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Clinical Value of Vascular Endothelial Growth Factor Combined with Interferon-γ in Diagnosing Malignant Pleural Effusion and Tuberculous Pleural Effusion*

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Summary: In order to investigate the clinical value of vascular endothelial growth factor (VEGF) combined with interferon-γ (IFN-γ) in diagnosing malignant pleural effusion and tuberculous pleural effusion, 42 cases of malignant pleural effusion and 45 cases of tuberculous pleural effusion in Tongji Hospital, from March 2004 to May 2005, were included. The carcinoembryonic antigen (CEA), VEGF and IFN-γ levels of pleural effusion were detected by using ELISA, and adenosine deaminase (ADA) activity was determined by using enzyme kinetic analytical method. The sensitivity, specificity, accuracy and area under the curve (AUC^{ROC}) of CEA and VEGF, VEGF/IFN-γ ratio, ADA and IFN-γ were measured by receiver operating characteristic curve (ROC). The results showed that CEA, VEGF levels and VEGF/IFN-γ ratio were significantly higher and the ADA and IFN-γ levels were significantly lower in malignant group than those in tuberculous group (P<0.01). The sensitivity, specificity, accuracy and AUC^{ROC} of VEGF/IFN-γ ratio (88.7%, 99.8%, 94.4%, 0.96 respectively) were higher than those of CEA (67.8%, 96.1%, 82.4%, 0.78 respectively) and VEGF (81.5%, 84.3%, 82.9%, 0.79 respectively). The sensitivity, specificity, accuracy and AUC^{ROC} of IFN-γ (85.7%, 96.4%, 90.9%, 0.94 respectively) were higher than those of ADA (80.2%, 87.6%, 83.8%, 0.81 respectively). It was concluded that VEGF/IFN-γ ratio and IFN-γ could be used as valuable parameters for the differential diagnosis of malignant pleural effusion and tuberculous pleural effusion.
Key words: vascular endothelial growth factor; interferon-γ; malignant pleural effusion; tuberculous pleural effusion

Sometimes it is so difficult for doctors to differentiate malignant pleural effusion and tuberculous pleural effusion that 20% to 30% patients can not be diagnosed correctly because of lacking high sensitivity and specificity methods. VEGF can promote angiogenesis, vascular hyperpermeability, tumorigenesis, tumors invasion and metastasis, which may accelerate the production of pleural fluid [1]. Lymphocytes are the cardinal cells in tuberculous pleural effusion, which can produce IFN-γ when they are stimulated by tuberculous antigen. IFN-γ plays an important role in the cellular immunity and the generation of pleural effusion in tuberculous pleuritis [2]. In this study, the levels of VEGF and IFN-γ were detected and compared with CEA and ADA in order to investigate the clinical value of VEGF, VEGF/IFN-γ ratio and IFN-γ in diagnosing malignant pleural effusion and tuberculous pleural effusion.

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1 MATERIALS AND METHODS

1.1 Subjects

The enrolled included 87 patients with pleural effusion in Tongji Hospital, from March 2004 to May 2005. There were 42 cases in malignant group whose malignant cells were found through pleural biopsy and cytological detections, including 22 males and 20 females with their age ranged from 32 to 76 years (mean 51.4±9.8 years). These 42 patients included 27 cases of lung cancer, 9 cases of mammary cancer, 4 cases of lymphoma and 2 cases of pleural mesothelioma. In tuberculous group, 45 patients including 25 males and 20 females with their age ranged from 18 to 72 years (mean 48.5±10.6 years) were all diagnosed through pleural biopsy, microbiological detection of sputum and pleural effusion in which mycobacterium tuberculosis was found. All patients received no special treatment before samples collection. Five mL effusion was got from each patient and centrifuged (2000 r/min) for 10 min at room temperature within 2 h. Supernatants were collected and stored at −20°C. The sex and age in two groups had no significant difference (P<0.05).

1.2 Reagents and Instruments

7170A automatic chemistry analyzer (Hitachi Co., Japan). NOVAPTH-MIPEADER enzyme labeling device (BIO-RAD Co., Netherlands). Human CEA, VEGF and IFN-γ ELISA kits (Jingmei Co., China).

Adenosine, glutamate dehydrogenase (GLDH) and reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) (Sigma Co., USA). α-ketoglutarate (Beijing Chemical Plant, China).

1.3 Detection of CEA, VEGF and IFN-γ Levels in Pleural Effusion by ELISA

The samples which were stored at −20ºC were thawed at room temperature. The test protocol of ELISA was performed according to the instruction of the ELISA detection Kit. At 450 nm of NOVAPTH-MIPEADER enzyme labeling device, absorbance (*A*) values of standard product and the samples were measured. A standard curve was drawn and the levels of CEA, VEGF and IFN-γ were calculated.

1.4 Determination of ADA Activity in Pleural Effusion by Enzyme Kinetic Analytical Method

Twenty μL sample was added to 360 μL ADA substrate solution containing 6 mmol/L adenosine, 1.1 mmol/L α-ketoglutarate, 0.28 mmol/L NADPH and 18 U/L GLDH. At 340 nm of 7170A automatic chemistry analyzer, the decreased rate of *A* values of NADPH was measured, and the activity of ADA was calculated.

1.5 Statistic Analysis

All the data were expressed as $\bar{x}\pm s$ and processed with SPSS 10.0 software. The results in different groups were compared with Student' *t*-test. $P<0.05$ was considered to be statistically significant. ROC was made by using SPSS 10.0 software. AUC^{ROC} was calculated and the cut-off point based on the maximum of sensitivity together with specificity was determined. With the cut-off point, the optimal critical value, sensitivity, specificity and accuracy were established.

2 RESULTS

2.1 Comparison of ADA, IFN-γ, CEA, VEGF Levels and VEGF/IFN-γ Ratio between Two Groups

The CEA and VEGF levels and VEGF/IFN-γ ratio in malignant group were significantly higher than those in tuberculous group ($P<0.01$). The ADA and IFN-γ levels in tuberculous group were significantly higher than those in malignant group ($P<0.01$, table 1).

Table 1 The ADA, IFN-γ, CEA, VEGF levels and VEGF/IFN-γ ratio of two groups

Groups	*n*	ADA (U/L)	IFN-γ (ng/L)	CEA (μg/L)	VEGF (ng/L)	VEGF/IFN-γ
Malignant	42	18.9±5.2*	41.7±8.3*	31.43±6.78*	1135.4±320.5*	28.28±4.72*
Tuberculous	45	53.4±12.3	153.7±36.4	8.09±2.02	676.7±216.9	4.43±0.78

*$P<0.01$ as compared with tuberculous group

2.2 The Value of VEGF and VEGF/IFN-γ Ratio in Diagnosing Malignant Pleural Effusion

The critical value, sensitivity, specificity, accuracy and AUC^{ROC} of CEA and VEGF and VEGF/IFN-γ ratio in diagnosing malignant pleural effusion were obtained by using ROC (fig. 1, table 2). Compared with CEA, VEGF had higher sensitivity (81.5%) and lower specificity (84.3%), while VEGF/IFN-γ ratio had both higher sensitivity (88.7%) and specificity (99.8%). Among them, VEGF/IFN-γ ratio had the highest AUC^{ROC} (0.96) and was the best parameter in diagnosing malignant pleural effusion.

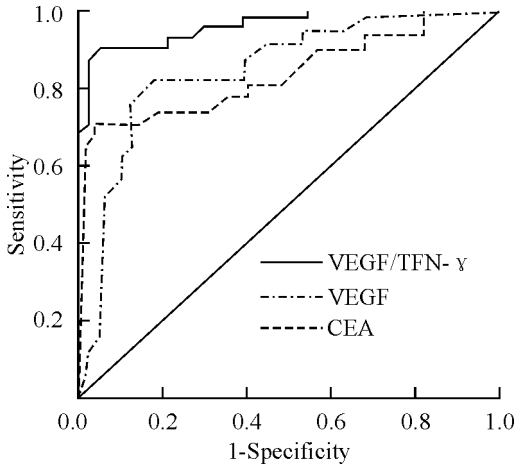

Fig. 1 The ROC of CEA, VEGF and VEGF/IFN-γ ratio in diagnosing malignant pleural effusion

Table 2 The value of CEA, VEGF and VEGF/IFN-γ ratio in diagnosing malignant pleural effusion

Parameters	AUC^{ROC}	Critical value	Sensitivity (%)	Specificity (%)	Accuracy (%)
CEA	0.78	15 μg/L	67.8	96.1	82.4
VEGF	0.79	945.7 ng/L	81.5	84.3	82.9
VEGF/IFN-γ	0.96	17.23	88.7	99.8	94.4

2.3 The Value of IFN-γ in Diagnosing Tuberculous Pleural Effusion

The sensitivity, specificity, accuracy and AUC^{ROC} of IFN-γ (85.7%, 96.4%, 90.9% and 0.94 respectively) were higher than those of ADA (80.2%, 87.6%, 83.8% and 0.81 respectively), indicating that IFN-γ was a better parameter than ADA in diagnosing tuberculous pleural effusion (fig. 2 and table 3).

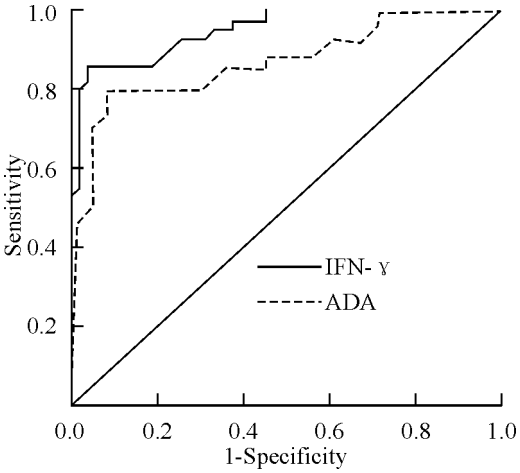

Fig. 2 The ROC of IFN-γ and ADA in diagnosing tuberculous pleural effusion

Table 3 The value of ADA and IFN-γ in diagnosing tuberculous pleural effusion

Parameters	AUC^{ROC}	Critical value	Sensitivity (%)	Specificity (%)	Accuracy (%)
ADA	0.81	40 U/L	80.2	87.6	83.8
IFN-γ	0.94	95.3 ng/L	85.7	96.4	90.9

3 DISCUSSION

Malignant disease and tuberculous pleuritis are the main cause of pleural effusion. Sometimes the distinction between them poses a diagnostic challenge to the physician. This is mainly due to the large proportion of cases in which no confirmatory diagnosis of pleural tuberculosis is achieved by microbiological methods, and the sensitivity of cytological detections for malignancy is inadequate. Closed needle biopsy of the pleura is more useful than microbiological methods in establishing tuberculosis as the etiology of the effusion, but for malignant effusions adds little diagnostic yield to fluid cytology. The question is whether this invasive procedure can be avoided as the first approach to distinguish these two etiologies.

At present, tumor markers of high sensitivity and specificity are scarce in diagnosis of malignant pleural effusions. CEA which is widely used in clinic has high specificity and low sensitivity. In this study it was found that CEA level in malignant group (31.43±6.78) was significantly higher than that in tuberculous group (8.09±2.02, P<0.01). The sensitivity, specificity and accuracy of CEA for diagnosis of malignant effusions were 67.8%, 96.1%, and 82.4% respectively, indicating that CEA was a tumor marker of high specificity and low sensitivity. Lots of researches proved that VEGF could promote angiogenesis and tumorigenesis. VEGF, mainly secreted by tumors combines with VEGF receptors (VEGFR) of tumor cells through autocrine mechanism, promotes proliferation and metastasis, and inhibits apoptosis of tumors. Additionally, VEGF can bind with VEGFR of vascular endothelia through paracrine, promote proliferation and migration of endothelia, and increase vascular permeability. VEGF facilitates malignant pleural effusions through the increased vascular permeability and exudation of plasma protein[3, 4]. In this study, VEGF level and VEGF/IFN-γ ratio in malignant group (1135.4±320.5 ng/L and 28.28±4.72 respectively) were significantly higher than those in tuberculous group (676.7±216.9 ng/L and 4.43±0.78 respectively, P<0.01). Compared with CEA, VEGF had higher sensitivity (81.5%) and lower specificity (84.3%), but VEGF/IFN-γ ratio had both higher sensitivity (88.7%) and higher specificity (99.8%). Among them, VEGF/IFN-γ ratio had the highest AUC^{ROC} (0.96) and was the best parameters in diagnosing malignant pleural effusion.

Although ADA has already been widely used to diagnose tuberculous pleural effusions, its sensitivity and specificity are not very high. Our study revealed that ADA level in tuberculous group (53.4±12.3 U/L) was significantly higher than that in malignant group (18.9±5.2 U/L, P<0.01). By using ROC, we calculated diagnostic efficiency of ADA upon tuberculous pleural effusions and found that the sensitivity, specificity and accuracy were 80.2%, 87.6% and 83.8% respectively. In recent years, many researches indicate that tuberculous pleuritis is a delayed hypersensitivity reaction against mycobacterial antigens in the pleural space where CD4+ T cells are recruited, activated and differentiate into Th1 cells which can secrete IFN-γ. Macrophages, monocytes and other inflammatory cells activated by IFN-γ secrete interleukin-1β, tumor necorosis factor α and transforming growth factor β which can initiate inflammatory cascade and improve microvascucular permeability, leading to pleural effusions[1]. Hiraki *et al*[5] detected IFN-γ level in 20 patients with tuberculous pleural effusion and found that concentration of IFN-γ was significantly higher in tuberculous than in other etiological effusions, such as malignant tumor, connective tissue disease, circulatory system disease, liver and renal disease. These studies indicate that IFN-γ is important for development of the disease. In this study, it was found that IFN-γ level in tuberculous group was 153.7±36.4 U/L. Because of different pathogenesis, IFN-γ level in malignant group was only 41.7±8.3 U/L, significantly lower than that of tuberculous group (P<0.01). The sensitivity, specificity, accuracy and AUC^{ROC} of IFN-γ for diagnosis of tuberculous pleural effusions were 85.7%, 96.4%, 90.9% and 0.94 respectively, which were higher than those of ADA, indicating that IFN-γ could substitute ADA for diagnosis.

To sum up, the results of this study suggested that to detect VEGF and IFN-γ levels of pleural effusions at the same time and to calculate VEGF/IFN-γ ratio can improve sensitivity and specificity, avoid misdiagnosis and leak-diagnosis in distinguishing malignant pleural effusion from tuberculous pleural effusion. So it is deserved to be used widely in clinical practice.

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