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The Differential Diagnostic Value of Interferon-γ, Interleukin-2, Tumor Necrosis Factor α and Adenosine Deaminase in Tuberculous and Malignant Pleural Effusion*

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ABSTRACT Objective: To explore the differential diagnostic value of interferon-γ (IFN-γ), interleukin-2 (IL-2), tumor necrosis factor α (TNF-α), and adenosine deaminase (ADA) level in the tuberculous and malignant pleural effusions. **Methods:** A total of eighty-eight specimens of pleural effusion were collected from 88 patients with pleural effusion in respiratorydepartment of affiliated Hospital of Medical College of Qingdao University and Qingdao Thoracic Hospital from September 2012 to March 2013. Among them, 46 pleural specimens were diagnosed as malignant, and the others were diagnosed as tuberculous. Enzyme-linked immunosorbent assay (ESISA) was used to detect the concentration of IFN-γ, IL-2, TNF-α and ADA in tuberculous and malignant pleural effusions. Receive operating characteristic curve (ROC) analysis was used to analyze whether there are significant differences between the two groups of patients with pleural effusion in concentration of IFN-γ, IL-2, TNF-α and ADA. **Results:** Tuberculous pleural effusion group of IFN-γ, IL-2, TNF-α and ADA was significantly higher than that of malignant pleural effusion group, and the difference was statistically significant (t = 8.118, 8.126, 8.066, 7.221; P<0.05); ROC analysis showed that pleural effusion of IFN-γ, IL-2, TNF-α and ADA in the diagnosis of the critical value of 201.45 pg/mL, 41.91 pg/mL, 21.55 pg/mL, 33.78 U/L, the sensitivity and specificity for IFN-γ, IL-2, TNF-α and ADA in pleural fluid were 91.3 %, 93.5 %, 91.2 %, 89.1 % and 91.0 %, 92.1 %, 89.9 %, 90.1 % , respectively.**Conclusion:** The IFN-γ, IL-2, TNF-α and ADA concentration in tuberculous and malignant pleural fluid are significant for the early diagnosis and differential diagnosis.

Key words: Tuberculous pleural effusion; Malignant pleural effusion; Cytokines

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Introduction

Pleural effusion is one of the most common diseases in department of respiration. Making the nature of the fluid clear is essential for the early diagnosis and treatment of diseases. The holdbacks pleural effusion can be caused by many kinds of diseases, lung cancer invading the pleura and tuberculous pleurisyis are the most common[1]. According to the latest research, the incidence of tuberculosis pleurisy shows a growing trend in pleural disease, accounted for 49.6% of the entire pleural diseases, and 29.6% of malignant pleural effusion [2]. Both tuberculous and malignant pleural effusions are exudates, which bring certain difficulties in definitive diagnosis.In recent years, the incidence of tuberculosis onset age has a tendency to increase [3], making it harder to identify tuberculous and malignant pleural effusion. At present, the method of distinguishing between tuberculous and malignant hydrothorax method is limited [4], the positive rate of pleural biopsy and finding the tumor cells, smearing acid fast stain of pleural effusion relatively lowand cause greater trauma; So, we are desperately looking for a simple and convenient method to identify tuberculous and malignant pleural effusions. In this study, the expression of IFN-γ, IL-2, TNF-α and ADA in pleural effusions were detected, and analyze its, value inidentifying two kinds of pleural effusions.

1 Materials and methods

1.1 Patients

Total of 46 patients who suffered from malignant pleural effusions, including 26 males, 20 females, 45.3 ± 17.5 years old, were admitted to respiratory department of affiliated Hospital of Medical College of Qingdao University from September 2012 to March 2013. Diagnostic basis: Patients with pleural effusion were definitively diagnosed with cancer by exfoliative cytologic examination, pleural-biopsy, electronic bronchoscope, CT guidance after skin lung puncturem ethod or thoracoscopy topathologic alhistology or cytology. Total of 46 patients who suffered from tuberculous pleural effusions, including 22 males, 20 females, 38.3 ± 16.8 years old, were admitted to Qingdao Thoracic Hospital from September 2012 to March 2013. Diagnostic basis: 1. Clinical

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symptoms: fever, sweats, malaise, anorexia, and other symptoms of pulmonary tuberculosis; 2. The characteristic tuberculous lesions can be detected by chest CT; 3. Positive of tuberculintest (PPD) or T-SPOT; 4. Seeing tuberculous nodules or caseous necrosis by pleuralbiopsy; 5. Detecting mycobacterium tuberculosis in the sputum, patients who were compatible with three above or accord with the fourth or the fifth one can be diagnosed with tuberculous pleural effusion. All patients were without any treatment.

1.2 Experiment materials

After admission, 10 mL of pleural effusion was collected during thoracentesis from untreated cases, and made them centrifuged at 3000 rpm for 10 min, under or dinary temperature. Then, preserved supernatant with EP tubal and frozen them at -80℃ as spare parts.

1.3 Method and reagent

Enzyme-linked immunosorbent assay (ESISA) was used to detect the concentration of IFN-γ, IL-2, TNF-α and ADA in tuberculous and malignant pleural effusions. Etectionkits were purchased from ABGENT company, Shanghai West Tang Bio-tech CO.LTD (imports packing). The detectionprocess is operated strictlyin accordance with productin structions.

1.4 Statistical analysis

All data were processed using the SPSS software 17.0 and presented as mean and standard deviation ($\bar{x}\pm s$). For parametric data, Students't -test was used to comparisons between mean values of two groups. P-values<0.05 were considered statistically significant. For the evaluation of the diagnostic of IFN-γ, IL-2, TNF-α and ADA, ROC were constructed and analyzed to determine the most accurate cut-off values for diagnosing TB pleurisyand to compare the performance of different diagnostic tests. Area under ROC curves (AUC) range from 1 to 0.5. If it were less than 0.7, accuracy of the diagnosis is low; high accuracy when exceeding 0.9, and the closer the AUC approaches to 1.0, the better diagnosis is.

2 Materials and methods

2.1 Comparison of concentrationandpositiverates with IFN−γ, IL−2, TNF−α and ADA in pleural effusion

Table 1 shows the mean concentration of IFN-γ, IL-2, TNF-α and ADA in two groups pleural effusion. The concentration of IFN-γ, IL-2, TNF-α and ADA were significantly higher in tuberculous pleural effusion than that in malignant pleural effusions, which have statistical significance. (T-value of Four groups were calculated separately; t=8.118, 8.126, 8.066, 7.221; P<0.05).

Table 1 The concentration of IFN-γ, IL-2, TNF-α and ADA in two different groups of patients with pleural effusion ($\bar{x}\pm s$)

Group	No	IFN-γ (pg/mL)	IL-2 (pg/mL)	TNF-α (pg/mL)	ADA (U/L)
TB	46	525.14± 378.91	83.37± 29.22	42.86± 19.90	60.35± 42.14*
Malignant	42	47.62± 41.98	38.28± 21.92	15.53± 9.69	22.63± 11.08

Note:*Compared with malignant pleural effusion group, we can conclude that P<0.05 (T-value of four groups above were calculated separately. t=8.118, 8.126, 8.066, 7.221;P=0.000, 0.000, 0.000, 0.000).

2.2 ROC curve analysis

1-specificity (false positive rate) in accordance with the abscissa, the ordinate sensitivity (true positive rate) for ROC curve of IFN-γ, IL-2, TNF-α and ADA for result analysis. AUC of IFN-γ, IL-2, TNF-α and ADA was respectively 0.946, 0.888, 0.931 and 0.940. AUC with 95 % confidence intervals was calculated, respectively, (0.896, 0.996), (0.811, 0.964), (0.876, 0.986), (0.883, 0.997). The diagnostic critical value of IFN-γ, IL-2, TNF-α and ADA was respectively 201.45 pg/mL, 41.91 pg/mL, 21.55 pg/mL, 33.78 U/L.

3 Discussion

Differential diagnosis of pleural effusion has always been one of most hardest problems in clinical work, although tuberculous and malignant pleural effusion are exudates, but due to the difference of the primary disease, its treatment and prognosis are entirely different. Therefore, determining the nature of pleural effusion in clinical work is most important. Tuberculous pleurisy, one of the most common etiologies of lymphocyte-predominant exudative pleural effusion, is pleural lesions caused by infection my-

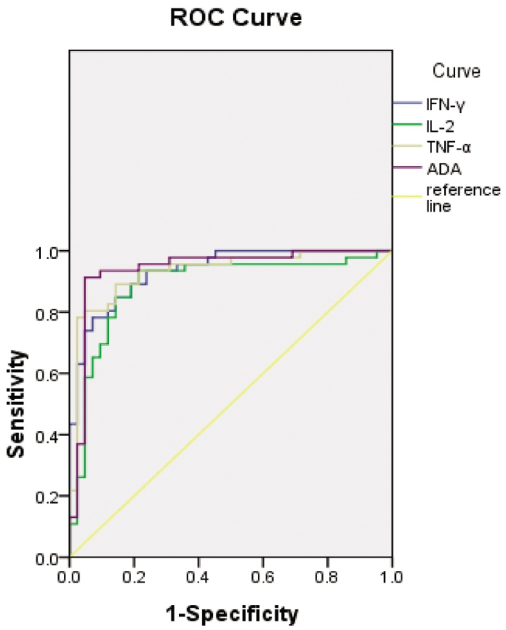

Fig. 1 ROC curves analyse for the differentiation of tuberculous and malignant effusions ofIFN-γ, IL-2TNF-α and ADA

cobacterium tuberculosis. Since its symptoms similar to malignant pleural effusion caused by lung cancer, it is difficult to identify the difference between them[5].

People infected with mycobacterium tuberculosis will cause the body's immune response, and CD4-positive T-lymphocytes (CD4+-T)are the major immune effector cells. Studies have found that local immune response enhanced after mycobacterium tuberculosis infecting pleura, which is led by helper T- lymphocyte cell (Th_1) type immune response[6]. The mechanism of the pleural effusion pleural inflammation was caused by delayed type hypersensitivity reaction of body to the mycobacterium tuberculosis. When infected by mycobacterium tuberculosis, the body will activate effector cells, increase their ability in killing mycobacterium tuberculosis. This process is led by Th1 type response and plays a protective immune response. Lymphocytes secrete a larger number of cytokines, such as IFN-γ, IL-2, IL-8, IL-12, TNF-α [7], etc. At the same time, IFN-γ, in turn, promote the differentiation of Th1 cellsand inhibit the differentiation of TH2 cell [8]. While in malignant pleural effusions, Th1 immune response function is relatively weak, mainly to TH2 immune response [9]. Some researchers believe that the body imposed Th2 immune response based is one of the mechanisms of tumor immune escape[10], and that the ability of produce IL-2 and respond of tumor cells significantly will decrease in cancer patients, resulting disease progression and tumor cells transfer[11].

In recent years, more and more researchers tend to look for cytokines associated with immune in pleural effusion, which has becoming one of hotspots in the diagnosis value of pleural effusion [12]. In a variety of inflammatory conditions, such as mycobacterium tuberculosis invade the pleura, cause Th1-dominating immune response, multiply and activate T-lymphocyte quickly. This process is mainly that Th0 trans form Th1 which will produce large amounts of cytokines of IFN-γ, IL-2, TNF-α[8], promotingphagocytosis of macrophages to mycobacterium tuberculosis[7]. A cytotoxic T lymphocyte (CTL) who is trans formed CD8-positive T-lymphocytes （$CD8^+$T）cells activated by IL-2 kill mycobacterium tuberculosis. Therefore, IFN-γ in tuberculous pleural effusion, IL-2 for high expression, and the experiment research shows that tuberculous pleural effusion of IFN-γ. The expression of IL-2 was obviously higher than that of malignant pleural effusion, consistent with other related research[13-16].

TNF-α of inflammatory mediators is a cytokine, who is produced by macrophages and lymphocytes, and has various biological activities, mainly for immune regulation, can stimulate monocyte, activate macrophages and enhance the phagocytic function[17]. Studies have shown that TNF-α of appropriate concentration has a protective function with body [18], but of higher concentration sisharmfulto body[18]. When the body is infected by mycobacterium tuberculosis, macrophages will be activated to produce large amounts of TNF-α, and together with other cytokines, kill mycobacterium tuberculosis. Therefore, TNF-α in tuberculous pleural effusion was highly expressed and tallied with experimental research, which showed the expression of TNF-α was significantly higher in tuberculous pleural effusion than in malignant pleural effusions, and with other related research[17, 19].

ADA has always been an important indicator of identification of tuberculous and malignant pleural effusions. Determination of ADA in pleural effusion has been confirmed as a useful supplemental diagnostic index for tuberculous pleurisy [20-22]. ADA is one of the important enzymes purine metabolisms of nucleic acid, mainly existed in neutrophils and lymphocytes, and the activity of ADA is closely related to the $CD4^+$ T [20]. When infected by mycobacterium tuberculosis, led by Th1 type response [6], the body will activate effector cells and activated lymphocytes will secrete larger amounts of ADA. Therefore, ADA in tuberculous pleural effusion was highly expressed [21], which tallied with experimental research that showed that the expression of ADA was significantly higher in tuberculous pleural effusion than in malignant pleural effusions, and with other related research[20-22].

Conventional methods for diagnosing tuberculous pleurisy have been proved insufficient. This experimental research showed that IFN-γ, IL-2, TNF-α and ADA levels were significantly higher in tuberculous pleural effusion than that in malignant pleural effusions, and their differences were significant ($P<0.05$). ROC analysis, the critical value of IFN-γ, IL-2, TNF-α and ADA in diagnosis of pleural effusion are respectively 201.45 pg/mL, 41.91 pg/mL, 21.55 pg/mL, 33.78 U/L, the sensitivity and specificity for IFN-γ, IL-2, TNF-α and ADA in pleural fluid were 91.3 %, 93.5%, 91.2%, 89.1 % and 91.0 %, 92.1 %, 89.9 %, 90.1 %, respectively. The results of this study that IFN-γ, IL-2, TNF-α and ADA can be used as an indicator to identify tuberculous and malignant pleural effusion, were similar to literatures reported in recent years [13-16, 21]. Therefore, IFN-γ, IL-2, TNF-α and ADA are important indicators for diagnosis of tuberculous pleuritis. This is of great significance to differential diagnosis of tuberculous and malignant pleural effusions.

This experimental research showed that the pleural effusion IFN-γ, IL-2, TNF-α and ADA contributed a lot to diagnosis of tuberculous pleural effusions, and that detection which combined the four were of great significance to differential diagnosis of tuberculous and malignant pleural effusions.

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细胞因子 IFN-γ, IL-2, TNF-α 和 ADA 对结核性和恶性胸腔积液鉴别诊断的价值 *

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摘要 目的：探讨细胞因子 γ- 干扰素（IFN -γ）、白介素 -2（IL-2）、肿瘤坏死因子 -α（TNF-α）和腺苷脱氨酶（ADA）对结核性和恶性胸腔积液的鉴别诊断的价值。**方法：**以 2012 年 9 月至 2013 年 3 月期间在青岛大学医学院附属医院呼吸科及青岛胸科医院未经治疗的胸腔积液患者为研究对象，其中恶性胸腔积液患者 46 例，结核性胸腔积液患者 42 例。采用双抗体夹心酶联免疫吸附测定法（ELISA）分别检测结核性和恶性胸腔积液患者中 IFN -γ、IL-2、TNF-α 及 ADA 的表达情况。并应用 ROC 曲线分析两组患者胸腔积液中 IFN -γ、IL-2、TNF-α 及 ADA 的表达差异及意义。**结果：**结核性胸腔积液组 IFN -γ、IL-2、TNF-α 及 ADA 的表达明显高于恶性胸腔积液组，差异有统计学意义（t=8.118、8.126、8.066、7.221; P=0.000、0.000、0.000、0.000，P<0.001）；ROC 曲线分析结果显示胸腔积液中 IFN -γ、IL-2、TNF-α 及 ADA 的诊断临界值为 201.45 pg/mL、41.91 pg/mL、21.55 pg/mL、33.78 U/L；诊断敏感度分别为 91.3 %、93.5 %、91.2 %、89.1 %；特异度分别为 91.0 %、92.1 %、89.9 %、90.1 %。**结论：**胸腔积液中 IFN -γ、IL-2、TNF-α 及 ADA 的表达对结核性和恶性胸腔积液诊断与鉴别诊断具有重要参考价值。

关键词：结核性胸腔积液；恶性胸腔积液；细胞因子

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