European Review for Medical and Pharmacological Sciences 2016; 20: 462-466

Cerebrospinal fluid IgG against TB-SA for diagnosis of tuberculous meningitis

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Abstract. – OBJECTIVE: A commercial enzyme linked immunosorbent assay (ELISA) detecting cerebrospinal fluid (CSF) IgG against TB-SA (CYPCO TB (IgG) ELISA assay) for diagnosing tuberculous meningitis (TBM) was evaluated retrospectively. Meanwhile, Pandy test was introduced to grade patients, improving its role in the detection of TBM.

PATIENTS AND METHODS: 187 consecutive cases with determined diagnosis were enrolled and divided into TBM group and Non-TBM group. CSFs were collected and sent for Pandy test, cytology, routine biochemistry tests, acid-fast smear and mycobacterial culture. The diagnostic parameters of the ELISA assay were evaluated. Differences in sensitivity between groups were estimated using a McNemar's test.

RESULTS: The overall sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of the ELISA assay were 31.6% (95% CI: 21.0-44.5%), 97.7% (95% CI: 93.4-99.2%), 85.8, 76.5, 13.7 and 0.7, respectively. McNemar's test showed the sensitivity of CYPCO TB (IgG) ELISA assay significantly positively correlated with the results of Pandy test ($p < 0.05$). The ten Pandy test "3+" patients were all TBM. Therefore, patients with "3+" Pandy test are highly suspected of TBM.

CONCLUSIONS: The commercial CYPCO TB (IgG) ELISA assay is able to rapidly confirm a diagnosis of TBM. The sensitivity of the ELISA assay is significantly positively correlated with the results of Pandy test. Patients with "3+" Pandy test are highly suspected of TBM.

Key Words:
Cerebrospinal fluid, Pandy test, Serodiagnosis, Tuberculous meningitis.

Introduction

Tuberculous meningitis (TBM) is the most lethal form of *Mycobacterium tuberculosis* (M.TB) infection. Approximately a third of patients die soon after presenting to hospital and many of those surviving are left with severe neurological sequelae[1-3]. Early recognition is believed to be able to reduce the burden of this disease, but this is hampered by the fact that it is often difficult to find bacteriological proof for TBM[4]. Currently, several molecular tests are evaluated, it's reported that the diagnosis of TBM continues to be a clinical challenge[4-6]. Therefore, routine biochemistry analysis (such as, total protein, glucose) with low specificity were still the main tool for diagnosing TBM[7-10].

A meta analysis reported that serological tests showed inconsistent and imprecise estimates of sensitivity and specificity in detection of tuberculosis[11]. The problem may contribute to several factors: M.TB burden, infection sites (pulmonary *vs* extra-pulmonary), antigen, immunoglobin type[11-14]. As a rapid and economic assay, serological test is still an attractive method for TB diagnosis. Since blood–brain barrier usually are disrupted in TBM, levels of CSF antibodies would increase. To detect CSF level of antibodies against M.TB may aid to diagnose TBM. The amount of globulins in CSF can be detected by the Pandy test, and the degree are qualitatively detected by turbidity or precipitates[15]. In the study, we evaluated a commercial assay detecting IgG against TB-SA (CYPCO TB (IgG) ELISA assay, Chengdou, Sichuan, China) in CSF for diagnosing TBM, retrospectively. Meanwhile, Pandy test was introduced to grade patients, improving the role of the ELISA assay in detection of TBM.

Patients and Methods

The study protocol was approved by the Ethics Committees of the Shandong Provincial Chest Hospital, written informed consent was waived for

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the retrospective nature. Between Jun, 2006 and May, 2014, 187 consecutive cases with determined diagnosis were enrolled; subsequently were divided into TBM group and Non-TBM group. For this study, TBM was defined as CSF microscopy or culture positive for M.TB. Patients were classified as 'Non-TBM' when an alternative diagnosis was established.

CSFs were collected from all patients, and sent for Pandy test, cytology, routine biochemistry tests, acid fast smear (Auramine O stain) and mycobacterial culture (Lowenstein-Jensen medium). CSF antibodies against M.TB TB-SA was detected by using CYPCO TB (IgG) ELISA assay (CYPCO, Chengdou, Sichuan, China), following the manufacturer's instruction. Levels of total protein (Leadman, Daxing, Beijing , China), glucose (Siemens, Tarrytown, NY, USA), lactate hydrogenase (Kehua, Xuhui, Shanghai, China), α-hydroxybutyrate dehydrogenase (Kehua, Xuhui, Shanghai, China), adenosine deaminase (Maker, Chengdou, Sichuan, China) and chloride ion (Siemens, Tarrytown, NY, USA) in CSF were assayed on an Advia 2400 chemistry analyzer (Siemens, Tarrytown, NY, USA).

Pandy Test was performed and the result was evaluated subjectively from zero to four plus, according to the density of the cloud produced. The criteria was as follows: "-", no cloud; "1+", faintly cloudy, smoky or hazy; "2+", turbidity clearly present, but newsprint easily read through tube; "3+", newsprint not easily read through tube; "4+", newsprint can't be seen through tub.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software. Data were expressed as mean ± standard deviation (SD). The statistical difference between the means was calculated compared using Mann-Whitney U test. The sensitivity, specificity, positive/negative predictive values and positive/negative likelihood ratios of the CYPCO TB (IgG) ELISA assay were evaluated. Differences in sensitivity between sub-groups were estimated using a McNemar's test. $p < 0.05$ was considered significant. All calculations were estimated at a 95% confidence interval (95% CI).

Results

The study participants included 129 Non-TBM patients (36.3 ± 18.3 years old, 73 males) and 57 TBM patients (34.5 ± 18.1 years old, 28 males). In the Non-TBM group, 52 patients were had viral meningitis, 13 cancer with brain metastases, 10 cryptococcal meningitis, 8 bacterial meningitis, 4 purulent meningitis and the remaining 42 had others' diseases. The results of Pandy test in Non-TBM group were as follows: 2+ (31 cases); 1+ (33 cases); negative (65 cases). In TBM group, only one case was confirmed by microscopy, the remaining was culture-confirmed.

Table I showed the characteristics of both groups. Mann-Whitney U analysis showed that CSF levels of white blood cell count, lymphocyte count, neutrophil count, total protein, glucose, lactate hydrogenase, α-hydroxybutyrate dehydrogenase, adenosine deaminase and chloride ion have significant difference between both groups (all $p < 0.01$).

The overall sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of CYPCO TB (IgG) ELISA assay were 31.6% (95% CI: 21.0-44.5%), 97.7% (95% CI: 93.4-99.2%), 85.8, 76.5, 13.7 and 0.7, respectively.

Pandy test, an assay to evaluate the CSF concentration of immunoglobins, was performed in our study. The results (Table II) showed that, no case was positive in CSF IgG against TB-SA among the "-" TBM patients, CYPCO TB (IgG) ELISA assay had a sensitivity of 0% (95% CI: 0-39.0%); among "1+" patients, it had a sensitivity of 9.1% (95% CI: 1.6-37.7%); among "2+" patients, it had a sensitivity of 36.7% (95% CI: 21.9-54.5%); among "3+" patients, it had a sensitivity of 60.0% (95% CI: 31.3-83.2%). McNe-

Table I. Clinical and cerebrospinal fluid data in TBM and Non-TBM groups.

	on-TBM	TBM
Number	129	57
Age (years)	36.3 ± 18.3	34.5 ± 18.1
Sex (male, %)	56,60%	49,10%
White blood cells (10^6/L)	63.4 ± 99.9	219.0 ± 229.3
Lymphocytes (%)	75.9 ± 27.7	58.0 ± 28.1
Neutrophils (%)	24.1 ± 27.7	42.0 ± 28.1
Total protein (g/L)	673 ± 533	1258 ± 619
Glucose (mmol/L)	2.55 ± 1.10	55.6 ± 230.4
Lactate dehydogenase (U/L)	31.5 ± 28.9	95.8 ± 94.5
α-hydroxybutyrate dehydrogenase (U/L)	25.0 ± 12.6	54.5 ± 45.8
Adenosine deaminase (U/L)	3.0 ± 3.5	6.6 ± 4.2
Chloride ion (mmol/L)	118.1 ± 6.9	108.5 ± 7.6

TBM: tuberculous meningitis.

Table II. CYPCO TB (IgG) ELISA assay in groups graded by Pandy test.

		CYPCO TB (IgG) ELISA assay		
		Negative	Positive	Total
Pandy test	-	6	0	6
	1+	10	1	11
	2+	19	11	30
	3+	4	6	10

mar's test showed the sensitivity of CYPCO TB (IgG) ELISA assay significantly positively correlated with the results of Pandy test ($p < 0.05$). It implied that the Pandy test was helpful to evaluate the diagnostic value of CSF IgG against TB-SA in detection of TBM. It is worth noting, that the ten Pandy test "3+" patients were all TBM. Therefore, patients with "3+" Pandy test are highly suspected of TBM.

Discussion

Our study showed the commercial assay detecting CSF IgG against TB-SA was a useful method in diagnosis of TBM. Meanwhile, there was significantly positive correlation between the results of Pandy test and CYPCO TB (IgG) ELISA assay. The Pandy test was helpful to evaluate the clinical value of the assay, data showed the sensitivity increased from zero in Pandy test "-" group to 60% in Pandy test "3+" group.

TBM is the most severe form of M.TB infection and delay of treatment is associated with high mortality and morbidity. Unfortunately, CSF microscopy has poor sensitivity, while mycobacterial culture need serveral weeks and, therefore, has a limited role in decisions about treatment of possible TB meningitis. Molecular tests have the potential to be the ideal tool in detection of TBM. Disappointedly, a systematic review showed that commercial PCR tests was a moderate sensitivity for diagnosis of TBM[16]. TB-SA, an acid phosphatase of M.TB, is coded by Rv3310, commercially known as M.TB specific antigen (TB-SA)[17]. In the past several years, numerous studies have demonstrated detection of antibodies against TB-SA is a rapid, simple, relatively sensitive and specific method for diagnosing tuberculosis[18-20]. Many M.TB antigens have been evaluated in detection of CSF antibodies, but the role of TB-SA in detection of CSF antibodies for diagnosis of TBM remains uncertain. In the present study, we showed the assay detecting CSF IgG against TB-SA had a moderate sensitivity and high specificity in detection of TBM. Pandy test, as an auxiliary assay, was helpful to improve the clinical use of the ELISA assay.

Yet, early diagnosis of TBM is notoriously difficult. The clinical features of TBM are nonspecific and currently available diagnostic tests lack sensitivity. As a consequence, treatment is given unnecessarily to some patients and delayed in many others[1]. Recently, several new TB assays have been evaluated. Feng GD et al evaluated modified Ziehl-Neelsen stain and ESAT-6 immunostain for detection of intracellular M.TB in CSF leukocytes, improving TBM diagnosis. The sensitivity were 82.9% for modified Ziehl-Neelsen stain and 75.1% for ESAT-6 immunostain, the specificity were 85.0% and 90.0%, respectively. The both assays improved the laboratory diagnosis of TBM[21]. Although the new tests shown promise in TBM diagnosis, further study need to be performed to validate their role in detection of TBM. Biochemical tests performed routinely on CSF were also evaluated in recent studies, such as glucose, total protein, cell counts, neutrophil predominance, and ADA[7-10]. These tests usually have low specificity and show limited diagnostic value. CSF ADA was a sensitive biomarker in detection of TBM, but the cutoff value of CSF ADA was low, the precision of chemistry analyzer may limit its use[22]. As we known, ELISPOT assays does not discriminate latent from active tuberculosis. In the past few years, ELISPOT assays using peripheral mononuclear cells and CSF mononuclear cells were evaluated in several studies, data supported it to be a useful adjunctive tool to the current tests for diagnosing TBM[23-25]. Other assays, such as detecting M.TB antigens, diagnostic scoring system, were also reported, but their diagnostic role still remains unclear[26-29].

This retrospective study can lead to selection bias, and thus to an inability to interpret or generalize results. Hence, a future approach will be to validate the diagnostic value of CYPCO TB (IgG) ELISA assay in detection of TBM prospectively. Meanwhile, there is urgently needed to evaluate a diagnostic model applying Pandy test and immunochromatographic tests (CSF albumin and CSF antibodies against M.TB antigens) in detection of TBM, the model maybe useful and easily performed in resource-limited settings.

Conclusions

The commercial assay detecting CSF IgG against TB-SA is able to rapidly confirm a diagnosis of TBM with an overall sensitivity and specificity of 31.6% (21.0-44.5%) and 97.7% (93.4-99.2%), respectively. The sensitivity of the ELISA assay significantly positively correlated with the results of Pandy test. Patients with "3+" Pandy test are highly suspected of TBM.

Acknowledgements

This work was supported in part by a grant from the Health Department of Shandong Province(NO.2011HZ085); in part by a grant from the Science and Technology Department of Jinan(NO.201303043).

Authors' Contributions

WMS and WXF conceived and designed the study. ZW supervised data collection. ZW and GXX collected data. WMS and WXF have been involved in the analysis and interpretation of data. WMS and WXF wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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