

Original Article

Evaluation of serum high sensitive C-reactive protein, procalcitonin, neopterin and leukocyte on different respiratory infectious disease in Chinese children

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Abstract: Objective: To investigate serum high sensitive C-reactive protein (hs-CRP), procalcitonin (PCT), neopterin (NP) and leukocyte (WBC) on different pathogens of respiratory infectious disease, and to provide antibiotic therapy with favorable evidences in Chinese children in the region of guangdong. Methods: 438 infants and children were diagnosed and divided into normal bacterial infection (group A1), suppurative tonsillitis (group A2), mycoplasma (Mp) infection (group B) and virus infection (group C). 50 healthy children were taken as control. Serum hs-CRP, PCT, NP, WBC and blood routine were detected and compared in all groups before and after antibiotic therapy. Results: Serum hs-CRP (mg/L) was higher in A1 (11.55±9.31), A2 (46.38±40.17) and B (6.25±2.64) groups than in control (1.39±1.76) group, significantly (P<0.05). Serum PCT (ng/mL) was higher in A1 (1.33±6.90), A2 (1.41±4.31), B (0.26±4.98) and C (0.18±7.10) groups than in control (0.05±5.75) group, significantly (P<0.05). Serum NP (nmol/L) in A1 (19.05±8.94), A2 (35.86±12.76), B (33.75±10.44) and C (43.51±15.90) groups was higher than in control (6.67±3.32) group, significantly (P<0.05). Serum WBC (1×10⁹/L) was higher in A1 (13.82±10.81) and A2 (13.64±10.55) groups than in control (7.45±3.30) group, significantly (P<0.05). The positive rate of hs-CRP in A1 (71.33%) and A2 (77.78%) groups were higher than in B (30.86%) and C (14.80%) group. The positive rate of PCT in A2 (100%) group was the highest; while in A1 (40.56%) group was higher than in B (32.10%) and C (17.35%) groups. The positive rate of NP in A1 (68.51%), A2 (72.22%), B (80.25%) and C (96.94%) increased gradually. The positive rate of WBC in A1 (51.75%) group was higher than in A2 (33.33%), B (34.57%) and C (15.31%) groups. The sensitivities and specificities of hs-CRP, PCT and WBC for bacterial respiratory infection were 72.05% and 100%, 47.20% and 98.00%, 49.69% and 96.00%, separately, while NP for viral were 96.94% and 100%. Conclusions: Detection of serum hs-CRP, PCT, NP and WBC was helpful for identification of bacterial, Mp pneumonia and virus infection. Using serum hs-CRP to evaluate bacterial respiratory infection was more sensitive than using serum PCT or WBC, independently, while using serum NP to evaluate viral respiratory infection was more sensitive. Combining serum hs-CRP, PCR, NP and WBC to evaluate different kinds of respiratory infection was more suitable clinically.

Keywords: C-reactive protein, procalcitonin, neopterin, leukocyte, respiratory infectious disease, children

Introductions

Respiratory infection was one of the large global burden of diseases. In 2010, lower respiratory tract infections accounted for 115,227,000 disability-adjusted life years (DALYs) [1]. According to Walker [2] et al., about 120 million were infected with pneumonia episodes and 1.2 million died for it, and 72% of the deaths were children with age under 2 years. The majority of the patients and the mortality occurred in Southeast Asia and Africa.

High sensitivity C-reactive protein (hs-CRP), as the marker of systemic inflammation, was used for analyzing and identifying bacterial infection. hs-CRP was consistently found to be related to the respiratory infections [3, 4], which was also found to be associated with lung function damage by Rasmussen [5] et al. Serum hs-CRP would not increased or just increase slightly when with virus infection, but would increase obviously when with direct trauma or bacterial infections. Depending on calcium, hs-CRP would combine with pathogens, injured tissues

Different respiratory infectious disease

and nuclear antigens. Different from the specific recognition of IgG to antigen, hs-CRP recognized the changed auto-antigens and exotic molecules according to pattern recognition. According to the phenomenon mentioned above, Du Clos [6] et al. proposed the basic theories including early-stage defense, signaling and activation of proinflammatory cells, humoral immunity and the activation of acquired immunity.

In previous studies, hs-CRP combined with different indexes was used for evaluating acute coronary syndrome (ACS) [7], carotid atherosclerosis (CAS) [8] and so on. Xie [9] et al. examined the serum PCT, CRP and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) for predicting the survival of the patients with early-onset stroke associated pneumonia (EOP). Zhao [10] and Pang [11] et al. investigated the serum hs-CRP in prehypertension combined with cardiovascular risk and hypertensive heart diseases accompanied with coronary artery disease. PCT, NP and CRP were both evaluated on differentiating bacterial from viral etiologies in patients with lower respiratory tract infections [12]. However, there was no relative report about serum hs-CRP combines with PCT, NP, and leukocyte (WBC) evaluated the different respiratory infectious disease in children.

PCT [13], NP [14] and leukocyte (WBC) [15] were also used for diagnosis of whether there was bacterial or virus infection, clinically.

In our study, we focused on the detection of serum hs-CRP combined with PCT, NP and WBC in the infants and children, who were diagnosed as with respiratory infectious diseases, aiming at Chinese children in the region of guangdong, in order to investigate the evaluation of hs-CRP combined with PCT, neopterin and WBC in the early diagnoses of respiratory infectious disease, and to reduce the abuse of antibiotics in infants and children.

Methods and material

Patients

438 children was diagnosed as respiratory infection according to the diagnostic criteria [16], and hospitalized in Second Clinical Medical College of Jinan University from January 1st,

2009 to December 31st, 2014. There were 64 newborns and 374 children, including 274 male and 164 female, age from 2 months to 11 years old. In the 438 patients, 161 patients were diagnosed as bacterial infection (group A) by sputum culture [17] and pharyngeal swab culture [18], including 143 cases with normal bacterial infection (group A1) and 18 cases with suppurative tonsillitis (group A2). 81 patients were diagnosed as mycoplasma (Mp) infection (group B) and 196 patients were diagnosed as virus infection (group C) by serologic confirming [19]. 50 healthy children were taken as control.

The results of bacterial culture showed that there were 65 cases with escherichia coli (40.37%, 65/161), 40 cases with klebsiella pneumonia (24.84%, 40/161), 31 cases with staphylococcus aureus (19.25%, 31/161), 9 cases with pseudomonas aeruginosa (5.59%, 9/161), and 16 cases with others (9.94%, 16/161).

Informed consent was obtained from all the parents of the patients. This investigation was approved by the medical ethics committee of Second Clinical Medical College of Jinan University.

Blood samples collection

After hospitalization, 2 ml of the venous blood sample was collected in ethylenediaminetetraacetic acid (EDTA)-K2 anticoagulative tube (Becton, Dickinson and Company, American) from each patient in 24 h. 3 ml of the venous blood sample was collected and the serum was separated for detections. After antibiotic therapy, the venous blood was collected according to previous methods

Detections

Mycoplasma pneumonia specific antibody (Mp-IgM) was detected by colloidal gold method and the relative agents were provided by Kanghua Biotech Co., Ltd. (Weifang, China). Serum virus was detected by immunofluorescence (IF) with respiratory virus screening kit (Merck Millipore, Shanghai, China). Serum bacteria were detected by biochemical medicine sensitivity experiments with automated microbiology identification and antibiotic susceptibility analysis system (Thermo Fisher Scientific, Inc. Shanghai,

Different respiratory infectious disease

Table 1. hs-CRP comparisons among groups before antibiotic therapy (X±SDs)

Groups	Cases (n=438)	hs-CRP (mg/L)	PCT (ng/mL)	NP (nmol/L)	WBC (1×10 ⁹ /L)
A1	143	11.55±9.31*	1.33±6.90*	19.05±8.94*	13.82±10.81*
A2	18	46.38±40.17*	1.41±4.31*	35.86±12.76*	13.64±10.55*
B	81	6.25±2.64*	0.26±4.98*	33.75±10.44*	8.39±4.61
C	196	1.84±2.03	0.18±7.10*	43.51±15.90*	7.58±6.02
Control	50	1.39±1.76	0.05±5.75	6.67±3.32	7.45±3.30

Note: *comparing to control group, P<0.05.

Table 2. Positive rate of hs-CRP, PCT and WBC among groups before antibiotic therapy

Groups	Cases (n=438)	hs-CRP		PCT		NP		WBC	
		Cases (n)	Rate (%)	Cases (n)	Rate (%)	Cases (n)	Rate (%)	Cases (n)	Rate (%)
A1	143	102	71.33	58	40.56	98	68.51	74	51.75
A2	18	14	77.78	18	100	13	72.22	6	33.33
B	81	25	30.86	26	32.10	65	80.25	28	34.57
C	196	29	14.80	34	17.35	190	96.94	30	15.31
Control	50	0	0	1	2.00	0	0	2	4.00

China). Blood routine examination was carried on with BC-5500 and relative reagents (Mindray Medical International Limited, Shenzhen, China).

hs-CRP assay

hs-CRP was detected with enzyme linked immunoassay (ELISA) kit (Kehua Bioengineering Co., Ltd. Shanghai, China) according to the specifications. The absorbance at 450 nm was measured using immune turbidimetry with AU400 automatic biochemistry analyzer (Olympus imaging China, Beijing). The calibration curve was linear between 0 and 10 mg/L with the detecting limit of 0.2 mg/L. The inter- and intra-assay variance was <10%. The healthy control was with the reference level of 10 mg/L.

PCT assay

PCT was detected with PCT ELISA kit (Thermo) according to the specifications. The functional assay sensitivity of PCT was 0.06 µg/L with the critical values for pneumonia of bacterial etiology was ≥0.15 µg/L [20]. The healthy control was with the reference level less than 0.5 ng/mL.

NP assay

NP was detected with ELitest® Neopterin-Screening kit (BRAHMS, Thermo) according to the specification. The absorbance at 405 nm

was measured using immune turbidimetry with AU400 automatic biochemistry analyzer (Olympus) with the detecting limit of 2 nmol/L. The inter- and intra-assay variance was <10%. The healthy control was with the reference level of 10 nmol/L [21].

WBC assay

WBC counting, as one of the blood routine examinations, was carried on with BC-5500 blood-counter system (Mindray). The healthy control was with the reference level of (3.5~9.5)×10⁹/L [22].

Statistical analysis

All the data were analyzed by SPSS 13.0 software. The measuring data were presented as mean ± standard deviations (X±SDs). Comparisons between groups were used t-test. A value of P<0.05 considered significant difference.

Results

hs-CRP, PCT, NP and WBC comparisons before antibiotic therapy

According to the results showed in **Table 1**, serum hs-CRP (mg/L) was higher in A1 (11.55 ±9.31), A2 (46.38±40.17) and B (6.25±2.64) groups than in control (1.39±1.76) group, significantly (P<0.05). hs-CRP in A2 group increased more obvious than in A1 and B groups.

Different respiratory infectious disease

Table 3. hs-CRP and WBC after antibiotic therapy (X±SDs)

Groups	Cases (n=211)	hs-CRP (mg/L)	NP (nmol/L)	WBC (1×10 ⁹ /L)
A1	143	5.49±6.31	-	10.78±8.44
A2	18	5.88±9.16	-	10.86±12.38
C	196	-	10.50±4.83	-
Control	50	1.39±1.76	6.67±3.32	7.45±3.30

Table 4. Sensitivities and specificities of hs-CRP, PCT and WBC for bacterial and NP for viral respiratory infection

	hs-CRP >10 mg/L	PCT >0.5 ng/mL	NP >10 nmol/L	WBC >10×10 ⁹ /L
Sensitivity (%)	72.05	47.20	96.94	49.69
Specificity (%)	100	98.00	100	96.00

There was no significant difference of hs-CRP (mg/L) between in group C (1.84±2.03) and control group.

Serum PCT (ng/mL) was higher in A1 (1.33±6.90), A2 (1.41±4.31), B (0.26±4.98) and C (0.18±7.10) groups than in control (0.05±5.75) group, significantly (P<0.05). PCT in A1 and A2 groups increased more obvious than in B and C groups.

Serum NP (nmol/L) in A1 (19.05±8.94), A2 (35.86±12.76), B (33.75±10.44) and C (43.51±15.90) groups was higher than in control (6.67±3.32) group, significantly (P<0.05). NP in C group increased more obvious than in A1, A2 and B groups, while in A2 and B groups increased more obvious than in A1 group.

Serum WBC (1×10⁹/L) was higher in A1 (13.82±10.81) and A2 (13.64±10.55) groups than in control (7.45±3.30) group, significantly (P<0.05). There were no significant difference of WBC (1×10⁹/L) between in B (8.39±4.61), C (7.58±6.02) groups and control group.

Positive rate of hs-CRP and WBC comparisons before antibiotic therapy

The value of hs-CRP >10 mg/L, PCT >0.5 ng/mL, NP >10 nmol/L and WBC >10×10⁹/L considered as positive. According to **Table 2**, the positive rate of hs-CRP in A1 (71.33%) and A2 (77.78%) groups were higher than in B (30.86%) and C (14.80%) group.

The positive rate of PCT in A2 (100%) group was the highest, while in A1 (40.56%) group was

higher than in B (32.10%) and C (17.35%) groups.

The positive rate of NP in A1 (68.51%), A2 (72.22%), B (80.25%) and C (96.94%) increased gradually.

The positive rate of WBC in A1 (51.75%) group was the highest, while in A2 (33.33%) and B (34.57%) groups were higher than in C (15.31%) group.

hs-CRP and WBC comparisons after antibiotic therapy

According to **Table 3**, comparing the serum hs-CRP (mg/L) before and after antibiotic therapy, there were differences in A1 (11.55±9.31 v.s. 5.49±6.31) and A2 (46.38±40.17 v.s. 5.88±9.16) groups before and after antibiotic therapy, the same was to serum WBC (1×10⁹/L) in A1 (13.82±10.81 v.s. 10.78±8.44) and A2 (13.64±10.55 v.s. 10.86±12.38) groups, significantly (P<0.05). However, comparing to control group, serum hs-CRP and WBC were both higher after antibiotic therapy. There was difference of serum NP (nmol/L) in C (43.51±15.90 v.s. 10.50±4.83) group before and after treatments, significant (P<0.05), but still higher than in control group (6.67±3.32).

Sensitivities and specificities for hs-CRP, PCT, NP and WBC

According to **Table 4**, the sensitivities and specificities of hs-CRP, PCT and WBC for bacterial respiratory infection were 72.05% and 100%, 47.20% and 98.00%, 49.69% and 96.00%, separately, while NP for viral were 96.94% and 100%. Using serum hs-CRP to evaluate bacterial respiratory infection was more sensitive than using serum PCT or WBC, independently.

Discussions

hs-CRP was synthesized in liver and transported to the blood, cerebrospinal fluid (CSF), hydrothorax and ascites and so on. hs-CRP was with the regulatory and agglutinating functions similar to IgG and its complement, which could promote the phagocytosis of macrophage and stimulated the expression of the tissue factors on the surface of monocyte [23]. Recently, hs-CRP was found to participate in the cytotoxicity

Different respiratory infectious disease

mediated by platelet [24]. On one hand, hs-CRP could oxidize and activate platelets by itself, on the other hand, hs-CRP could combined with platelet activated factor (PAF) to inhibit the release of arachidonic acid, which would inhibit the neutrophil induced by PAF and the production of peroxide.

Oh [25] et al. found that when with bacterial infection, inflammatory cell infiltrated and released endogenous transmitters to stimulate hepatocyte to promote the synthesis of hs-CRP, and the serum PCT, as a marker of bacterial infection [26], would also change. At the early stage of bacterial infection, serum hs-CRP would increase rapidly, with tendency positive to the severity of infection. However, the amount of WBC was normal or just increased slightly. In our study, at the early stage of bacterial infection, the positive rate of hs-CRP in A1 and A2 groups were 71.33% and 77.78%, separately, the PCT were 40.56% and 100%, and the WBC were 51.75% and 33.33%. Comparing the serum hs-CRP, PCT and WBC in bacterial respiratory infection, the sensitivities and specificities of hs-CRP, PCT and WBC for bacterial respiratory infection were 72.05% and 100%, 47.20% and 98.00%, 49.69% and 96.00%, separately, which indicating that serum hs-CRP was more sensitive to evaluate bacterial respiratory infection.

However, there were large individual differences between each child, especially the emotion of the infants and children with infectious disease would induced large fluctuation of serum WBC, which would result in the false positive of WBC. hs-CRP was not influenced by emotion easily, as well as PCT. Therefore, combining serum hs-CRP with PCT and WBC was more suitable for early diagnosis of bacterial infection.

In healthy people, the content of serum hs-CRP was very low (<3 mg/L). But in those with inflammation or acute injury, hs-CRP increased in 4 to 6 hours and reached the peak in 36 to 50 h, approximately 100 to 1,000 times of healthy people. hs-CRP was not easily affected by sex, age, anemia, hyperglobulinemia, even the temperature, and would return to normal with remission [27, 28]. Although WBC was the indicator of bacterial infection, WBC failed to support with effective information in infants or children for the not significant changes.

The main pathogens in respiratory infection in pediatrics department included bacteria, Mp pneumonia and virus. NP was the predictive marker of virus infection [29], which was sensitive for early assessment of acute respiratory syndrome both in adults and children. When with virus infection, the serum NP would increase more than 10 nmol/L [21]. In our study, the sensitivity of NP for viral respiratory infection was 96.94%, which was accorded to and indicated the fact that NP played an important role in diagnosis of virus infection. With the limitations including not the separation of virus or detection of calcitonin zymogen, and the long time of bacteria culture, hs-CRP combined with PCT NP could be the diagnostic and treating indicator at the early infectious stage.

From the results mentioned in **Table 1**, hs-CRP in the infants and children with bacterial or Mp infection were both higher than in healthy infants and children (control group), significantly, while in those with virus infection was not significantly different from the control. There were significant differences of the positive rate of hs-CRP among the bacterial (A1, 71.33%; A2, 77.78%), Mp (30.86%) and virus (14.80%) infection, which was similar to PCT (A1, 40.56%; A2, 100%; B, 32.10%; C, 17.35%) and NP (A1, 68.51%, A2, 72.22%; B, 80.25%; C, 96.94%) among each group. These phenomenon indicated that hs-CRP combined with PCT could be the index to identify and distinguish different infections induced by different kinds of pathogen, while hs-CRP combined with NP could identify and distinguish infections induced by bacteria or virus, accorded to previous researches [30, 31].

Additionally, before antibiotic therapy on bacterial infection, serum hs-CRP (mg/L) was higher in A1 (11.55±9.31) and A2 (46.38±40.17) groups comparing to the control (1.39±1.76) group, significantly (P<0.05). After therapy, serum hs-CRP (mg/L) both decreased in A1 (5.49±6.31) and A2 (5.88±9.16) groups, which indicated that hs-CRP could be the index for infection control, accorded to previous researches [32, 33].

In conclusion, serum hs-CRP combined with PCT, NP and WBC detection was helpful for identifying and distinguishing bacterial, Mp pneumonia and virus infection, as well as providing rational use of antibiotics with favorable

evidences. However, our study only focused on the infants and children in the region of Shenzhen guangdong with limited quantity of samples and large individual and regional differences. There were differences of the sensitivities and specificities of serum hs-CRP, PCT, NP and WBC for diagnosing different kinds of respiratory infection. Therefore, identification of different kinds of respiratory infectious disease still needed to accord to the clinically practical situations and analyzed comprehensively.

Disclosure of conflict of interest

None.

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Different respiratory infectious disease

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