Level of antibodies against mycobacterial glycolipid in the effusion for diagnosis of tuberculous pleural effusion

Takeshi Morimoto*, Shingo Takanashi, Yukihiro Hasegawa, Koji Fujimoto, Koichi Okudera, Akihito Hayashi, Kageaki Taima, Ken Okumura

Second Department of Internal Medicine, Hirosaki University School of Medicine, Zaifu-cho 5, Hirosaki, 036-8562, Japan

Received 1 September 2005; accepted 31 January 2006

KEYWORDS
Tuberculous pleural effusion; Lipoarabinomannan (LAM); Tuberculous glycolipid (TBGL); Adenosine deaminase (ADA); Interferon-γ (IFN-γ)

Summary
Background: Diagnosing tuberculous pleural effusion (pTB) is often difficult because the culturing of tubercle bacilli results in a negative test in the majority of cases. Serological tests for the detection of antibodies to tuberculous glycolipid (TBGL) and lipoarabinomannan (LAM) have been introduced for the diagnosis of pulmonary tuberculosis. We examined the levels of these antibodies, adenosine deaminase (ADA) and interferon-γ (IFN-γ) in the pleural effusion and compared their diagnostic values in pTB.

Methods: We studied 65 patients with pleural effusion. Of those, 19 patients were diagnosed as having pTB according to our broad case definition. The etiologies in the other 46 patients were malignant effusion, transudative effusion and miscellaneous diseases. Determiner TBGL antibody™ (D-TBGL-Ab) and MycoDot™ were used for the detection of anti-LAM and anti-TBGL antibodies, respectively, in the pleural effusion.

Results: The sensitivity of ADA was 78.9% (15/19) and the specificity 97.8% (45/46). The sensitivity of IFN-γ was 84.2% (16/19) and the specificity 93.5% (43/46). The sensitivities of D-TBGL-Ab and MycoDot™ were both 52.6% (10/19) and their specificities were 95.7% (44/46) and 97.8% (45/46), respectively. When DTBGL-Ab (cutoff point: 2.0 U/ml) and ADA activity (cutoff point: 57 IU/l) were combined, the sensitivity was 94.7% (18/19) and the specificity 93.5% (43/46).

Conclusions: In the diagnosis of pTB, D-TBGL-Ab and MycoDot™ each have low sensitivity but high specificity. When D-TBGL-Ab is used in combination with ADA, the
Introduction

Many diseases cause pleural diseases,1,2 and tuberculosis (TB) remains the most common cause of pleural effusion in many countries.3 The diagnosis of tuberculous pleural effusion (pTB) is made by demonstrating the existence of tubercle bacilli in the sputum, the pleural fluid, or the pleural biopsy specimen, or by demonstrating the existence of granulomas in the pleura.4,5 In many cases, however, it is difficult to diagnose pTB. There are many biological markers which have been proposed to increase the diagnostic sensitivity to pTB.6 So far, adenosine deaminase (ADA) activity has been considered the most useful marker, although it is not elevated in all pTB patients. Recent studies have shown that interferon-γ (IFN-γ) is elevated in pTB, and it is thought to be a more sensitive diagnostic tool than ADA.4,7,8 However, the elevation of either ADA or IFN-γ is not a specific reaction to TB.6

Recently, new serological tests for the detection of antibodies against tuberculous glycolipid (TBGL), including trehalose-6,6'-dimycolate (TDM) and lipoarabinomannan (LAM), a glycolipid common in mycobacteria, have been developed for the diagnosis of pulmonary TB.9,10 The positive and negative predictive value of serological diagnostic testing using glycolipids, however, may be influenced greatly by the prevalence of the disease in each country.11 Because one of the mechanisms of pTB is a hypersensitive response to mycobacterial protein, we thought that measuring the level of antibodies against the glycolipid of TB in the pleural effusion might be a more useful diagnostic approach than serological examination.3 In this study, we examined the level of antibodies against TBGL in the pleural effusion. We also examined the levels of ADA activity and IFN-γ, and compared the diagnostic value of these tests.

Methods

Patients

We examined 65 patients with pleural effusion who were referred to Hirosaki University Hospital. They were 49 men and 16 women, and their mean age was 60 years, ranging from 20 to 81 years. All patients had undergone a clinical work-up for pleural effusion, including pleurocentesis, and the pleural effusion samples were kept at −80 °C.

The diagnosis of pTB was made when acid-fast bacilli were detected in the pleural effusion or in other biological materials by microscopy, when Mycobacterium tuberculosis was detected upon culturing, or when granulomatous inflammatory change with necrosis was found in the pleural biopsy specimen. The patients who responded to 3- or 6-month empirical anti-TB therapy and did not have any other disease were also considered to have pTB. According to these criteria, 19 patients were diagnosed as having pTB (pTB group) and the other 46 as not having pTB (non-pTB group). The non-pTB group consisted of 33 patients with malignant pleural effusion and lung or other cancer, 4 with parapneumonic effusion, 2 with collagen disease, 6 with congestive heart or renal failure, and 1 with postoperative pleural effusion after coronary-artery bypass grafting.

Measurement of ADA activity and IFN-γ level in the pleural effusion

The ADA activity and IFN-γ level were determined for all samples. The ADA activity was measured by enzyme assay using the UV-RATE method (MIZUHO MEDY. Co., Ltd., Saga, Japan). The IFN-γ level was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA).

Detection of antibodies against TDM and other minor glycolipids of the cell wall of Mycobacterium tuberculosis in the pleural effusion

Determiner TBGL antibody (D-TBGL-Ab) (Kyowa-Medex Co., Ltd., Tokyo, Japan) detects the antibody against TDM, trehalose 6-mycolate, 2,3-diaclytrehalose, phenolic, TBGL, etc., all of which derive from the glycolipids of TB. TDM is a characteristic cord factor of the mycobacterial membrane, including TB. D-TBGL-Ab is a quantitative analysis kit for rapid serodiagnosis. We used 400 μl of sample diluent, and 10 μl of pleural fluid instead of serum.
Detection of the antibody against LAM in the pleural effusion

MycoDot® (Mossman Associates Inc., Blackstone, MA, USA) detects the antibody against LAM. If the anti-mycobacterial antibody is detected in the specimen, colloidal gold particles will specifically aggregate to the antigen location on the test comb to give a colored dot indicative of a positive reaction. We used 100 μl of the sample diluent, and 100 μl of pleural fluid instead of serum.

Statistical analysis

All data are expressed as means (and ranges), unless otherwise stated. The data were compared using the Mann-Whitney and Chi-square tests between the pTB group and the non-pTB group. We determined all cutoff points by likelihood ratio (LR) analysis, except in the case of MycoDot®. Receiver operating characteristic (ROC) curve analysis was used to compare the usefulness of each marker.

Results

ADA activity and IFN-γ level

The mean ADA activity was 68.8 IU/l (range: 12.9–125.9 IU/l) in the pTB group and 16.0 IU/l (range: 3.4–88.0 IU/l) in the non-pTB group (P < 0.0001) (Fig. 1A). The mean IFN-γ was 1453.9 pg/ml (range: 0–3832.7 pg/ml) in the TB group and 51.7 pg/ml (range 0–626.0 pg/ml) in the non-TB group (P < 0.0001) (Fig. 1B).

Determiner TBGL antibody® and antibody against LAM

The mean D-TBGL-Ab was 4.97 U/ml (range: 0–32.36 U/ml) in the pTB group and 0.64 U/ml (range: 0–2.97 U/ml) in the non-pTB group (P = 0.0004) (Fig. 1C). In terms of the MycoDot® test, there were 10 positive samples in the pTB group, while there were 45 negative samples and 1 positive sample in the non-pTB group (P < 0.0001) (Fig. 1D).

Sensitivity, specificity and the ROC curve

The sensitivity, specificity and positive and negative predictive value determined at each of the three levels are shown in Table 1 for all tests used in this study. The best cutoff points were determined by LR. When the ADA cutoff point was set at 57.0 U/ml, the sensitivity was 78.9% and the specificity 97.8%. When the IFN-γ cutoff point was set at 248 pg/ml, the sensitivity was 84.2% and the specificity 93.5%. When the D-TBGL-Ab cutoff point was set at 2.0 U/ml, the sensitivity was 52.6% and the specificity 95.7%. As for MycoDot®, the diagnostic sensitivity for pTB was 52.6% and the specificity 97.8%.

The ROC curves for the ADA activity, IFN-γ level and D-TBGL-Ab level are shown in Fig. 2. ROC curve analysis showed that ADA activity was the best indicator among the three markers. To obtain a better diagnostic value, we combined the results of the tests two by two and compared the resulting diagnostic precisions (Table 1). It was found that when D-TBGL-Ab (cutoff point: 2.0 U/ml) and ADA activity (cutoff point: 57 IU/l) were combined, the sensitivity was 94.7% (18/19) and the specificity 93.5% (43/46). This combination was the most sensitive and specific for the diagnosis of pTB.

Discussion

Recently, serological diagnostic kits for TB diagnosis, such as D-TBGL-Ab and MycoDot®, have become available. The reported sensitivity and specificity of D-TBGL-Ab are 66–90% and 95–99%,9,10,12–16 respectively. The sensitivity and specificity of MycoDot® are reported to be 41–90% and 84–100%,11,17–23 respectively. The range of sensitivity in both of these tests is wide because the diagnostic value of these tests is influenced greatly by the prevalence of TB in the population being studied. Actually, populations which have undergone prior bacillus Calmette-Guerin (BCG) vaccination tend to show positive responses to the serologic test for TB,11 which in turn results in decreased specificity. Thus, these serological tests for TB might not be useful.

In many areas of the world, TB still remains the most common cause of pleural effusion. However, the culturing of sampled effusion yields negative results in the majority of patients with pTB. In our study, the positive reactions of MycoDot® were judged visually, and 10 out of 19 samples were positive in this study. D-TBGL-Ab and MycoDot® were found to have high specificities, but the sensitivities of these tests were lower than we expected. Low levels of anti-mycobacterial antibody in the pleural effusion have been reported previously.24 The pleural fluid in patients with pleural TB dose not usually contain a large antigen load because of the small number of organisms. And
this can explain the low sensitivity of these serological tests.

There have been many reports on the usefulness of ADA activity and IFN-γ level in the diagnosis of pTB. These reports have shown that the range of sensitivity and specificity of ADA activity are 69–100% and 72–100%, respectively, whereas the sensitivity and specificity of the IFN-γ level are 80–100% and 90–100%, respectively. Our data were consistent with these reports. We
Antibodies of Mycobacterium in pleural effusion

Further compared the sensitivity, specificity and the area under the ROC curve among these tests, and this comparison showed that ADA activity was the most useful diagnostic tool.

A positive correlation between ADA activity and the IFN-\(\gamma\) level in the pleural effusion of our present subjects has been observed. ADA is found in monocytes and macrophages, while IFN-\(\gamma\) is produced by activated CD4\(^+\) T lymphocytes\(^{3,8}\). These cells constitute the chain of response to mycobacterial infection.\(^{6}\) However, not only specific antigens, such as tubercle bacilli but also non-specific antigens stimulate CD4\(^+\) T lymphocytes and elevate the IFN-\(\gamma\) level. Therefore, the elevation of ADA activity and IFN-\(\gamma\) in the pleural effusion is not specific to TB. In fact, we observed elevated ADA activity in a patient with thymoma.

The D-TBGL-Ab test and MycoDot\(^{©}\) test were found to be specific to TB cases, although a few positive results were obtained in non-pTB cases. Therefore, we examined the diagnostic value of the combination between each of these antibody tests and ADA activity or the INF-\(\gamma\) level. When D-TBGL-Ab (cutoff point: 2.0 U/ml) and the ADA activity (cutoff point: 57 U/ml) were combined, the sensitivity was 94.7%, and the specificity 93.5%, the positive predictive value was 85.7%, and the negative predictive value 97.7%. This combination was the most effective in diagnosing pTB in our study. However, when studying large populations where TB is endemic, we should consider the expense involved in administering these tests. Measurement of the ADA activity in pleural fluid is the most cost-effective method. When the diagnosis of pTB is uncertain, the D-TBGL-Ab test or the MycoDot\(^{©}\) test using pleural effusion instead of serum is recommended.

In conclusion, the measurement of anti-LAM antibodies and anti-TBGL antibodies in the pleural effusion is useful for the diagnosis of pTB. In addition to measuring the ADA activity in the pleural effusion, measuring the anti-TBGL antibody levels in the pleural effusion seems to be an excellent diagnostic method for the diagnosis of pTB.

References


Figure 2 Receiver operating characteristic (ROC) curve for the diagnosis of tuberculous pleural effusion and area under the curves (AUC). ADA = adenosine deaminase; IFN-\(\gamma\) = interferon-\(\gamma\); D-TBGL-Ab = Determiner TBGL antibody\(^{©}\).


