Clinical Biochemistry 45 (2012) 215–218

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

The diagnostic value of apolipoprotein E in pediatric patients with invasive bacterial infections

Chuanqing Wang [a], Yi Wang [b], Aimin Wang [a], Pan Fu [a], Yi Yang [c,*]

[a] The Clinical Microbiology Lab, Department of Nosocomial Infection Control, Children's Hospital of Fudan University, 399 Wanyuan Road, Shanghai 201102, China
[b] Department of Neurology, Children's Hospital of Fudan University, 399 Wanyuan Road, Shanghai 201102, China
[c] Institute of Pediatrics, Key Laboratory of Neonatal Diseases, Ministry of Health, Children's Hospital of Fudan University, 399 Wanyuan Road, Shanghai 201102, China

ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 11 November 2011
Accepted 14 November 2011
Available online 2 December 2011

Keywords:
Apolipoprotein E
Bacterial meningitis
Sepsis
Diagnostic value
Pediatric patients

ABSTRACT

Objective: To evaluate the potential diagnostic value of apolipoprotein E (ApoE) measurements in pediatric patients with invasive bacterial infections.

Design and method: A total of 185 pediatric patients were enrolled in this study, including 94 patients with confirmable infections and 91 patients without confirmable infections. Serum and cerebrospinal fluid (CSF) ApoE levels were measured by immunoturbidimetry. The diagnostic values of ApoE were evaluated by the receiver operating curve (ROC) method.

Results: ApoE levels in CSF were significantly increased in patients with bacterial meningitis, and serum ApoE was markedly elevated in patients with sepsis or with bacterial meningitis compared with patients with other infections and uninfected children. The optimal ApoE cutoff value for CSF was >1.7 mg/L with 85% sensitivity and 100% specificity and was >42 mg/L in serum with 80% sensitivity and 93% specificity.

Conclusion: ApoE detection provided a novel diagnostic marker for invasive bacterial infections in pediatric patients.

Introduction

The diagnosis of bacterial infections can be difficult in some cases. The clinical presentation of infections caused by different pathogenic microorganisms is not specific in some cases. The diagnostic values of some indicators of bacterial infections are limited because the indicators show varying sensitivity at different stages of a disease [1]. Hence, the use of antimicrobial agents, particularly in the initial phase of the treatment of an infection, has become a common phenomenon despite the lack of sufficient evidence regarding their efficacy for a given bacterial infection [2]. The overprescription of antibiotics is ineffective, and it contributes to the development of resistance in the pathogens [3]. Moreover, overprescription also increases treatment costs and the probability of developing antibiotic-associated diseases [4].

Bacterial meningitis (BM) and sepsis are invasive conditions that lead to high mortality and disability in neonates and children [5,6]. Obtaining a quick diagnosis is necessary for providing optimal treatment. Bacteria cultures are the best screening technique in the case of a bacterial infection. However, the overprescription of antibiotics has undermined the sensitivity of this technique. Moreover, the hematologic markers that help in the early diagnosis of bacterial infections usually overlap in infections caused by different bacteria [7].

Apolipoprotein E (Apo E), a 34-kDa protein that is synthesized at high levels by hepatocytes and at low levels by astrocytes in the brain, has been observed to increase in the serum of patients with sepsis [8–10], but there are no reports on the changes of ApoE concentrations in the cerebrospinal fluid (CSF) of patients with encephalic bacterial infections. We hypothesized that the elevation of ApoE levels in serum or in CSF may be a potential marker for the diagnosis of invasive bacterial infections in pediatric patients. In this study, we aimed to detect the variations in ApoE levels in the serum and CSF of BM and sepsis patients and to evaluate the diagnostic value of ApoE in children with invasive bacterial infections.

Methods

Subjects

This study was conducted from January to July 2010 at the Children's Hospital of Fudan University, Shanghai, China. The study protocol was approved by the hospital's ethics committee.

Pediatric patients with acute pyrexia (axillary temperature > 38 °C) accompanied by neurological symptoms were enrolled in this study. BM cases were defined as those presenting with clinical symptoms of meningitis; the causative bacteria were identified directly by culturing blood or CSF and indirectly by an antigen test or Gram staining the CSF samples. In addition, CSF pleocytosis (CSF white blood cell count > 15×10^6/L for infant patients and > 30×10^6/L for neonatal

* Corresponding author. Fax: +86 21 64931883.
E-mail address: yyang@shmu.edu.cn (Y. Yang).

0009-9120/$ – see front matter © 2011 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2011.11.009

patients) was analyzed [11–13]. Aseptic meningitis (AM) cases were defined as those presenting with clinical symptoms of meningitis and showing CSF pleocytosis and a negative bacteriological profile in the CSF or blood [11,12]. Cases of confirmed sepsis were defined as those showing a positive blood culture, except for the growth of organisms considered to be contaminants, such as coagulase negative staphylococcus.

Laboratory measurements

The following tests were performed on all patients. The C-reactive protein (CRP) levels in the peripheral blood samples were detected by immunoturbidimetry (QuikRead, Orion Diagnostic Inc., Finland). The procedure has followed the protocol provided by the kit. The peripheral blood white blood cell (WBC) counts were performed by automatic blood analyzer (XS-800i, Sysmex, Japan). The CSF WBC count was tested by direct counting method [12]. The CSF protein levels were tested by one-step method (Wako Pure Chemical Industries, Ltd., Japan). The CSF glucose levels were tested by oxidase assay (keHua Bioengineering Corporation, China). The CSF chloride levels were tested by chloride ion selective electrode method (HITACHI, Japan) [12]. The *Streptococcus pneumonia* antigens in the CSF samples were tested by immunochromatographic membrane assay (Binax Now *Streptococcus pneumonia* Test, Binax, Inc., USA). The enterovirus RNA in the CSF samples were detected by using the commercial licensed kit (Da An Gene Co. Ltd, China) and carried out by real-time RT-PCR assay in our clinical virology laboratory. The blood samples were cultured by blood culture machine (BacT/ALERT 3D 240, France) and the CSF samples were cultured by blood agar and chocolate agar (Biomerieux, Shanghai, Ltd. China). The bacteria were identified by VITEK 60 system (Biomerieux, France).

The ApoE levels in the serum and CSF samples were tested by immunoturbidimetry (ApoE Auto N DAIICHI; Daiichi).

Statistical methods

The results obtained by the analysis of different variables are expressed as the mean ± standard deviation (±SD) or the mean with 95% confidence interval (CI). The variables in different groups were compared by a one-way analysis of variance (ANOVA). ApoE cutoff values in CSF and serum samples were calculated by using the receiver operating characteristic (ROC) curve method. The diagnostic value was evaluated on the basis of the area under the ROC curve (AUC). The level of significance was set at $p<0.05$, and the statistical analyses were conducted using SPSS 16.0.

Results

A total of 185 pediatric patients were included in this study; their clinical characteristics are shown in Table 1. The strains isolated from the blood of patients with sepsis included staphylococci (n = 12), enterobacteria (n = 6), *Enterococcus* (n = 8), *Streptococcus* (n = 6), and other organisms (n = 4). The bacteria from the CSF and blood samples of patients with BM included staphylococci (n = 11), *S. pneumoniae* (n = 6), *Escherichia coli* (n = 5), *Enterococcus* (n = 3), and *Listeria monocytogenes* (n = 1).

Table 1
Clinical characteristic of the different patient groups.

	BM	AM	Sepsis	Control	*p* value
Patient number	26	32	36	91	0.000
Male (%)	67	32	42	41	0.06
Age (median) (range)	3.9 m (1 m–11 y)	4 y (2 m–10 y)	2.9 m (1 m–5 y)	1 y (1 m–13 y)	0.000
Fever time (day)[a]	2.5 ± 1.5	3.2 ± 2.0	2.7 ± 2.0	3.1 ± 1.4	0.64

BM — bacterial meningitis, AM — aseptic meningitis, m — month, y — year.
[a] Fever time before admission.

The CSF ApoE levels were significantly increased in the BM group compared with the other three groups ($p<0.0001$). The serum ApoE levels in the sepsis group and the BM group were significantly higher than those in the AM group and the control group ($p<0.0001$) (Fig. 1). Regression analysis showed a significant correlation between the variation in the CSF ApoE levels and the time course of BM ($R=0.75$, $p<0.001$), but there was no correlation between the variation of serum ApoE levels and the course of sepsis ($R=0.16$; $p=0.35$) (Fig. 2). In addition, to investigate the impact of serum ApoE on CSF ApoE levels, we analyzed the correlation between these two sources of ApoE. The results showed that the changes in the serum ApoE and CSF ApoE levels in patients with sepsis were not correlated ($R=-0.084$; $p=0.71$).

To evaluate the diagnostic value of ApoE for invasive bacterial infections in pediatric patients, a ROC analysis was performed (Fig. 3). Cutoff values were obtained from the ROC analysis. The optimal cutoff value of CSF ApoE was > 1.7 mg/L. At this cutoff value, the sensitivity of the CSF ApoE measurement was higher than the CSF protein test, the

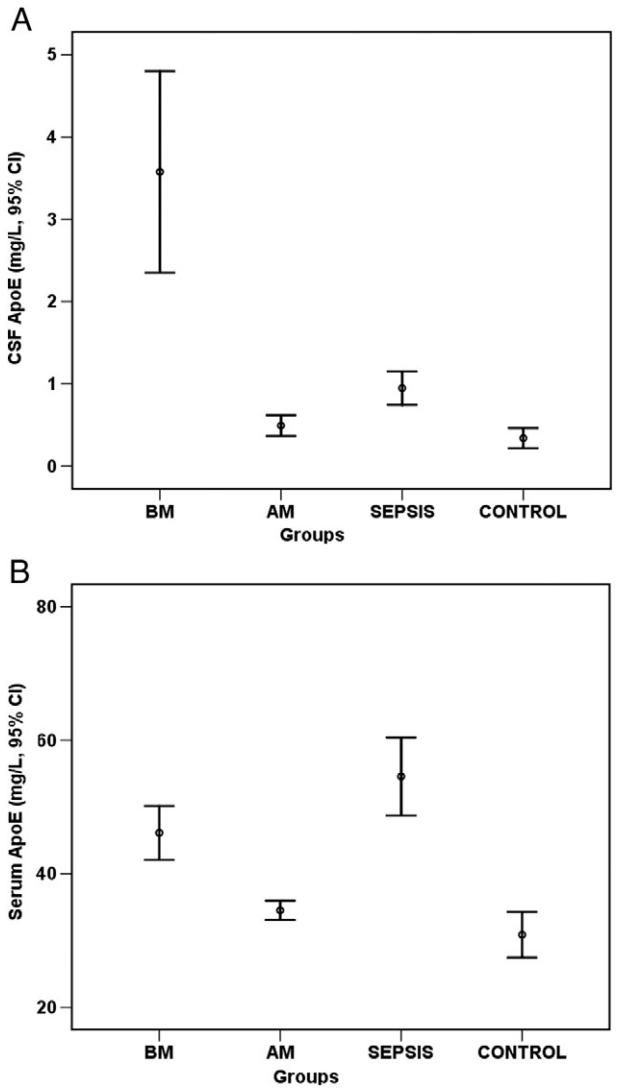

Fig. 1. Apolipoprotein E (ApoE) levels in the cerebrospinal fluid (CSF) and serum from patients of different groups. (A) CSF ApoE levels were 3.6 mg/L (95% CI, 1.04–0.14) in the BM group with onset ≤6 days, 0.6 mg/L (95% CI, 0.1–0.01) in the AM group, 0.9 mg/L (95% CI, 0.15–0.03) in the sepsis group, and 0.3 mg/L (95% CI, 0.08–0.01) in the control group ($p<0.0001$). (B) Serum ApoE levels were 55.1 mg/L (95% CI, 7.94–4.01) in the sepsis group, 48.8 mg/L (95% CI, 5.72–3.50) in the BM group, 34.6 mg/L (95% CI, 3.83–2.68) in the AM group, and 31.2 mg/L (95% CI, 4.53–2.06) in the control group ($p<0.000$).