

Original Article

Evaluation of heparin-binding protein and/or procalcitonin levels in the diagnosis of bacterial intracranial infection using receiver operating characteristic (ROC) curve value

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Abstract: Objective: The aim of this study was to investigate the receiver operating characteristic (ROC) curve value to evaluate heparin-binding protein (HBP), procalcitonin (PCT), and HBP+PCT levels in the cerebrospinal fluid, and find a more valuable indicator for the early diagnosis of bacterial intracranial infections (BIIs). Methods: Patients (94 total) with intracranial infections were divided into bacterial intracranial infection (BII; n = 40) and non-bacterial intracranial infection (NBII; n = 54) groups, while 40 patients without intracranial infections were used as negative controls. HBP and PCT levels in the cerebrospinal fluid were tested in all cases. Glasgow scores were recorded for the BII group, and the correlation between HBP/PCT levels and Glasgow scores was analyzed. Results: There were significant differences in HBP levels between the BII, NBII, and control groups ($P < 0.05$), but not in PCT ($P > 0.05$) levels, and there was a positive correlation between HBP levels and Glasgow scores in BII group ($P < 0.05$). In the BII-control group, the area under the curve (AUC; 0.928) for HBP was significantly greater than that for PCT (0.802, $P < 0.05$), and the combined AUC for HBP and PCT was not greater than the AUC for HBP ($P > 0.05$) alone. In the BII-NBII group, the AUC for HBP was significantly greater than that for PCT ($P < 0.05$), and the AUC for the combination of HBP and PCT was not greater than that for HBP ($P > 0.05$). Conclusion: Cerebrospinal fluid HBP levels showed better sensitivity and specificity than PCT levels in the diagnosis of BII, and were related with disease severity.

Keywords: Heparin binding protein, procalcitonin, bacterial intracranial infection

Introduction

Bacterial intracranial infections (BII), including bacterial meningitis and encephalitis, are serious infectious diseases with high mortality and morbidity, and early diagnosis and timely treatment improves their prognosis [1]. However, the symptoms are similar to non-bacterial intracranial infection (NBII) caused by *Mycobacterium tuberculosis*, viruses, and other pathogens. Although bacterial culture has been treated as the gold standard for diagnosis of bacterial intracranial infection, it needs long time with low positive rate of diagnosis which could not meet the clinical needs for quick and definite diagnosis [2, 3], and routine cerebrospinal fluid tests, such as white blood cell (WBC), total protein, glucose, and C-reactive protein (CRP)

assays, do not detect pathogens. Recently, it was reported that procalcitonin (PCT) and heparin-binding protein (HBP) are valuable markers for the diagnosis of bacterial infections, and could be used for the differential diagnosis of BIIs [4, 5]. The purpose of this study was to investigate the diagnostic value of HBP and/or PCT in BIIs, with the goal of finding a valuable indicator for early diagnosis.

Materials and methods

Subjects and inclusion/exclusion criteria

Inclusion criteria: cerebrospinal fluid (CSF) WBC > 10 cells/ μ L with any of the following three items: (1) temperature (T) $\geq 38^{\circ}\text{C}$ for more than 3 days; (2) meningeal irritation (+); (3) blood

HBP and PCT for the diagnosis of bacterial intracranial infection

Table 1. Age and gender in different groups (n, %)

Clinical features	BII	NBII	Control	P
Number of cases	40	54	40	
Age	35.9 ± 16.8	36.9 ± 17.6	36.2 ± 17.2	0.708
Gender (Male, %)	23 (57.5%)	31 (57.4%)	19 (47.5%)	0.537

Table 2. HBP and PCT of CSF in different groups

Groups	HBP (ng/L)	PCT (ng/mL)
BII	88.1 ± 38.2	0.124 ± 0.034
NBII	30.1 ± 14.6	0.108 ± 0.019
Control	23.56 ± 11.2	0.095 ± 0.026
F	5.613	0.986
P	< 0.001	0.523

WBC > 10 × 10⁹ cells/L; or CSF culture was positive. There were 94 cases that met the inclusion criteria from January-December 2016 in Weifang People's Hospital, Xuanwu Hospital Affiliated to Capital Medical University, and Qianfoshan Hospital of Shandong Province. The patients included with intracranial infection were divided into a bacterial infection group (BII) and a non-bacterial infection group (NBII, eg. viral infection, tuberculosis infection, cryptococcal infection, etc.). Inclusion criteria for the BII group were [6]: (1) CSF WBC > 1000 cells/μL and neutrophil ratio > 75%; (2) CSF culture or Gram staining positive; (3) CSF glucose < 2.5 mmol/L or glucose ratio in CSF/blood < 0.4; other patients were included in the NBII group. Furthermore, 40 patients without intracranial infection (CSF WBC < 10/μL) were used as a negative control group. These patients suffered from disorders including schizophrenia, diabetes insipidus, spinal cord compression, and myelitis. Patients with chronic kidney disease, respiratory failure, heart failure, tumors, infectious disease, and immune dysfunction (systemic lupus erythematosus, rheumatoid arthritis, etc.) were excluded. The patients provided informed consent, and the medical Ethics Committees of the hospitals listed above approved this study.

CSF detection

The CSF of all patients in the BII and NBII groups was tested within 72 hours. CSF was collected through: (1) lumbar puncture; (2) lumbar pool drainage; (3) ventricular drainage; (4) ventricular mirror examination, using aseptic processing procedures. If there was a drainage tube, the CSF in the tube was drained first. WBC and glucose tests were performed within

2 hours by the bovine counting plate and hexokinase methods (Abbott Laboratories, Chicago, USA), respectively. The CSF was centrifuged at 200 × g for 5 minutes to obtain the supernatant, which was then stored at -80°C for HBP and PCT detection.

HBP was detected by enzyme linked immunosorbent assay (ELISA) using a rabbit anti-human HBP antibody (LS-C74497), provided by Lifespan Bioscience (Seattle, USA); PCT was detected by electrochemiluminescence using a cobas e 411 analyzer (Roche Diagnostics, Mannheim, Germany). To assess disease severity, physicians with standardized training recorded Glasgow scores for all patients within 72 hours of infection. The Glasgow Scale is used to give reliable and objective assessment of conscious state of patients. The highest score is 15, indicating consciousness is awake. Generally, disturbance of consciousness was classified as: minor, 12-14; moderate, 9-11; severe, 8 points or less. The lower the score, the more severe the disturbance of consciousness.

Statistical analysis

Normality was tested by one-sample Kolmogorov-Smirnov test. Normally distributed data is represented by the mean ± standard deviation (SD) and was compared using analysis of variance (ANOVA). Comparison of rates among multiple groups was conducted by ANOVA. The correlation between HBP, PCT and Glasgow score was analyzed by Pearson correlation coefficient. The area under the receiver operating characteristic (ROC) curve (AUC) was analyzed using MedCalc software. Differences were considered statistically significant when *P* < 0.05. Data were analyzed in SPSS Statistics 18.0.

Results

General information

There were no differences in age and gender between the BII, NBII, and control groups (Table 1).

HBP and PCT levels in the CSF

HBP was significantly higher in the BII group than in the NBII and control groups (*P* < 0.01) and there were no differences in PCT between the groups (*P* > 0.05, Table 2).

Table 3. AUC of HBP, PCT and PRE-1 in the BII group and the control group

	AUC	SE	95% CI	P
PRE-1	0.946	0.0275	0.855-0.988	*P = 0.6304
HBP	0.928	0.0374	0.831-0.979	*P = 0.0123
PCT	0.802	0.0563	0.679-0.894	#P = 0.0427

Compared with PRE-1 *P < 0.05, Compared with HBP #P < 0.05.

Pearson correlation analysis

Glasgow scores in the BII group positively correlated with HBP in the CSF (Pearson correlation coefficient = 0.785, $P < 0.05$). Among the cases, 12 had scores of 1-4 points, 16 cases had scores of 5-8 points and 12 cases had scores > 8. There was no relationship between the Glasgow score and PCT in the CSF (Pearson correlation coefficient = 0.342, $P > 0.05$).

ROC curve of the BII and control groups

The BII and control groups were defined as dependent variables, and HBP and PCT were defined as independent variables. Logistic regression was used to calculate the regression equation of HBP (X_1) and PCT (X_2), Logit $P = -10.404 + 0.097X_1 + 54.081X_2$, providing a new combined indicator (PRE-1, the combination of HBP and PCT) to diagnose BII. The ROC was established by HBP, PCT, and PRE-1, and the AUCs for HBP (0.928) and PRE-1 (0.946) were significantly greater than that of PCT (0.802, $P < 0.05$), while no difference was found between HBP and PRE-1 ($P > 0.05$, **Table 3; Figure 1A**).

ROC curve of the BII and NBII groups

The BII and NBII groups were defined as dependent variables, and HBP and PCT were defined as dependent variables. Logistic regression was used to calculate the regression equation of HBP (X_1) and PCT (X_2), Logit $P = -12.48 + 0.079X_1 + 63.076X_2$, providing a new combined indicator (PRE-2) to diagnose BII. The ROC was established by HBP, PCT, and PRE-2, and the AUCs for HBP (0.890) and PRE-2 (0.914) were significantly greater than PCT (0.720, $P < 0.05$), while no difference was found between HBP and PRE-2 ($P > 0.05$, **Table 4; Figure 1B**).

Discussion

Intracranial infection is a common neurological disease caused by a number of pathogens.

Currently, the mortality and morbidity of BII are very high. The incidence of BII in developed countries is approximately 5 per 100,000 adults, which is 10 times higher than in developing countries [7]. Meningeal irritation sign is the main clinical manifestation of bacterial intracranial infection and other types of infections in acute attack. It is very difficult to differentiate the types of intracranial infection by clinical manifestations alone, which always leads to confusion of differentiation of bacterial infection and non-bacterial infection in early stage [8].

The diagnosis of intracranial infections depends on examination of the CSF [2], including cytologic, protein, glucose, and chloride assays, CSF smears, and bacterial cultures, and polymerase chain reaction (PCR) [9]. Although the sensitivity of these tests is very high, their specificity is generally insufficient for differential diagnosis. In contrast, the specificity of CSF smears, bacterial cultures, and PCR are high, the detection rates are low, with a high possibility of false negatives. Bacterial culture is the gold standard for the diagnosis of BII, but this requires at least 3 days, which is too long for early diagnosis. Therefore, it has been important to find a new indicator with high sensitivity and specificity to quickly identify BII.

HBP is expressed in the azurophil granules of neutrophils and its expression is extremely low in healthy individuals but increases after bacterial infection [10, 11]. Previous studies have found that the HBP level is related to the diagnosis and prognosis of bacterial infections [12-15].

Linder et al. [5] reported that the AUC of HBP in the diagnosis of BII was 0.994 with cut-off value of 20 ng/mL, with 100% sensitivity and 99.2% specificity. In this study, the AUC of HBP in the diagnosis of BII was 0.928 with cut-off value of 34.3 ng/mL, and the sensitivity and specificity were 86.7% and 82.3%, respectively. The differences may result from the small sample size in this study. In addition, some patients had been treated before admission, the CSF was collected at different times, and patients were not classified according to disease severity. However, the results indicate that HBP is related with the severity of BII, and is a better prognostic factor for organ dysfunction than PCT, CRP, and WBC levels (AUC = 0.80) [15].

HBP and PCT for the diagnosis of bacterial intracranial infection

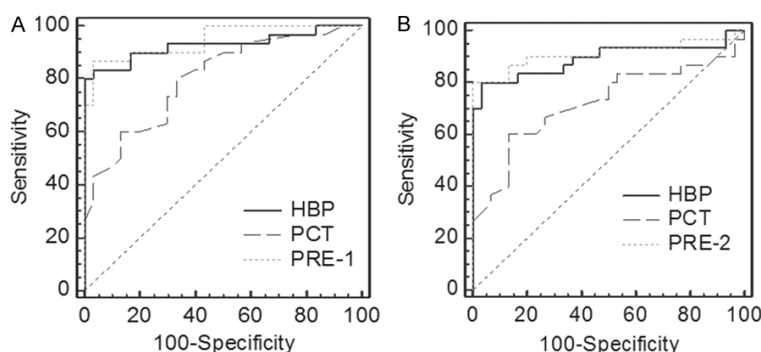


Figure 1. A: ROC of HBP, PCT and PET-1 in the BII and the control group; B: ROC of HBP, PCT and PRE-2 in the BII and the non-BII groups.

Table 4. AUC of HBP, PCT and PRE-2 in the BII group and the non-BII group

	AUC	SE	95% CI	P
PRE-2	0.914	0.0321	0.809-0.969	*P = 0.2659
HBP	0.890	0.0539	0.782-0.956	*P = 0.007
PCT	0.720	0.0631	0.589-0.828	#P = 0.0364

Compared with PRE-2 *P < 0.05, Compared with HBP #P < 0.05.

PCT is normally secreted by thyroid C cells, and many cell types (liver, lung, kidney, fat, muscle, etc.) release PCT during systemic inflammatory responses [16]. Serum PCT is an important diagnostic indicator of bacterial sepsis [17], and increases with prolonged bacterial infection. Because PCT has a high molecular weight, precluding its passage through the blood-cerebrospinal fluid barrier, the cerebrospinal fluid PCT level of BII patients may reflect the infection situation [18]. The results of the study showed that the AUC of PCT was lower than those of HBP and PRE-1. When the cut-off value was 0.1 ng/mL, the sensitivity and specificity were 80.0% and 66.7%, respectively, meaning that the diagnostic value was poor, consistent with the results of Hu et al. [19]. This may be because few brain cells would release PCT, and PCT in the blood could not pass through the blood-cerebrospinal fluid barrier. Other studies have found that PCT is an important predictor of intracranial infection after craniocerebral surgery [2], which may be related to brain injury, stress responses, bleeding, and an incomplete blood-brain barrier. Furthermore, Jereb et al. [20] studied central nervous system infections, and found that at a PCT cut-off value of 0.5 ng/mL, the positive predictive values for

bacterial and viral infections were 100% and 74%, respectively, which is inconsistent with the present study. Therefore, whether the level of CSF PCT increases in patients with BIIs remains controversial.

Correct diagnosis in the early stages of intracranial infection is important to improve prognosis. In this study, the AUC of HBP was higher than PCT and PRE-2 in the BII and NBII group, and when the cut-off

value of HBP was 51 ng/mL, the sensitivity and specificity for BIIs were 80% and 96.67%, respectively. At the same time, the AUC of PCT was 0.720, which was not high enough to diagnose BII. This indicates that HBP is of great clinical value in the early differential diagnosis of intracranial infection.

However, there are some limitations in this study. The sample size was small, and although some studies have reported increased blood PCT in BIIs [4], blood PCT levels were not tested. Furthermore, *M. tuberculosis* intracranial infection was not distinguishable from bacterial intracranial infection, as HBP levels were the same [21].

In summary, HBP is valuable in the differential diagnosis of BIIs and NBIs, and also is related to disease severity. Evaluation using a combination of HBP and PCT levels was not better than HBP levels alone in the diagnosis of BIIs.

Disclosure of conflict of interest

None.

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