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Evaluation of a diagnostic flow chart applying medical thoracoscopy, adenosine deaminase and T-SPOT.TB in diagnosis of tuberculous pleural effusion

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Abstract. – OBJECTIVE: To evaluate a diagnostic flow chart applying medical thoracoscoy (MT), adenosine deaminase (ADA) and T-SPOT.TB in diagnosis of tuberculous pleural effusion (TPE) at a high TB burden country.

PATIENTS AND METHODS: 136 patients with pleural effusion (PE) were enrolled and divided into TPE and Non-TPE group. MT (histology), PE ADA and T-SPOT.TB were conducted on all patients. ROC analysis was performed for the best cut-off value of PE ADA in detection of TPE. The diagnostic flow chart applying MT, ADA and T-SPOT.TB was evaluated for improving the limitations of each diagnostic method.

RESULTS: ROC analysis showed that the best cut-off value of PE ADA was 30U/L. The sensitivity and specificity of these tests were calculated respectively to be: 71.4% (58.5%-81.6%) and 100% (95.4-100.0%) for MT, 92.9% (83.0-97.2%) and 68.8% (57.9-77.9%) for T-SPOT.TB, and 80.0% (69.6-88.1%) and 92.9% (82.7-98.0%) for PE ADA. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value of the diagnostic flow chart were 96.4% (87.9-99.0%), 96.3% (89.6-98.7%), 25.714, 0.037, 97.4 and 94.9, respectively.

CONCLUSIONS: The diagnostic flow chart applying MT, ADA and T-SPOT.TB is an accurate and rapid diagnostic method in detection of TPE.

Key Words:
Tuberculous pleural effusion, Diagnostic flow chart, Medical thoracoscopy, Adenosine deaminase, T-SPOT.TB.

Introduction

Tuberculosis (TB) remains as an important health problem worldwide. In 2012, it was estimated that 8.6 million people developed active TB and 1.3 million died because of this infection[1]. Extrapulmonary (EPTB) was estimated at 10-20% of TB patients, and tuberculous pleural effusion (PE) was the most common form of EPTB[2]. Most TB PEs manifest as an acute illness, massive PE causes severe dyspnea and markedly affects the prognosis of patients. Therefore, patients with PE need to be promptly and accurately diagnosed and immediately treated.

PE adenosine deaminase (ADA) has been widely evaluated as a good biomarker in detection of tuberculous pleural effusion (TPE)[3]. Recently, several limitations of ADA in detection of TPE have been reported. Because of increasing in parapneumonic pleural effusions (PPEs), the ADA assay had limited diagnostic value in low TB burden countries, and pediatric population, for detection of TPE[4-6]. A retrospective study showed that mycobacterial load affected ADA levels of TPE, it implied using ADA would detect smear- or culture- positive TPE patients more easily, but negative TPE patients may be missed[7]. T-SPOT.TB has very high sensitivity in detection of TPE, and is widely used in investigate the prevalence of TB infection[8-11]. But T-SPO.TB has no capability to discrimination of active and latent TB infection. Poor specificity in detection of TPE was reported in several studies[8,12]. Medical thoracoscopy (MT), as an invasive examination, had perfect specificity and moderate sensitivity[13]. For accurate diagnosis of TPE, a diagnostic flow chart constructed to take full advantage of these technologies may be useful.

In this work, we aimed to evaluate a diagnostic flow chart applying MT (histology), ADA and T-SPOT.TB in diagnosis of TPE at a high TB burden country.

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Patients and Methods

Patients

This study was approved by the Shandong Provincial Chest Hospital Review Board. Written consent was not required by study participants since data was collected as a part of routine program monitoring and evaluation. Between April 2012 and May 2015, 146 consecutive PE patients with examinations (MT, pleural ADA and T-SPOT.TB) were obtained based on medical records. 5 PE patients were excluded for uncertain causes, 5 for loss of follow-up. Finally, 136 patients with determined diagnosis were enrolled.

TPE was diagnosed by isolation of M.TB from PE or pleural tissue. Malignant PE was diagnosed if the cytology or pleural biopsy specimen revealed underlying malignancy. PPE was defined as any exudative effusion (criteria established by Light et al[14]) associated with bacterial pneumonia, lung abscess or bronchiectasis.

Medical Thoracoscopy

Thoracoscopy was standardized in accordance with current European practice previously described[15]. Briefly, thoracoscopy was performed in the lateral decubitus position under local anesthesia with 1% lidocaine and analgesia and sedation using propofol or general anesthesia. A 7-mm trocar was then inserted, and a 0° telescope was inserted through it and connected to a video camera. The pleural space was carefully inspected through the thoracoscope (Richard Wolf rigid thoracoscopy; Knittlingen, Germany). Abnormal (suspicious) areas were biopsied, and sent for histologic examination by two experienced pathologists.

ADA

The ADA activity was measured in PE by kinetic method employing xanthine oxidase peroxidase[16] on automated clinical chemistry analyzer using commercially available kits (Maker, Sichuan, China), intra-assay and inter-assay coefficient of variability were ≤ 5% and ≤ 10%.

T-SPOT.TB

T-SPOT.TB assay (Oxford Immunote Ltd., Edinburgh, UK) was performed according to the manufacture instructions. Briefly, peripheral blood mononuclear cells was separated from a whole blood sample and incubated with the antigens (ESAT-6 and CFP10). The secreted cytokine by sensitized T cell was captured by specific antibodies on the membrane. Finally, the cytokine was detected by a chromogenic spot assay. Following manufacture instructions, the result of the testing was categorized "positive" or "negative" by spot count.

Statistical Analysis

Statistical analysis was carried out using SPSS 17.0 software and MedCalc Version 8.0.1.0. Continuous variables are expressed as mean (SD) while categorical variables are expressed as number and group percentages. Differences in ADA levels between groups were analyzed using Mann-Whitney U-test. Receiver operator characteristic (ROC) analysis was performed to evaluate sensitivity and specificity of diagnostic methods. Positive and negative predictive values, positive and negative likelihood ratios of diagnostic methods were also determined. A p value < 0.05 was considered statistically significant.

Results

Patient Characteristics

136 patients were divided into, 1) TPE group, included 56 cases, aged 39.5 ± 15.9 years old, 62.5% of them were male; 2) Non-TPE group, included 80 cases (47 MPEs, 22 PPEs and 11 other causes), aged 52.5 ± 11.4 years old, 70.0 % of them were male. Pleural levels of total protein, LDH, amylase and ADA in TPE group were significant higher than in Non-TPE group (all $p < 0.01$). Total bilirubin and glucose in PE of TPE group were lower than of Non-TPE group (all $p < 0.01$). Table 1 shows detailed characteristics of the both groups.

Evaluation of Individual Diagnostic Methods

The area under ROC curve of ADA in detection of TPE was 0.873 (95% CI: 0.805, 0.924). Under the best cut-off value of 30U/L, the ADA assay had a sensitivity of 80.0% (69.6-88.1%) and a specificity of 92.9% (82.7-98.0%). MT showed high specificity 100% (95.4-100.0%) and moderate sensitivity 71.4% (58.5-81.6%). T-SPOT.TB had good sensitivity 92.9% (83.0-97.2%) and poor specificity 68.8% (57.9-77.9%). Table II shows the diagnostic performance of individual, or combined diagnostic methods and the diagnostic flow chart.

Evaluation of Combined Diagnostic Methods

The both methods (MT or ADA, MT or T-SPOT.TB) were evaluated in detection of TPE, for maximizing the sum of specificity and sensitivity of combined methods. Our results showed, (1) the

sensitivity of ADA or MT increased to 100% (93.6-100%); (2) the sensitivity of T-SPOT.TB or MT increased to 96.4% (87.9-99.0%); (3) both of combined methods decreased the specificity of the MT.

Evaluation of the Diagnostic Flow Chart

Figure 1 showed the diagnostic flow chart applying ADA, MT and T-SPOT.TB for diagnosis of TPE. By the method, only two TPE cases were misdiagnosed as Non-TPE, three Non-TPE cases were as TPE. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value of the diagnostic flow chart were 96.4% (87.9-99.0%), 96.3% (89.6-98.7%), 25.714, 0.037, 97.4 and 94.9, respectively.

Discussion

We evaluated a diagnostic flow chart applying MT, ADA and T-SPOT.TB in detection of TPE. To our best knowledge, the study was the first time to report a diagnostic flow chart in detection of TPE. Meanwhile, our data suggested the flow chart was an accurate and rapid method in diagnosis of TPE.

Recently, several reports focused on diagnosis of TPE. Valdés et al[17] constructed a polytomous model based on demographic parameters and different biological markers to detect malignant or tuberculous pleural effusion, the model correctly classified a high proportion of TPE patients (85.8%). Shi et al[18] reported IL-27 had good performance in detection of TPE, when combined with IFN-γ and ADA, the sensitivity or specificity would increase up to 100%[19]. Another two reports[20,21] suggested IL-27 had the capability to improve the diagnostic role of ADA in diagnosis of TPE. Currently, several other biomarkers is also evaluated for diagnosis of TPE, such as SOD2, IL-33, and YKL-40[22-26].

ADA, as a rapid and cheap biomarker in detection of TPE, has been widely evaluated. Up to now, there are four major limitations restricting its use in

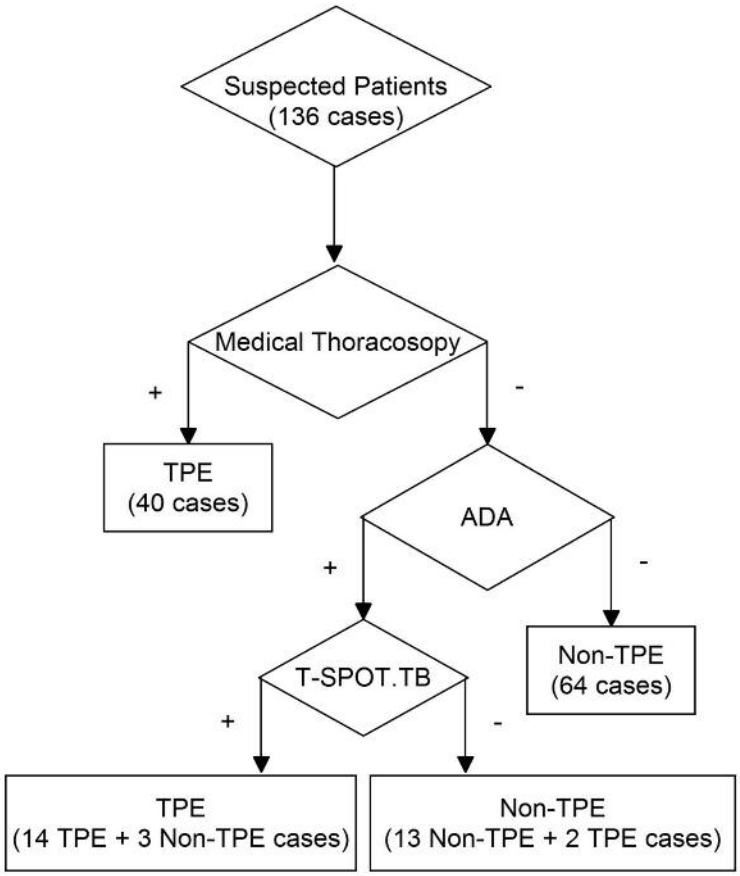

Figure 1. Diagnostic flow chart applying Medical Thoracoscopy, ADA and T-SPOT.TB in detection of TPE.

Table I. Patients characteristics of TPE and Non-TPE group.

	TPE	Non-TPE
Number	56	80
Age	39.5 ± 15.9	52.5 ± 11.4
Male	35 (62.5%)	56 (70%)
Total proetin (g/L)	47.4 ± 5.6	44.9 ± 12.0
Total bilirubin (μmmol/L)	9.09 ± 3.31	13.97 ± 9.34
Glucose (mmol/L)	3.97 ± 2.76	5.12 ± 3.61
LDH (U/L)	1343 ± 2444	903 ± 1012
ADA (U/L)	59.6 ± 27.1	22.3 ± 21.2
Amylase (U/L)	28.3 ± 7.6	54.7 ± 169.8
WBC (106/L)	3.57 ± 1.98	4.54 ± 6.52
Lymphocyte (%)	80.3 ± 32.2	69.5 ± 36.6
Neutrophil (%)	19.7 ± 32.2	30.5 ± 36.6

TPE, tuberculous pleural effusion; LDH, lactate dehydrogenase; ADA, adenosine deaminase; WBC, white blood cell.

clinical practice: (1) several factors affect levels of pleural ADA, such as age, lymphocytes, and TB burden[4,7,27,28]; (2) levels of pleural ADA not only increase in TPE patients, but also in PPEs, lymphoma, empyema, legionnaires' disease, pleural brucellosis and mycoplasma pneumoniae pneumonia[4,5,29-32]; (3) other biomarkers are necessary to aid improving the performance of ADA in detection of TPE, such as IL-33, IL-27, and lymphocyte proportion[19,25,33]; (4) the best cut-off value of pleural ADA may vary depending on the incidence of TPE[4,34,35]. Until now, ADA has been a good screening assay in detection of TPE, although the four points limited its use.

Interferon gamma release assays are *in vitro* immunologic diagnostic tests used to identify M.TB infection. One such assay is an enzyme-linked immunosorbent spot assay, commercially known as T-SPOT.TB. Meta analysis showed that T-SPOT.TB assay was superior, in comparison with the tuberculin skin test, for detecting confirmed active TB disease and latent tuberculosis infection[36,37]. Two reports from China[12,38] showed that the T-SPOT.TB assay have high sensitivity in detection of TPE. Therefore, the negative result can be read as exclusion criteria of TPE.

MT is a minimally invasive endoscopic procedure used in the diagnosis of pleural disease. Its diagnostic value has been proven in many studies. However, MT occasionally fails to reveal an abnormal pathologic result[39,40]. For example, in our study, only 71.4% of TPE were diagnosed by MT (histology). As limitations showing previously, T-SPOT.TB and ADA couldn't reach the goal of accurate diagnosis of TPE. To achieve high sensitivity and specificity in detection of TPE, a diagnostic flow chart by clever use of MT, T-SPOT.TB and ADA was constructed. Our work showed the flow chart had high sensitivity and excellent specificity (all close to 100%) for diagnosing TPE.

First limitation of the study was the smaller sample size. Secondly, it was difficult to assess the utility of diagnostic assays such as IP-10 and IFN-γ in this report, as they were performed in few patients. Thirdly, the diagnostic performance of MT would vary depending on size and quality of abnormal pleural tissues.

Conclusions

Our data suggests the flow chart applying MT, ADA and T-SPOT.TB is an accurate and rapid method in detection of TPE. At the help of the flow chart, TPE patients could be easily identified and given effective treatment.

Table II. Diagnostic performance of individual or combined methods and diagnostic flow chart in detection of tuberculous peleural effusion.

Diagnostic Methods	Senstivity	Specificity	PLR	NLR	PPV	NPV
MT	71.4% (58.5%-81.6%)	100% (95.4-100.0%)		0.286	100	71
T-SPOT.TB	92.9% (83.0-97.2%)	68.8% (57.9-77.9%)	2.971	0.104	81	87.2
ADA (30 U/L)	80.0% (69.6-88.1%)	92.9% (82.7-98.0%)	11.2	0.22	94.1	76.5
ADA or MT	100% (93.6-100%)	80.0% (70.0-87.3%)	5		87.7	100
T-SPOT.TB or MT	96.4% (87.9-99.0%)	68.8% (57.9-77.9%)	3.086	0.052	81.5	93.1
Diagnostic flow chart	96.4% (87.9-99.0%)	96.3% (89.6-98.7%)	25.714	0.037	97.4	94.9

MT, Medical Thoracoscopy; ADA, adenosine deaminase; PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

Authors' contributions

HY and HTR conceived and designed the study. HY collected data. ZW supervised data collection. WMS and WXF have been involved in the analysis and interpretation of data. WMS and WXF wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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