Head-to-Head Comparison of TB-LAMP, Mycobacterial Culture and Adenosine Deaminase for Diagnosis of Pleural Tuberculosis in China

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Abstract: **Objectives:** The objective of the prospective single-center study was to investigate the diagnostic performance of Loop-Mediated Amplification test (TB-LAMP), mycobacterial culture and adenosine deaminase (ADA) for diagnosing pleural tuberculosis (TB) from the pleural effusions in a TB-endemic setting. **Methods:** We retrospectively analyzed patients suspected of having pleural TB in Weifang between March 2018 and October 2019. The PE samples were evaluated by smear microscopy, mycobacterial culture, TB-LAMP and ADA assay. **Results:** Overall, 170 patients with suggestive of pleural TB were retrospectively reviewed in this study, of which 125 were diagnosed as pleural TB. Among 125 pleural TB cases, 52 cases were identified by TB-LAMP, resulting in a sensitivity of 41.6%. When combing MGIT and TB-LAMP, 13 additional positive cases were detected compared to MGIT culture alone, demonstrating a sensitivity of 56.8%. The mean ADA levels were corelated with age, and the mean ADA value of <35 years group was significantly higher than that of ≥70 years group (p=0.0214). **Conclusion**: In conclusion, our data demonstrate the promising effectiveness of TB-LAMP in detection of MTB in concentrated PE specimens. The ADA levels are decreased with advanced age, highlighting the urgent need for confirmation of different cut-off values for various age group.

Keywords: Pleural Tuberculosis; Diagnosis; Lamp; Adenosine Deaminase

Introduction

Pleural tuberculosis (TB) is one of the most common forms of extrapulmonary tuberculosis across different regions around the world, as well as a common cause of pleural effusions (PEs) in TB-endemic countries (Pang et al., 2019). Because of the paucibacillary nature of TBP in pleural effusions, it remains a diagnostic challenge highlighting the urgent need of highly accurate tools for correctly diagnosing TBP patients (Solari et al., 2018). The Loop-Mediated Amplification test for TB (TB-LAMP) is endorsed by the World Health Organization to facilitate the detection of pulmonary TB patients (WHO, 2016). However, the current data is restricted to sputum samples only, which hamper the potential use of TB-LAMP in various EPTB samples. Therefore, the prospective single-center study was conducted to investigate the diagnostic performance of TB-LAMP, mycobacterial culture and adenosine deaminase (ADA) for diagnosing pleural TB from the pleural effusions in a TB-endemic setting.

Materials and Methods

We retrospectively analyzed patients suspected of having pleural TB in the Second People's Hospital of Weifang

between March 2018 and October 2019. The pleural effusion (PE) samples were evaluated by Ziehl-Neelsen smear microscopy, mycobacterial culture in the the Mycobacterial Growth Indicator Tube (MGIT) 960 automated system (Becton Dickinson), TB-LAMP and adenosine deaminase assay as previously described (Liu et al., 2020). The clinically diagnosed pleural TB was used as the reference standard to assess the sensitivity, specificity, positive predict value (PPV), and negative predictive value (NPV).

Results

Overall, 170 patients with suggestive of pleural TB were retrospectively reviewed in this study, of which 125 were diagnosed as pleural TB based on laboratory, histology and/or clinical evidence. Among 125 pleural TB cases, 58 were detected by MGIT culture testing, yielding a sensitivity of 46.4% (95% confidence interval (95% CI: 37.7%-55.1%). For TB-LAMP, 52 cases were identified as pleural TB cases, resulting in a sensitivity of 41.6% (95% CI: 33.0%-50.2%). There was no significant difference observed in sensitivity between MGIT culture and TB-LAMP (p=0.45). When combing MGIT and TB-LAMP, 13 additional positive cases were detected compared to MGIT culture alone, demonstrating a sensitivity of 56.8% (95% CI: 48.1%-65.5%). In addition, 44 out of 45 non-pleural TB cases could correctly identified by TB-LAMP, yielding a specificity of 97.8% (95% CI: 93.5%-100.0%) (Table 1).

Table 1.Comparison of diagnostic efficacy between MGIT culture and TB-LAMP in the diagnosis of tuberculous pleurisy

Method	Clinical diagnosis		Sensitivity （%）	Specificity （%）	Positive predictive value （%）	Negative predictive value （%）
	Tuberculous pleurisy （125）	Non tuberculous pleurisy （45）				
LAMP						
Positive	52	0	41.6%	100%	100%	38.1%
Negative	73	45				
MGIT culture						
Positive	58	0	46.4%	100%	100%	40.2%
Negative	67	45				
LAMP and MGIT culture						
Positive	71	0	56.8%	100%	100%	45.5%
Negative	54	45				

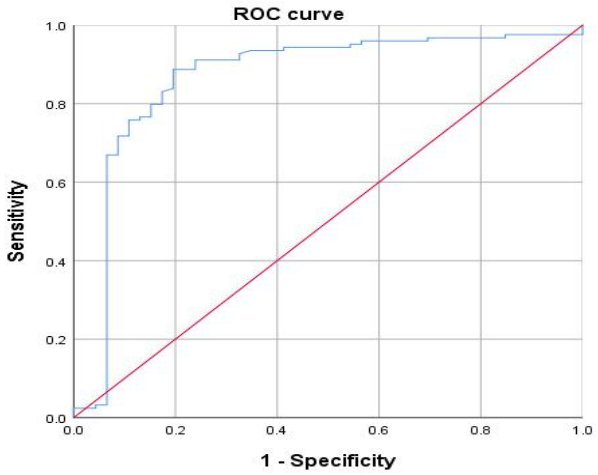

Figure 1A depicts the ROC curve calculated for the cut-off value for distinguishing pleural TB and non-pleural TB cases. A threshold of 17.85 U/l was determined to best differential diagnosis of pleural TB. Using this temporary value, the ADA assay had a sensitivity of 91.2% and a specificity of 66.7%. We further analyzed the ADA concentrations stratified to different age groups (Fig. 1B). The mean ADA level was decreased with advanced age, ranging from 42.11±16.24 for <35 years group to 29.95±23.17 for ≥70 years group. Statistical analysis revealed that the mean ADA value of <35 years group was significantly higher than that of ≥70 years group (p=0.018).

Figure 1A.the ROC curve calculated for the cut-off value for distinguishing pleural TB and non-pleural TB cases

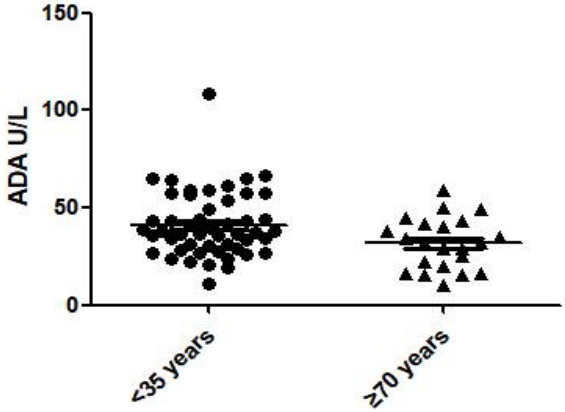

Figure. 1B. the ADA concentrations stratified to different age groups

Discussion

In this study, our data firstly demonstrate the promising performance of TB-LAMP in detection of MTB in PE specimens. A meta-analysis of recent evaluation studies reported that the pooled sensitivity of Xpert was low at 17.0% for diagnosis of pleural TB compared with a composite reference standard, which was significantly lower than that of TB-LAMP (WHO, 2013). In contrast, a serial of pervious reports have repeatedly confirmed that Xpert outperforms TB-LAMP in detecting MTB from sputum specimens (WHO, 2013; WHO, 2016). Compared with the Xpert assay using hemi-nested PCR, the TB-LAMP had higher amplification efficiency, with DNA amplified 10^9-10^{10} times in 30 min (Parida et al., 2008). A study of analytic sensitivity demonstrated that the detection limit of Xpert was 131 CFU/ml (Blakemore et al., 2010), whereas the combined use of isothermal amplification and fluorescence assay yielded a detection limit of 5-50 CFU/ml for TB-LAMP (Iwamoto et al., 2003). Despite the higher analytic sensitivity, TB-LAMP loads the maximal 60 μl of clinical specimens, only one twentieth of that loaded in Xpert (approximate 1 mL). The sampling issues may contribute to

the decreased efficacy of TB-LAMP in sputum samples. In the present study, the concentrated PE samples rather than the original samples were used for TB-LAMP, which may be a potential explanation for these conflicting evaluation results. In addition, we also observe that a proportion of pleural TB cases missed in automated MGIT system yielded positive results by TB-LAMP, reflecting the paucibacillary nature of PE samples. Our results indicate that the parallel use of mycobacterial culture and TB-LAMP will bring additional benefit of correctly diagnosing pleural TB patients.

The ADA in pleural effusion is considered as a useful maker for the differential diagnosis of pleural TB (Abrao et al., 2014). In our analysis, using a cut-off level of 18 U/l, the pleural TB cases yielded a specificity of 67%, which was significantly lower than previous observations (Keng et al., 2013). Notably, we found that the ADA levels were decreased with advanced age. Similar results were reported by Abrao and colleagues that age was correlated with pleural ADA levels in the Brazil population (Abrao et al., 2014). As a common biochemical indicator, the ADA production appears to be influenced by factors associated with the anti-TB immune. In view of the fact that aging is associated with declines in immune system function (Weyand and Goronzy, 2016), the immunosenescence in both innate and adaptive immunity may be an important reason for the decreased ADA production in the old age group. Therefore, the different cut-off levels should be set for various age groups to help clinicians interpret the ADA results.

In conclusion, our data demonstrate the promising effectiveness of TB-LAMP in detection of MTB in concentrated PE specimens. The ADA levels are decreased with advanced age, highlighting the urgent need for confirmation of different cut-off values for various age group. Further studies are warranted to evaluate the performance of TB-LAMP in other forms of concentrated extrapulmonary specimens.

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Ethical approval statement

This study was approved by the Ethics Committee of the Second People's Hospital of Weifang. All data were kept anonymous in analysis process.

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