marker for TBPE), but remain vigilant for a 20.8% (1 minus PPV) chance of mistaking non-tuberculous diseases as TBPE.
- When pfADA level is <26.5 U/L with early negative findings in pleural fluid for malignancy and non-tuberculous infection, tuberculosis is highly unlikely, but caution should be exercised because of a 3.9% (1 minus NPV) chance of mistaking TBPE for another disease.
- Other investigations are always indicated when the clinical progress is incompatible with the working diagnosis.

在香港成人病例中，以腺苷脫氨酶水平作診斷結核性胸腔積液的優化研究

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引言：胸腔積液腺苷脫氨酶（pfADA）水平可用於快速診斷結核性胸腔積液。本文旨在估算pfADA在本地用作診斷結核性胸腔積液的截取值，並探討如何優化其效用。

方法：本研究回顧香港一組公立醫院內的病歷紀錄，分析2011年1月1日至12月31日期間，用Diazyme測試劑檢測的pfADA水平。分析中考慮各種不同情景，包括有否使用Light的標準去篩選，以及有沒有考慮胸水和胸膜活檢的早期（2週內）結果。對於每種情景，我們使用ROC曲線來找出pfADA的截取值，並估計其陽性和陰性預測值。

結果：可供研究的病歷紀錄有860份。胸腔積液的病因包括：充血性心臟衰竭、慢性腎功能衰竭、或肝腎疾病導致的低蛋白血症共246例（28.6%）；惡性腫瘤198例（23.0%）；非結核性感染168例（19.5%）；結核性胸腔積液157例（18.3%）；以及其他原因91例（10.6%）。所有結核性胸腔積液的pfADA水平均為≤100 U/L。當分析僅限於pfADA水平為≤100 U/L及早期胸水中沒有惡性腫瘤和非結核性感染的689例時，陽性預測值顯著增加，但陰性預測值未明顯降低。使用同樣方法，即使額外使用Light的標準篩檢，或附加考慮封閉的胸膜活檢，都不能進一步提高預測值。因此，我們估算pfADA的診斷截取值為26.5 U/L，其敏感性為87.3%，特異性93.2%，陽性預測值79.2%，陰性預測值96.1%，準確性91.9%。性別、年齡和共病並沒有明顯影響結核性胸腔積液的預測值。

結論：在毋須使用Light的標準篩選，僅把分析限制在pfADA水平為≤100 U/L及考慮早期胸水結果的情況下，我們找到pfADA的本地診斷截取值。

Introduction

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism, with its primary function in the development and maintenance of the immune system. There are at least two ADA isoforms: ADA1 and ADA2. Whereas ADA1 is found in most body cells (especially lymphocytes and macrophages), ADA2 is predominantly found in the human plasma and serum, and co-exists with ADA1 in macrophages. Absence of ADA1 causes severe combined immunodeficiency. Serum ADA2 level is increased in collagen vascular disease,[1,2] and most cancers.

Many studies have suggested that pleural fluid adenosine deaminase (pfADA) is useful in the diagnosis of tuberculous pleural effusion (TBPE).[3-13] The merits of using pfADA include its low cost, short turnaround time, and high sensitivity and specificity.[3,12] Notwithstanding possibly better sensitivity and specificity for detecting TBPE by combining ADA1 or ADA2 in pleural fluid (PF) with other PF biomarkers such as tumour necrosis factor–alpha, interleukin 27, interferon-gamma and dipeptidyl peptidase IV,[14-17] it may not be cost-effective to combine pfADA with other PF biomarkers.[18] Although ADA2 is predominantly increased in TBPE, and ADA1 is more commonly associated with pleural effusion due to pyogenic bacteria,[19] determination of ADA1 and ADA2 may not provide a diagnostic advantage over the use of total pfADA.[20]

A standardised and automated method (Diazyme commercial kit; Diazyme Laboratories, San Diego [CA], United States) has been developed to determine pfADA activity. The test performance of pfADA has largely been evaluated by including all cases with pleural effusion, and estimating its sensitivity and specificity with reference to an optimal cut-off value. Some studies fine-tuned the test performance by restricting the analysis to subjects with lymphocytic exudates[9,13] or to young adults.[21] In Hong Kong, pfADA has been measured centrally by the Chemical Pathology Laboratory at the Princess Margaret Hospital using the Diazyme commercial kit. In the absence of a diagnostic cut-off value established from local data, pfADA level of ≥30 U/L has been used territory-wide in Hong Kong for detecting TBPE. This is with reference to a retrospective Thai study of 59 (33.1%) patients with TBPE among 178 patients with predominantly exudative lymphocytic pleural effusion.[22] It suggested a sensitivity of 82% and specificity of 91% for pfADA level of ≥30 U/L, as measured by the Diazyme commercial kit.[22] Corresponding estimates of positive predictive value (PPV) and negative predictive value (NPV) were 81.4% and 90.8%, respectively.[22]

Although pfADA rapidly detects TBPE, it is often assessed alongside other tests that include sputum bacteriology for acid-fast bacilli (AFB) and other pathogens, sputum cytology, PF bacteriology for AFB and other pathogens, PF biochemistry, PF cytology, and pleural biopsy. Restricting analysis to patients with exudative pleural effusion may help optimise the utility of pfADA for detecting TBPE. Excluding patients with an early diagnosis of non-tuberculous disease, notably malignancy and non-tuberculous infection, may also help improve the utility of pfADA for TBPE.[5,9,23-26]

Diagnostic test accuracy depends on sensitivity and specificity, which are relatively stable, and pre-test probability that can be enhanced by selecting appropriate patients. In this study, we aimed to optimise its utility by increasing the pre-test probability, and establish a local diagnostic pfADA cut-off value for adult TBPE. Additionally, we evaluated whether the prediction of TBPE using the pfADA cut-off value was affected by sex, age, or co-morbidity.

Methods

We retrospectively searched a centralised computerised database for consecutive PF specimens tested for ADA from 1 January 2011 to 31 December 2011 and assembled a cohort of patients with exudative pleural effusion. These patients were all

managed at a cluster of public hospitals that served a large population in western Kowloon of Hong Kong. At least 90% of patients with pleural effusion had PF tested for ADA. We considered pfADA alongside early (within 2 weeks) findings in PF and pleural biopsy, with and without applying Light's criteria[27] in multiple scenarios. For each scenario, we used the receiver operating characteristic (ROC) curve and the Youden Index (the point of maximal summation of sensitivity and specificity estimates) to identify an optimal pfADA diagnostic cut-off value for TBPE, and estimated the corresponding PPV and NPV. The Youden Index maximises the difference between the true-positive rate (sensitivity) and the false-positive rate (1 minus specificity), thereby maximising the correct classification rate. When the Youden Index comprised more than 1 point, we also considered the point at minimal distance between the ROC curve and the coordinate with 100% specificity and 100% sensitivity.

The following data were collected by review of medical records that had been created and maintained by clinicians who were unaware of the study hypothesis: sex, age (at the time of initial diagnosis), smoking history, drinking history, co-morbidity (chronic obstructive pulmonary disease, diabetes mellitus, chronic renal failure), use of immunosuppressive treatment for at least 1 month in the past year, nature of PF (exudate vs transudate by Light's criteria), sputum AFB smear and culture, PF AFB smear and culture, PF bacterial and fungal stain, PF culture of other bacteria or fungus, pleural biopsy findings, other significant findings related to initial or definitive diagnosis, the early diagnosis (within 2 weeks after checking pfADA), and the definitive diagnosis (by 1 year after checking pfADA).

This study was conducted in accordance with the amended Declaration of Helsinki, and approved by the Kowloon West Cluster Research Ethics Committee (IRB approval number: KW/EX-13-139(69-17)) and the Department of Health Ethics Committee (IRB approval number: L/M 400/2013).

Definitions

The exudative versus transudative nature of PF was established by reference to Light's criteria that classify PF as exudative in the presence of any one of the following: ratio of protein in PF to serum >0.5, ratio of lactate dehydrogenase (LDH) in PF to serum >0.6, and PF LDH level of >200 IU/L.[27]

A definitive diagnosis of TBPE was made when *Mycobacterium tuberculosis* complex was isolated in culture of PF or parietal pleura, or any one of the following in the absence of an alternative diagnosis by 1 year after pfADA checking: (i) granulomatous inflammation of parietal pleura, (ii) culture-proven pulmonary tuberculosis (TB) with pleural effusion and compatible response to TB treatment, (iii) a clinical diagnosis of TBPE with compatible response to TB treatment, or (iv) AFB and/or positive findings from nucleic acid amplification tests in PF or parietal pleura. An early diagnosis of TBPE was made when pleural biopsy showed granulomatous inflammation in the absence of an alternative cause, or rarely, the presence of AFB or positive findings from nucleic acid amplification tests in PF or parietal pleura.

Parapneumonic effusion refers to any pleural effusion secondary to pneumonia or lung abscess.[28] The PF is often exudative with a predominance of neutrophils.[28] It can be 'simple' (with sterile exudate) or 'complicated' (with progression to a fibrinopurulent state), characterised by pH <7.2, glucose level of <2.2 mmol/L, and LDH level of >1000 IU/L.[29] Empyema thoracis is a complicated parapneumonic effusion with frank pus.[28] A definitive diagnosis of simple non-tuberculous parapneumonic effusion was made if the PF was exudative and sterile with LDH level of ≤1000 IU/L and if there was a compatible clinical response to empirical antibiotic treatment, in the absence of an alternative diagnosis by 1 year after the first attempt of diagnostic thoracentesis. Without an identifiable non-tuberculous pathogen, we considered it impossible to confidently make an early diagnosis of simple non-tuberculous parapneumonic effusion. A definitive diagnosis of complicated non-tuberculous parapneumonic effusion, or empyema thoracis in the presence of frank pus or compatible radiological signs on chest computed tomographic scan was made if the PF was exudative with a non-tuberculous pathogen (demonstrated by positive stain/culture) in PF or parietal pleura, or LDH level of >1000 IU/L, and compatible clinical response to empirical antibiotic treatment and/or drainage, in the absence of an alternative diagnosis by 1 year after the first attempt of diagnostic thoracentesis. An early diagnosis of complicated non-tuberculous parapneumonic effusion, or empyema thoracis in the presence of frank pus or compatible radiological signs, was made when a non-tuberculous pathogen could be identified in PF or parietal pleura.

Malignant pleural effusion refers to the presence of malignant cells in PF and/or parietal pleura.[30] A definitive diagnosis of malignant pleural effusion was made if malignant cells were found in PF and/or parietal pleura, or clinical/radiological findings were compatible with malignant pleural effusion in the absence of an alternative diagnosis by 1 year after the first attempt of diagnostic thoracentesis. An early diagnosis of malignant pleural effusion was made when malignant cells could be demonstrated in PF or parietal pleura.

Statistical analysis

Chi squared test (for categorical data), Fisher's exact test (for categorical data), McNemar's test (for paired data), Student's *t* test (for continuous variables

normally distributed), and Mann-Whitney *U* test (for continuous variables not normally distributed) were used as appropriate to evaluate the association between TBPE and the pfADA cut-off as well as demographic factors and co-morbidity. Factors with a P value of <0.25 by univariate analysis were forced into a logistic regression model after considering multicollinearity.

Laboratory methods

Throughout the study period, ADA activity was measured by the same automated method, the Diazyme commercial kit in the Beckman Coulter UniCel DxC 800 Synchron Clinical System. The automated Diazyme method has been validated.[31]

Results

Search from the computerised database of ADA assay from 1 January to 31 December 2011 identified a total of 903 independent PF specimens from 903 patients. We evaluated 860 patients with pleural effusion and pfADA after excluding 42 cases that were peritoneal rather than PF and one case with no medical record. Table 1 shows their definitive diagnoses and the corresponding pfADA value. Pleural effusion was caused by congestive heart failure, chronic renal failure, hypoalbuminaemia, or nephrotic syndrome/nephropathy with membranous glomerulonephritis in 246 (28.6%) cases, malignancy in 198 (23.0%), non-tuberculous infection (simple non-tuberculous parapneumonic effusion, complicated non-tuberculous parapneumonic effusion other than empyema, and non-tuberculous empyema thoracis) in 168 (19.5%), TBPE in 157 (18.3%), and miscellaneous or unknown causes in 91 (10.6%). By Light's criteria, 626 (72.8%) cases were classified as exudative, 222 (25.8%) as transudative, and 12 (1.4%) as indeterminate (lack of data).

Among the 198 patients with malignant pleural effusion, an early diagnosis could be established by detecting malignant cells in PF in 136 (68.7%), including 21 also detected by pleural biopsy (20 closed and 1 open), and seven (3.5%) by pleural biopsy alone (5 closed and 2 open). Malignant pleural effusion was caused by lung cancer in 152 (76.8%) patients, lymphoid or haematological malignancy in 12 (6.1%), unknown primary in nine (4.5%), gastric cancer in five (2.5%), ovarian cancer in four (2.0%), breast cancer in four (2.0%), liver cancer in two (1.0%), pancreatic cancer in two (1.0%), and one (0.5%) each by cancer in the nasopharynx, tongue, oesophagus, unspecified gastrointestinal tract, kidney, urinary bladder, prostate, and nerve.

Among the 168 patients with non-tuberculous infection, infection was bacteriologically confirmed by PF culture in 21 (12.5%) cases with non-tuberculous empyema thoracis (including 19 with early diagnosis), and 10 (6.0%) with complicated non-tuberculous parapneumonic effusion (including 9 with early diagnosis).

Among the 157 patients with TBPE, the diagnosis was (1) bacteriologically confirmed by PF culture in 62 (39.5%) including four also confirmed

TABLE 1. Definitive diagnosis of pleural effusion among a cohort of 860 patients with pfADA level measured

Diagnosis	No. (%) of patients	Median (range) pfADA (U/L)*
Malignant pleural effusion	198 (23.0)	9.7 (<2.4 to 245)
Tuberculous pleural effusion	157 (18.3)	44 (<2.4 to 86)
Congestive heart failure†	139 (16.2)	4.1 (<2.4 to 25)
Simple non-tuberculous parapneumonic effusion	97 (11.3)	11 (<2.4 to 52)
Uncertain diagnosis	72 (8.4)	9.4 (<2.4 to 104)
Chronic renal failure or uraemic pleuritis‡	59 (6.9)	4.6 (<2.4 to 30)
Non-tuberculous empyema thoracis	42 (4.9)	48 (8.9 to 343)
Cirrhosis with ascites or hypoalbuminaemia other than cirrhosis§	42 (4.9)	4.6 (<2.4 to 13)
Complicated non-tuberculous parapneumonic effusion other than empyema	29 (3.4)	25 (2.4 to 79)
Liver abscess or parapharyngeal abscess or postoperative subphrenic collection	8 (0.9)	11 (9.5 to 180)
Nephrotic syndrome or nephropathy with membranous glomerulonephritis	6 (0.7)	3.2 (<2.4 to 8.9)
Pneumothorax or haemothorax or haemopneumothorax	5 (0.6)	12 (3.9 to 22)
Connective tissue disorder or serositis	3 (0.3)	21 (8.1 to 23)
Meigs syndrome	2 (0.2)	4.6 (4.4 to 4.8)
Pleuroperitoneal fistula	1 (0.1)	<2.4

Abbreviation: pfADA = pleural fluid adenosine deaminase

* Readings of <2.4 U/L could not be accurately quantified

† 14 Patients had chronic renal failure

‡ 4 Patients had end-stage renal failure on chronic ambulatory peritoneal dialysis

§ 2 Patients had hepatocellular carcinoma, 2 had congestive heart failure, and 2 had chronic renal failure

by pleural tissue culture and 26 also suggested by pleural biopsy; (2) bacteriologically confirmed by pleural tissue culture in 12 (7.6%) including four also confirmed by PF culture and nine also suggested by pleural biopsy; (3) histologically suggested by pleural biopsy in 74 (69 closed and 5 open; 47.1%) including 26 also confirmed by PF culture and nine also confirmed by pleural tissue culture; (4) clinically suggested by pulmonary TB in 44 (28.0%) including 26 solely by clinical correlation with radiological progress; and (5) clinically suggested by culture-proven TB ascites in one (0.6%). Sputum AFB smear was positive in nine (5.7%) patients, with *M tuberculosis* complex isolated in the sputum culture of 51 (32.5%). An early diagnosis could be established in 65 (41.4%), using pleural biopsy in 64 and polymerase chain reaction in PF in one. The majority (n=152) of patients with TBPE were Chinese.

Among 90 patients with TBPE and closed pleural biopsy performed, TBPE was detected by pleural biopsy in 69 (76.7%) and pfADA cut-off level in 85 (94.4%). The difference was statistically significant (P<0.005 by McNemar's test).

Table 2 shows the distribution of pfADA levels stratified by the nature of PF and tuberculous versus non-tuberculous pleural effusion. The prevalence (pre-test probability) of TBPE was significantly higher among exudative (24.3%) than transudative (2.3%) cases. All cases with TBPE had pfADA level of ≤86 U/L. With pfADA level of >86 U/L, all cases (n=18) with exudative non-tuberculous pleural effusion had pfADA level of >100 U/L of whom 13 patients had non-tuberculous empyema thoracis, two had lymphoma, one had plasmacytoma, one had liver abscess, and one had an uncertain diagnosis.

Figure 1 shows how we proceeded to increase the pre-test probability of TBPE by first excluding transudates, and then stepwise excluding non-tuberculous patients to further increase the pre-test probability. For each scenario, the pfADA cut-off value was tabulated alongside estimates of sensitivity, specificity, PPV, and NPV. Restricting analysis to 461 patients with exudative pleural effusion, pfADA level of ≤100 U/L, and early negative findings for non-tuberculous infection and malignancy in PF significantly increased PPV from 66.3% to 79.5% and non-significantly reduced NPV from 97.1% to 94.5%. Further excluding seven patients with an early diagnosis of malignancy by pleural biopsy resulted in no change to PPV and a non-significant decrease in NPV. Figure 2 shows an alternative approach that disregards Light's criteria. Restricting analysis to 689 patients with pfADA level of ≤100 U/L and early negative findings for non-tuberculous infection and malignancy in PF also significantly increased PPV from 66.3% to 79.2% and non-significantly reduced NPV from 97.1% to 96.1%. Further excluding seven patients with an early diagnosis of malignancy by pleural biopsy resulted in no change to PPV and a non-significant decrease in NPV from 96.12% to 96.07%. With no significant difference in PPV (P=0.938) or NPV (P=0.279) between the two approaches, the utility of pfADA may be optimised by applying a diagnostic cut-off among patients with pfADA level of ≤100 U/L and early negative findings for malignancy and non-tuberculous infection in PF, without considering Light's criteria or pleural biopsy. As such, pfADA level of ≥26.5 U/L, ascertained from the ROC curve using the Youden Index, detected TBPE with a sensitivity of 87.3%, specificity of 93.2%, PPV of 79.2%, NPV of 96.1%, and accuracy of 91.9% (Fig 3).

Table 3 shows different causes of pleural effusion above and below the diagnostic pfADA cut-off value of 26.5 U/L among the 689 patients with pfADA level of ≤100 U/L, and early negative findings for malignancy and non-tuberculous infection in PF. It is noteworthy that in the seven (4.0%) patients with pfADA level of ≥26.5 U/L and 64 (12.4%) patients with pfADA level of <26.5 U/L, the diagnosis was uncertain. Among 157 patients with TBPE, 137 (87.3%, sensitivity) tested positive (pfADA ≥26.5 U/L), with false-negative results in 20 (12.7%, the false-negative rate or 1 minus sensitivity). Among 532 patients with non-tuberculous pleural effusion, 496 (93.2%, specificity) tested negative (pfADA <26.5 U/L), with false-positive results in 36 (6.8%, false-positive rate or 1 minus specificity).

TABLE 2. Distribution of pfADA levels stratified by the nature of pleural fluid and tuberculous versus non-tuberculous pleural effusion

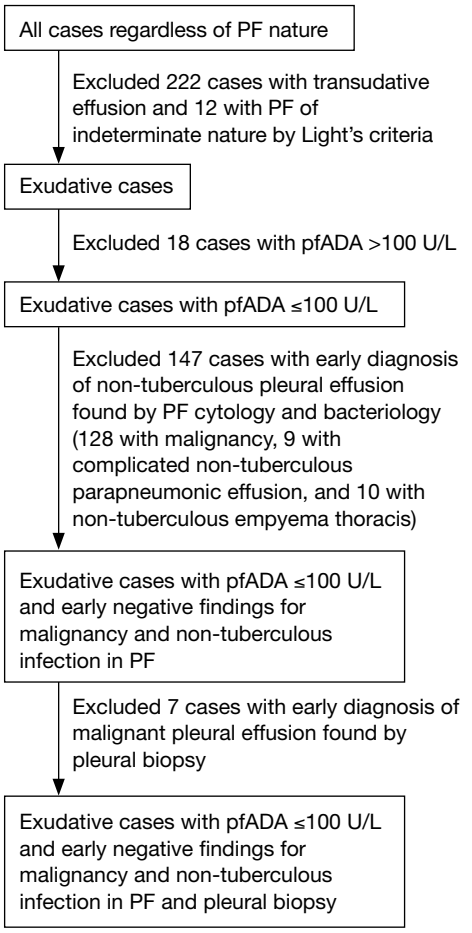
Nature of pleural fluid by Light's criteria	Non-tuberculous pleural effusion		Tuberculous pleural effusion		Total
	No. of patients	Median (range) pfADA (U/L)*	No. of patients	Median (range) pfADA (U/L)*	
Exudate	474	11.0 (<2.4 to 343)†	152	44.0 (5.3 to 86.0)	626
Transudate	217	4.0 (<2.4 to 13)	5	13.0 (<2.4 to 45)	222
Indeterminate (lack of data)‡	12	8.9 (<2.4 to 300)	0	Not applicable	12

Abbreviation: pfADA = pleural fluid adenosine deaminase

* Readings of <2.4 U/L could not be accurately quantified

† For those with non-tuberculous pleural effusion and pfADA >86 U/L, all had pfADA >100 U/L

‡ Diagnoses included malignant pleural effusion in 7, non-tuberculous empyema thoracis in 1, pneumothorax in 1, simple non-tuberculous parapneumonic effusion in 2, and hypoalbuminaemia due to causes other than cirrhosis in 1



	n	N	pfADA cut-off (U/L)	Pr (%)	Sens (%)	Spec (%)	PPV (95% CI) [%]	NPV (95% CI) [%]
All cases regardless of PF nature	157	860	25.5	18.3	87.9	90.0	66.3 (59.7-72.4)*	97.1 (95.5-98.2)†
Exudative cases	152	626	27.5	24.3	88.2	87.8	69.8	95.9
Exudative cases with pfADA ≤100 U/L	152	608	26.5	25.0	89.5	89.9	74.7	96.2
Exudative cases with pfADA ≤100 U/L and early negative findings for malignancy and non-tuberculous infection in PF	152	461	26.5	33.0	89.5	88.7	79.5 (72.8-84.9)*	94.5 (91.2-96.6)†‡
Exudative cases with pfADA ≤100 U/L and early negative findings for malignancy and non-tuberculous infection in PF and pleural biopsy	152	454	26.5	33.5	89.5	88.4	79.5 (72.8-84.9)	94.3 (91.0-96.6)‡

FIG 1. Optimising predictive values of pfADA by first considering Light's criteria

Abbreviations: CI = confidence interval; n = cases with tuberculous pleural effusion; N = denominator; NPV = negative predictive value; PF = pleural fluid; pfADA = pleural fluid adenosine deaminase; PPV = positive predictive value; Pr = prevalence; Sens = sensitivity; Spec = specificity

* Change is statistically significant (P=0.004)

† Change is statistically non-significant (P=0.051)

‡ Change is statistically non-significant (P=0.943)

Among 173 patients who tested positive, 137 (79.2%, PPV) were true-positive, and 36 (20.8%, 1 minus PPV) were false-positive with non-tuberculous diseases mistaken for TBPE: 10 with complicated non-tuberculous parapneumonic effusion, 10 with non-tuberculous empyema thoracis, seven with uncertain diagnosis, four with malignant pleural effusion, four with simple non-TB parapneumonic effusion, and one with chronic renal failure. Among 516 patients tested negative, 496 (96.1%, NPV) were true-negative, and 20 (3.9%, 1 minus NPV) were false-negative with TBPE mistaken for non-tuberculous pleural effusion. Among 689 test results, 633 (91.9%) were accurate and comprised 137 true-positive and 496 true-negative results.

A logistic regression model that considered sex, age, and co-morbidity alongside the pfADA diagnostic cut-off value identified pfADA level of ≥26.5 U/L as the only significant predictive variable of TBPE (Table 4).

Discussion

As a limited surrogate marker for TBPE, pfADA level must be interpreted alongside other clinical, radiological, and laboratory findings that help exclude non-tuberculous diseases, thereby increasing the pre-test probability of TBPE and the PPV. Using local data as measured by the Diazyme commercial kit, we demonstrated that TBPE was unlikely when pfADA level was >100 U/L. Restricting analysis to patients with pfADA level of ≤100 U/L and early (within 2 weeks) negative findings for malignancy and non-tuberculous infection in PF significantly increased the PPV and non-significantly reduced the NPV. Using this approach, neither additionally restricting

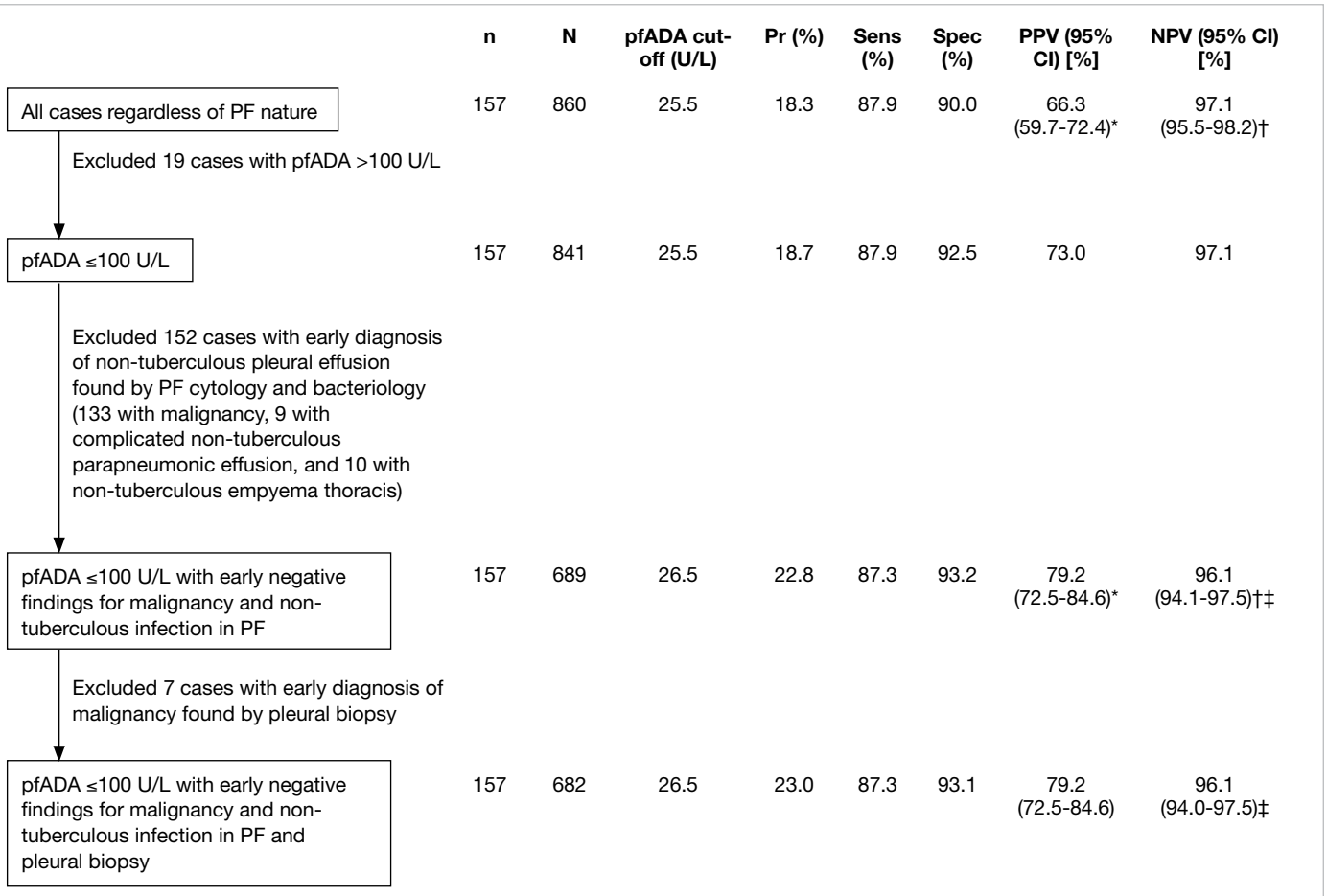


FIG 2. Optimising predictive values of pfADA without considering Light's criteria

Abbreviations: CI = confidence interval; n = cases with tuberculous pleural effusion; N = denominator; NPV = negative predictive value; PF = pleural fluid; pfADA = pleural fluid adenosine deaminase; PPV = positive predictive value; Pr = prevalence; Sens = sensitivity; Spec = specificity

* Change is statistically significant (P=0.005)

† Change is statistically non-significant (P=0.364)

‡ Change is statistically non-significant (P=0.965)

analysis to exudates by Light's criteria, nor adding closed pleural biopsy, would further enhance the predictive value of pfADA for TBPE. This might be explained by the fact that pfADA level of >13 U/L excluded all non-tuberculous transudative cases (Table 2), and that pfADA was significantly more sensitive than closed pleural biopsy for TBPE. A recent study, which demonstrated a need to suspect empyema or lymphoma when the pfADA level was extremely high,[32] corroborated our findings regarding the low likelihood of TBPE when pfADA level was >100 U/L. Furthermore, we demonstrated that the prediction of TBPE using the pfADA diagnostic cut-off value was not affected by sex, age, or co-morbidity. Another study that developed a predictive model for TBPE also failed to show any significant association between TBPE and either age or sex.[33]

Among patients with pfADA level of ≤100 U/L, when pfADA level is ≥26.5 U/L with early negative findings in PF for malignancy and non-tuberculous infection, it is probably appropriate to manage the patient as a case of TBPE, without additionally performing pleural biopsy (also a surrogate marker for TBPE). Nonetheless, it is important to remain vigilant due to a 20.8% (1 minus PPV) chance of mistaking non-tuberculous diseases for TBPE and prescribing unnecessary TB treatment. When pfADA level is <26.5 U/L with early negative findings in PF for malignancy and non-tuberculous infection, TB is highly unlikely. Again caution should be exercised in the presence of a 3.9% (1 minus NPV) chance of mistaking TBPE for other diseases. Tuberculosis is potentially fatal although effective treatment can reduce morbidity and mortality. Yet standard TB treatment is not without harmful side-effects that include hepatotoxicity. This occurs in 1% to 3% of patients on average and becomes more prevalent among the elderly people and those with underlying liver disease.[34,35] Additionally, treating

non-tuberculous disease as TB may also delay the diagnosis of other diseases including malignancy. It is important to balance the benefits of TB treatment against the risks when using a pfADA cut-off value to diagnose TBPE. In general, if the test suggests TBPE, and the risk of morbidity or mortality from untreated TB is substantial, it is prudent to promptly start TB treatment, and closely monitor treatment progress, with further investigations for other diseases conducted concurrently or as soon as treatment response is considered suboptimal. Other investigations are always indicated when the clinical progress is incompatible with the working diagnosis.

A major drawback of this study was its retrospective nature and related selection and misclassification bias. Selection bias may be modest as public hospitals provide approximately 90% of hospital care in Hong Kong, and we included every consecutive and non-duplicated PF sample from all patients managed during the study period in a large public hospital cluster in which at least 90% patients with pleural effusion had PF tested for ADA. Misclassification bias may occur. Efforts made by clinicians to confirm TB disease may be selectively affected by knowledge about the association between pfADA and TB. Non-tuberculous infection could have been misclassified as TB, thereby overestimating

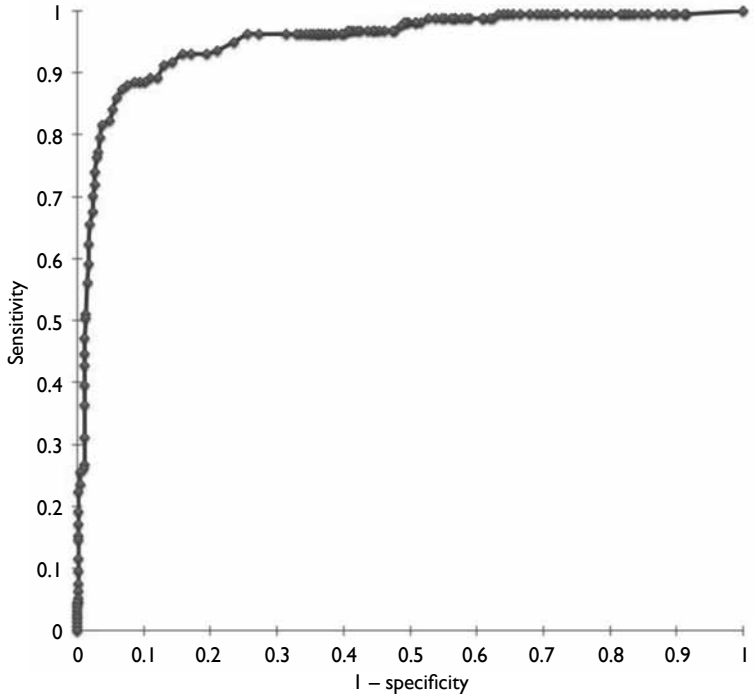


FIG 3. Receiver operating characteristic curve based on data of 689 patients with pleural fluid adenosine deaminase level of ≤100 U/L and early (within 2 weeks) negative findings for malignancy and non-tuberculous infection in pleural fluid

TABLE 3. Different causes of pleural effusion above and below the diagnostic pfADA cut-off value among 689 patients with pfADA level of ≤100 U/L, and early (within 2 weeks) negative findings for malignancy and non-tuberculous infection in pleural fluid

Cause of pleural effusion	No. (%) of patients	
	pfADA <26.5 U/L (n=516)	pfADA ≥26.5 U/L (n=173)
TBPE	20 (3.9)	137 (79.2)
Complicated non-tuberculous parapneumonic effusion	10 (1.9)	10 (5.8)
Non-tuberculous empyema thoracis	9 (1.7)	10 (5.8)
Uncertain diagnosis	64 (12.4)	7 (4.0)
Malignant pleural effusion	57 (11.0)	4 (2.3)
Simple non-tuberculous parapneumonic effusion	93 (18.0)	4 (2.3)
Chronic renal failure or uraemic pleuritis	58 (11.2)	1 (0.6)
Congestive heart failure	139 (26.9)	0
Cirrhosis with ascites or hypoalbuminaemia due to other causes	42 (8.1)	0
Liver abscess or parapharyngeal abscess or postoperative subphrenic collection	7 (1.4)	0
Pneumothorax or haemothorax or haemopneumothorax	5 (1.0)	0
Connective tissue disorder or serositis	3 (0.6)	0
Meigs syndrome	2 (0.4)	0
Nephrotic syndrome or nephropathy	6 (1.2)	0
Pleuroperitoneal fistula	1 (0.2)	0

Abbreviations: pfADA = pleural fluid adenosine deaminase; TBPE = tuberculous pleural effusion

TABLE 4. Univariate and multiple logistic regression analyses of tuberculous versus non-tuberculous exudative pleural effusion among 461 patients with exudative pleural effusion, pfADA level of ≤100 U/L, and early (within 2 weeks) negative findings for malignancy and non-tuberculous infection in pleural fluid

Variable	Univariate analysis, No. (%) of patients*			Multiple logistic regression analysis†
	Non-tuberculous (n=309)	Tuberculous (n=152)	P value	Adjusted OR (95% CI)
Sex				
Male	190 (61.5)	109 (71.7)	0.03	0.7 (0.3-1.5)
Female	119 (38.5)	43 (28.3)		
Median (IQR) age upon enrolment (years)	74 (58-83)	65 (43-79)	<0.001	0.99 (0.98-1.01)
pfADA ≥26.5 U/L				
No	274 (88.7)	16 (10.5)	<0.001	59.8 (31.7-112.9)
Yes	35 (11.3)	136 (89.5)		
Smoking				
No history	204 (66.0)	86 (56.6)	0.05	1.1 (0.5-2.1)
Current smoker or ex-smoker	105 (34.0)	66 (43.4)		
Drinking				
No history	267 (86.4)	127 (83.6)	0.41	-
Habitual drinker, ex-drinker, or social drinker	42 (13.6)	25 (16.4)		
Chronic obstructive pulmonary disease				
No	279 (90.3)	142 (93.4)	0.26	-
Yes	30 (9.7)	10 (6.6)		
Diabetes mellitus				
No	230 (74.4)	130 (85.5)	0.01	0.8 (0.4-1.9)
Yes	79 (25.6)	22 (14.5)		
Chronic renal failure				
No	245 (79.3)	132 (86.8)	0.05	0.8 (0.3-1.8)
Yes	64 (20.7)	20 (13.2)		
Immunosuppressive treatment				
No	302 (97.7)	149 (98.0)	1.0	-
Yes	7 (2.3)	3 (2.0)		

Abbreviations: CI = confidence interval; IQR = interquartile range; OR = odds ratio; pfADA = pleural fluid adenosine deaminase

* Unless stated otherwise

† Variables with P<0.25 by univariate analysis were forced in during multiple logistic regression analysis

PPV or underestimating NPV. On the other hand, TBPE could also have been misclassified as non-TB, thereby underestimating PPV or overestimating NPV. Another possible source of misclassification bias was uncertain diagnosis (Table 3), which was considered as non-tuberculous during analysis. Of note, TBPE labelled as uncertain diagnosis could have caused an underestimation of PPV or overestimation of NPV. Nonetheless, the lack of a definitive diagnosis by 1 year might suggest a low likelihood of TBPE, thereby reducing the impact of this misclassification bias.

We have established a pfADA diagnostic cut-off value for TBPE by restricting analysis to patients with pfADA level of ≤100 U/L, and considering early PF findings for malignancy and non-tuberculous infection, but not Light's criteria.

Declaration

All authors have disclosed no conflicts of interest.

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