Iran J Public Health, Vol. 45, No.4, Apr 2016, pp.435-440

Original Article

Predictive Role of Serum Tumor Markers in Diagnosis of Pulmonary Tuberculosis

*Jingjing MA [1], Dan XIA [1], Jing HU [1], Rui FU [1], Lijun XU [1], Ying ZHANG [1], Mengying ZHANG [1], Benhe LI [1], Jianghua YANG [2], *Yufeng WEN [1]*

1. *Dept. of Preventive Medicine, School of Public Health, Wannan Medical College, Wuhu City, China*
2. *Dept. of Infectious Diseases, Yijishan Hospital, Wuhu City, China*

***Corresponding Author:** Email: wenwent2008@163.com

(Received 25 Oct 2015; accepted 16 Feb 2016)

Abstract

Background: The diagnosis of pulmonary tuberculosis (PTB) is complicated and time-consuming currently. There was association of PTB with serum tumor markers. In this study we aimed to evaluate the predictive role of serum CA125, CA199 and CEA as diagnostic tools for PTB.

Methods: This study was designed as a case-control study with 565 subjects who visited the Yijishan Hospital from Jun to Dec in 2014.This case-control study matched as for age and sex with 113 cases and 452 controls. Serum CA125, CA199 and CEA levels were detected by electrochemiluminescence instrument. The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was performed to evaluate the diagnostic value on PTB.

Results: Serum levels of CA125, CA199 and CEA in PTB patients were significantly higher than those in control group (P<0.001). There was no significantly different of three tumor markers between initial treatment group and re-treatment group. The logistic regression analysis showed that CA125 was an impact factor to PTB. The ROC analysis revealed that AUC of CA125 was 0.966 (95%CI: 0.951-0.981), the sensitivity, specificity in serum and cut-off were 95.6%, 85.0% and 10.30 U/ml, respectively.

Conclusion: The serum CA125 has potential good diagnostic performance for PTB.

Keywords: CA125, CA199, CEA, Pulmonary tuberculosis, Receiver-operating, Characteristic curve

Introduction

Tuberculosis is a leading cause of death, especially in low and middle income countries (1). China is one of the 22 high TB burden countries. The latest estimates included in the global TB report of 2014 that there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths, among them China accounted for 11% new tuberculosis (2). Nevertheless, most deaths from TB are preventable by early diagnosis and treatment. Totally 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment (2). However recent studies showed extensive delay in TB diagnosis and treatment were severe (3). Hence, the crucial point to eradicate the disease is improving the diagnosis of tuberculosis.

In high prevalence of TB areas, the sputum smear microscopy test, with a sensitivity of 50%-70%, is available and affordable (4). Although smear-negative cases are less infectious than smear-positive cases, at least 17% of TB cases are affected by smear-negative cases (5). The *Mycobacterium* culture is the golden standard for TB diagnosis, but the detection results cost a long time and have a low positive rate, the sensitivity of

43%–83% (6). PCR test has found an increasingly wide utilization in TB diagnosis, but in developing areas its utilization remains limited because of the high cost of detection and the deficiency of special equipment and professional staffs (7, 8). Therefore, the cheap, accurate and rapid diagnostic tests for TB are much-needed (9).

In recent years, serum tumor markers were an effective and non-invasive diagnostic tool (10), and became a common clinical method for screening tumor (11). Interestingly, a study in 1995 reported that CA125 was increased in PTB (12), since then several studies reported that the serum levels of CA125, CA199, CEA in PTB were higher than those in normal population (13-16). The results provided a clue that tumor markers had potential clinical value for the diagnosis of PTB possibly. However, the study on the predictive power of tumor markers and which tumor marker has higher sensitivity and specificity in diagnosis of PTB are uncleared.

In this study, we aimed to evaluate the sensitivity, specificity and cut-off of serum tumor markers to PTB diagnosis by ROC, and expected to evaluate the correlations between serum tumor markers and clinically relevant parameters of PTB.

Materials and Methods

Study subjects

This study was designed as a case-control study with 565 subjects who visited the Yijishan Hospital from Jun to Dec in 2014. Case group was selected from subjects with PTB diagnosed by physicians according to reference (17). Cases were excluded if there was a change in diagnosis during the treatment period, and the smokers were not enrolled in this study. Control group was selected from individuals who underwent physical examination in Center of Health Examination of Yijishan Hospital during the same period. If the subjects with systemic infections, history of pulmonary tuberculosis, closely contact with PTB patients, tumor, neoplastic diseases and gynecological disorders were excluded from control group. We also excluded the subjects who were in inflammatory conditions (confirmed by standard diagnostic criteria) with clinical features that could overlap the TB. For example, subjects with differential diagnosis of PTB, and other severe respiratory infections representing patients who had non-tuberculous destructive pulmonary pathology.

The case group was matched age and sex with control group. The distribution of PTB cases and controls was not intended to reflect a particular population or epidemiological setting, but to encompass a broad range of symptomatically overlapping clinical presentations. The information including demographic characteristics was extracted from the electronic medical record. The protocol of this study was approved by Medical Ethics Committee of Wannan Medical College, all participants had been informed about the study and gave their consent.

Serum tumor marker detection

All participants were characterized by sputum smear microscopy and culture including subsequent antigen or molecular confirmation of *M. Tuberculosis*. Serum samples were collected within 2 days of the first presentation and before initiation of treatment for PTB patients.

Three milliliter venous blood was collected from the patients fasted for 12 h and then centrifuged and stored at −70 °C until assayed. Serum CEA, CA125 and CA199 were detected by Roche electrochemiluminescence instrument at Department of Nuclear Medicine of Yijishan Hospital. All detection reagents were the corresponding bundled reagents, and its operation was in strict accordance with the manual. The normal reference values were as follows: CEA≤5ng/ml, CAl25≤35 U/ml, and CAl99≤ 37 U/ml.

Statistical analysis

Before analysis, means (SD) or median (25th percentile, 75th percentile) was calculated for continuous variables and proportions for categorical variables. Chi-square test was conducted to compare the differences of demographic characteristics between case and control groups. And the mean concentrations of CA125, CA199 and CEA between initial treatment and retreatment, case group

and control group were tested by Kruskal-Wallis method of nonparametric test. In our study, the concentrations data of CA125, CA199 and CEA like other references are not normal distribution, and then we selected the non-parametric tests. Furthermore, the Mann-Whitney U test was applied to determine the abnormal rates of three tumor markers between case group and control group. Followed was identified the related serum tumor markers to PTB by logistic regression analysis. Then the ROC curve analysis was conducted to assess the difference between areas under the curve (AUC) which belonged to respective tumor marker and to evaluate the diagnostic sensitivity and specificity at the optimal cut-off. $P<0.05$ was considered to indicate a statistically significant difference. Data management and all analyses were performed using R software program, version 3.0.0 (http://www.R-project.org).

Results

Demographic characteristics

The case group was completed on 113 cases, including 58 PTB initial treatment patients (37 men, 21 women, age ranged from 16 to 81 yr, average age: 38.16±18.86) and 55 retreatment patients (35 men, 20 women, age ranged from 19 to 91 yr, average age: 52.18±21.22). Matched controls were 452 healthy people (288 men, 164 women, age range 16 to 91 yr old, average age: 45.17±20.97). There were no significant differences of age, sex and smoking between case and control group. In terms of drink and marital status, there were significantly different between two groups (Table 1).

The analysis of concentration levels and abnormal rates of three tumor markers between case and control groups

The concentration of serum CA125, CA199 and CEA in case group were found significantly higher than those in control group ($P<0.001$) (Table 2). We also accessed the abnormal rates of three tumor markers between two group, the abnormal rates of CA125 (Case group: 48.67% vs Control group: 0.22%) and CEA (Case group: 7.08% vs Control group: 0.88%) in case group were significantly higher than in control group ($P<0.001$). The abnormal rate of CA199 was no significant different between two groups (Table 3).

Table 1: Demographic characteristics of participants

Variables		Case group (n, %)	Control group (n, %)	χ^2	*P. value*
Sex	Male	72 (63.72)	288 (63.72)	0.00	1.000
	Female	41 (36.28)	164 (36.28)		
Age	16~44	58 (51.33)	232 (51.33)	0.00	1.000
	45~60	23 (20.35)	92 (20.35)		
	>60	32 (28.32)	128 (28.32)		
Marital status	Married	56 (49.56)	297 (65.71)	10.06	0.002
	Single	57 (50.44)	155 (34.29)		
Smoke	yes	0 (0.00)	1 (0.22)		1.000
Drink	yes	89 (78.76)	139 (30.75)	86.57	<0.001

Table 2: The comparison of concentration levels of three tumor markers between case and control group

Variables	Case group (*n*=113)	Control group (*n*=452)	*Z*	*P. value*
CA125 (U/ml)	33.71 (18.70, 68.86)	5.72 (3.87, 8.14)	402.64	<0.001
CA199 (U/ml)	9.09 (4.94, 13.55)	6.06 (4.18, 8.21)	20.68	<0.001
CEA (ng/ml)	1.89 (1.17, 3.11)	1.02 (0.68, 1.70)	52.33	<0.001

The analysis of concentration levels of three tumor markers between initial treatment and retreatment group

To understand whether the retreatment of PTB effect the three tumor markers, we test the levels of serum CA125, CA199 and CEA, there were no significant differences between initial treatment group and retreatment group.

Evaluation the association of three tumor markers with PTB

The logistic regression analysis was performed to evaluate the association of three tumor markers with PTB, PTB (case group=1, control group=0) was selected as dependent variable, and CA125, CA199 and CEA were selected as independent variables. The results showed that CA125 was significantly related to PTB (OR = 1.133, $P<0.001$) (Table 4).

Table 3: The comparison of abnormal rates of three tumor markers between case and control group (n, %)

Variables	Case group (n=113)	Control group (n=452)	χ^2	*P. value*
CA125	55 (48.67)	1 (0.22)	237.67	<0.001
CEA	8 (7.08)	4 (0.88)	16.69	<0.001
CA199	2 (1.77)	5 (1.11)	0.33	0.568

Table 4: The evaluation of association of three tumor markers with PTB by binary logistic regression

Factors	β	*SE*	*Wald*χ^2	*P*	OR (95%CI)
CA125	0.115	0.015	57.311	<0.001	1.133 (1.101-1.166)
CEA	0.200	0.126	2.529	0.112	1.222 (0.954-1.564)
CA199	-0.001	0.007	0.033	0.855	0.999 (0.985-1.013)
Constant	-3.493	0.281	154.625	<0.001	

The analysis of predictive value of three tumor markers by ROC

The ROC analysis revealed that for CA125, the AUC whose 95% confidence interval ranges from 0.951 to 0.981 was 0.966, and the cut-off value was 10.30, with the sensitivity, specificity 0.956 and 0.850, respectively.

Discussion

In this study we investigated the diagnostic value of three common clinical serum tumor markers, the CA125, CA199 and CEA in case group were found significantly higher than those in control group, but only CA125 was the positive association factor with PTB, the value of predictive power (AUC) was 0.966, the cut-off, sensitivity and specificity of CA125 were 10.30 U/ml, 0.956 and 0.850, respectively.

CA125 was identified by molecular cloning (18) and presents in the tracheal, bronchial, bronchiolar, terminal bronchiolar epithelium (19), as a cell surface glycoprotein involved in promoting ovarian cancer cell growth, and it was considered as a specific biological marker for ovarian cancer. However, our study suggested that CA125 was significantly higher PTB patients both in treatment and retreatment, and had higher sensitivity and specificity compare to another Study (15). The possible reason was that severe PTB was associated with more bronchial epithelial cells destruction, which induced the elevated CA125 level in PTB patients (16). A study in Germany explained that it was the inflammatory mesothelial cell proliferation, which induced the secretion of CA125 in patients with TB (20).

CA199 is another common clinical tumor marker in diagnosis pancreatic cancer (21) and gastric cancer (22). We selected CA199 and tested the diag-

nosis of predictive value to PTB, since there several case reports showed that CA199 was increased in a PTB patient (23) and in an endobronchial TB patient (24). However, this study found that CA199 had no significant to PTB in multivariables logistic aggression analysis. Canturk Tasci, et al. also reported that there were no statistically significant in CA199 values of PTB group before treatment when compared with that of the healthy control group, and the difference found in CA199 levels before and after treatment in patients with PTB was not statistically significant ($P<0.08$) (25). The reason has been unknown, whatever the results of case report may influenced by some casual factors.

A report in 1993 demonstrated that elevated serum CEA was found in 16.9% of patients with active pulmonary tuberculosis (26). Especially in female, a report showed that among 6 to 7 female patients with miliary tuberculosis, CEA had elevated (27). These finds were similar to our results, but in our study, CEA in male (1.35) was significantly higher than that in female (0.89), and the AUC of CEA was only 0.710. Thus diagnosing PTB with CEA is not a good method.

Differentiating pulmonary tumor from PTB is difficult, in our study there 48.67% PTB case were abnormal according to CA125 normal reference, interestingly, the cut-off in ROC curve of CA125 was 10.30, and it was significantly lower than its normal references of tumor ≤ 35U/ml. In other words, it may be a potential bio-marker which can differentiate the pulmonary tumor from PTB.

The present study has several limitations. Firstly, the patients in case group of this study were recruited only with PTB, thus the results may not be applicable in other type TB. Secondly, both case and control groups, we excluded patients according to imaging findings and clinical experiences instead of tissue biopsy, it was inevitable that the PTB and tumor patients incubation period were enrolled this study. Thirdly, all patients in our study were characterized by sputum smear microscopy and culture including subsequent antigen or molecular confirmation of *Mycobacterium TB* to determine the reference standard, so these results may be not applicable in smear-negative PTB. The mechanism of the association of the serum tumor markers with PTB remains to be further explored.

Conclusions

Serum CA125 has good diagnostic performances for PTB, this diagnostic accuracy would surpass that of other available immediate diagnostic options, and could yield a result much more rapidly than cultures.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgments

The project was supported by the National Innovation Entrepreneurship Training Program for Undergraduate of China (Grant NO. AH20140368010). The authors declare that they have no conflict of interest.

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