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Short Communication

Clinical significance of the serum biomarker index detection in children with Henoch-Schonlein purpura

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ABSTRACT

Objective: To explore a panel of serum biomarkers for laboratory diagnosis of pediatric Henoch-Schönlein purpura (HSP).

Methods: The blood white blood cells (WBC) and serum levels of serum amyloid A (SAA), interleukin 6 (IL-6), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin E (IgE), C-reactive protein (CRP), complement component 3 (C3), complement component 4 (C4), and ASO (anti-streptolysin O) were detected in 127 patients with Henoch-Schonlein purpura (HSP), 110 cases of septicemia patients, and 121 healthy volunteers. The diagnostic ability of biomarkers selected from HSP and septicemia patients was analyzed by ROC curve. By designing the calculation model, the biomarker index was calculated for laboratory diagnosis of HSP and differential diagnosis between HSP and septicemia.

Results: The levels of serum WBC, CRP, IL-6 and SAA in the septicemia patients were significantly higher than those in the control group ($p < 0.05$). Compared with the healthy individuals, serum levels of WBC, CRP, IL-6, SAA, IgA and IgM were significantly increased in patients with HSP ($p < 0.05$). The area under the curve (AUC) of SAA, IgA, IgM, WBC, IL-6, and CRP in the patients with HSP was 0.964, 0.855, 0.849, 0.787, 0.765, and 0.622, respectively. The values of SAA, IgA, IgM, WBC, IL-6, and CRP in septicemia patients were 0.700, 0.428, 0.689, 0.682, 0.891, and 0.853, respectively. Biomarker index = $(SAA + IgA/4000 + IgM/4000) \times 0.4^{\frac{CRP\ (mean\ value)}{CRP\ (i)}}$. The biomarker index in HSP patients was significantly higher than that of the healthy controls. However, the biomarker index in septicemia patients was significantly lower than the control.

Conclusion: The biomarker index of HSP patients is higher than that of the control group. While in the infectious disease represented by septicemia, it is decreased. The detection of biomarker index could exclude the interference of infection as the auxiliary examination to HSP patients.

1. Introduction

Henoch-Schonlein purpura (HSP) is an IgA-mediated vascular allergic hemorrhagic disease that usually involves the skin, joints, gastrointestinal tract and kidneys [1]. Most common clinical manifestations are skin rashes and petechiae, accompanied by abdominal pain, joint swelling, proteinuria, and other symptoms [2]. The exact cause of HSP is still unknown [3]. An abnormal immune response to an infection is believed to play a pivotal role in many cases through the cytokines and immunoglobulin [4]. Other cases of HSP have been associated with vaccinations, foods, drugs, and chemicals. Up to now, no definitive laboratory test is available for the diagnosis of HSP disorder [5]. Auxiliary examinations are often used to exclude other disease and evaluate complications. There have been a large number of reports on the relationship between c-reactive protein (CRP), procalcitonin (PCT), interleukin 6 (IL-6), etc. and clinical manifestations in HSP patients [6]. In our previous study, serum amyloid A (SAA), CRP, IL-6, white blood cell (WBC), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin E (IgE), immunoglobulin G (IgG), anti-streptolysin O (ASO), complement component 3 (C3), complement component 4 (C4) and ferritin were analyzed and assessed by ROC (receiver operating characteristic) curve. Among them, SAA was found to be the most valuable biomarker for laboratory diagnosis of patients with HSP [7]. However, the levels of SAA, WBC, and CRP, which have clinical significance for the diagnosis of HSP, have also increased in serum when the body is infected [8,9]. By detecting these biomarkers for use in the adjuvant procedures will reduce the specificity of the laboratory diagnosis of HSP. Biomarker panel could improve the diagnostic accuracy of HSP, and might help the differential diagnosis of it [10,11]. However, simultaneous detection of multiple biomarkers also increases the

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Table 1
Comparison in the levels of serum biomarkers between HSP, septicemia patients and healthy volunteers.

Biomarker	Healthy volunteers (n = 121)	HSP patients (n = 127)	Septicemia patients (n = 110)
WBC ($\times 10^9$/L)	7.726 ± 2.23*$^{\Delta}$	11.03 ± 4.19*	10.98 ± 7.20$^{\Delta}$
IgA (mg/L)	1195.98 ± 385.10*	2251.07 ± 993.23*	1127.03 ± 581.11
IgE (IU/mL)	115.895 ± 183.04	157.85 ± 211.09	118.61 ± 196.32
IgG (mg/L)	10,125.61 ± 1396.3	9394.39 ± 2329.53	9081.04 ± 2015.69
IgM (mg/L)	744.98 ± 195.76*	1187.78 ± 362.46*	655.65 ± 475.25
C3 (mg/L)	1111.80 ± 182.73	1174.10 ± 223.09	1229.6 ± 207.62
C4 (mg/L)	261.20 ± 60.93	256.37 ± 70.89	327.31 ± 71.93
ASO (IU/mL)	42.38 ± 30.14	54.96 ± 50.60	48.65 ± 48.65
Ferritin (ng/L)	80.53 ± 54.77	71.75 ± 38.06	79.62 ± 39.67
CRP (ng/L)	4.66 ± 4.19*$^{\Delta}$	16.35 ± 32.44*	72.23 ± 86.86Δ
IL-6 (pg/mL)	5.35 ± 2.48*$^{\Delta}$	38.44 ± 97.46*	44.25 ± 93.11Δ
SAA (μg/mL)	1.83 ± 0.88*$^{\Delta}$	6.06 ± 5.07*	2.23 ± 0.36Δ

*HSP VS control ($p < 0.05$); Δ: Sepsis group VS control ($p < 0.05$).

patient's financial burden, especially for some candidates without significant value. Several researchers focus on improving the sensitivity and specificity of the test items, yet it still remains to be elucidated.

In view of the interference of the infection factors for the diagnosis of HSP, a systemic inflammatory response (SIRS) disease, also called sepsis, caused by various pathogenic bacteria is selected as a reference. As the toxemia can cause tissue organ cell degeneration, microvascular embolism, tissue necrosis and bleeding, the levels of infectious biomarkers in sera will be significantly increased [12,13]. Recently, there was a lack of systematic study to evaluate the sensitivity and specificity for differential diagnosis of HSP and septicemia patients.

In this study, we analyzed the biomarkers of HSP and sepsis patients, and evaluated them as auxiliary test. The biomarker index was calculated according to a formula, which was finally used to improve the specificity of lab test for patients with HSP.

2. Material and methods

2.1. Patients

One hundred and twenty one healthy volunteers with a median age of 7.2 (2–13 years of age) and 127 HSP patients with a median age of 7.3 (2–14 years) were recruited from Tianjin Children's Hospital. One hundred and ten cases of sepsis, including 64 males and 46 females with the age of 1 to 10 years were also collected in the study. Among them, there were 54 cases with gram-negative bacteremia, and another 56 cases were gram-positive. This study has been cleared by our Institution Ethics Review Board for human studies and all patients have signed an informed consent.

2.2. Specimen collection

The venous blood (2–3 ml) was obtained from the patients and healthy individuals in the early morning. After centrifugation, the serum was separated and stored at − 80 °C prior to analysis.

2.3. Biomarker detection

Serum IL-6 and IgE were detected using Roche COBASE601 electrochemical luminescence detector (Roche, Germany), according to the manufacturer's instructions. IgA, IgM, IgG, C3, C4, and ASO were measured by Beckman Kurt IMMAGE800 specific protein analysis system (Beckman Coulter Co.,), according to the manufacturer's instructions. WBC was counted by XisenMi Kang XS-800i automatic five-class blood analyzer. Serum CRP and ferritin levels were measured using the Nycocard READER II Calibrator (Axis-Shield PoC AS, Norway) and the Unicel DXI 800 Immunoassay System (Beckman Coulter, Inc.), respectively. SAA was detected by a commercial ELISA kit (Shanghai Xin Le Biotechnology Co., Ltd., China).

2.4. Statistical processing

Statistical analysis was performed using SPSS19.0 Statistics software. The non-parametric test and unpaired *t*-test were used to analyze the data of each group. A two-sided P value < 0.05 was considered statistically significant. For the biomarkers with statistical significance, the ROC curves were used to evaluate the value and the optimal threshold of the test indicators. The area under the curve (AUC) could distinguish between non-informative ($AUC \leq 0.5$), less accurate ($0.5 < AUC \leq 0.7$), moderately accurate ($0.7 < AUC \leq 0.9$), highly accurate ($0.9 < AUC < 1$) and prefect tests ($AUC = 1$) [14].

3. Results

3.1. Serum biomarker levels in patients with HSP and sepsis

The levels of serum WBC, CRP, IL-6 and SAA in the septicemia patients were significantly higher than those in the control group ($p < 0.05$). Compared with the healthy individuals, serum levels of WBC, CRP, IL-6, SAA, IgA, and IgM were significantly increased in patients with HSP ($p < 0.05$) (Table 1). There was no statistical significance in other biomarkers between HSP or sepsis patients and healthy control.

3.2. The AUC values of selected biomarkers in HSP and sepsis patients

In order to evaluate the diagnostic ability of serum biomarkers, the AUC values of them in patients with HSP and septicemia patients were analyzed by ROC curve. The AUC values of SAA, IgA, IgM, WBC, IL-6, and CRP in the patients with HSP were 0.964, 0.855, 0.849, 0.787, 0.765, and 0.622, respectively. The values of SAA, IgA, IgM, WBC, IL-6, and CRP in sepsis were 0.700, 0.428, 0.689, 0.682, 0.891, and 0.853, respectively (Fig. 1A～B).

3.3. Biomarker index in patients with HSP

$$\text{Biomarker index} = (\text{SAA} + \text{IgA}/4000 + \text{IgM}/4000) \times 0.4\frac{\text{CRP (mean value)}}{\text{CRP (i)}}$$

The biomarker index was calculated by using the formula above in patients with HSP and septicemia patients and healthy controls [15,16]. The biomarker index of HSP patients was significantly higher than that of the control group (2.999 ± 2.42 vs. 1.40 ± 0.81; $p < 0.05$). However, the septicemia patients group was significantly lower than the control group (0.552 ± 0.76 vs. 1.40 ± 0.81; $p < 0.05$). Furthermore, the clinical significance of biomarker index in HSP patients was analyzed by ROC curve. The AUC value of biomarker index was