

Evaluation of high sensitivity C-reactive protein assay in cerebrospinal fluid on the Dimension RxL analyzer

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Abstract

Introduction: Low sensitivity and specificity in traditional laboratory tests became insufficient for accurate diagnostics and initiation of proper treatment of patients infected with bacterial meningitis. High sensitivity C-reactive protein (hsCRP) may be an appropriate supplement for rapid diagnosis of bacterial meningitis. The subject of our investigation was the determination of C-reactive protein in cerebrospinal fluid (CSF) during acute bacterial meningitis.

Methods: HsCRP was analysed by a sensitive immunoturbidimetric assay using the Dimension RxL analyser (Siemens). Cerebrospinal fluid concentrations of C-reactive protein have been measured in 20 patients (age range, 1 to 50 years) presenting with acute bacterial meningitis and also in a non-infected, non-inflamed control group (n=25).

Results: The accuracy and precision of the method proved to be satisfactory. Repeatability of serial sampling for hsCRP described by coefficient of variation were CV=2.1-4.5%. This assay hsCRP in cerebrospinal fluid demonstrates adequate performance characteristics for routine clinical use. Elevated levels of CRP were found in 95% patients with bacterial meningitis. The mean CRP value in 25 uninfected control group was 0.25 mg/L (range 0.10-0.55). The mean CRP for patients with bacterial meningitis was 21.4 mg/L (range 0.40-100).

Conclusions: A sensitive assay for CRP in CSF would be a useful adjunct to conventional investigation of acute infective meningitis.

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Keywords: High sensitivity C-reactive protein, cerebrospinal fluid, bacterial meningitis

Introduction

C-reactive protein (CRP) is the acute phase protein, historically one of first to be recognised. Intraindividual variation is a major limitation of the assay when the assay is used for directing therapies. Intraindividual variations of the CRP levels are from 30% to 60% (1). Serial measurement maybe required to estimate true mean of CRP depending on the intended application in any specific individual. CRP seems to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infection, histolytic disease, and a variety of other disease states (2). CRP is composed of five identical, non-glycosylat-

ed, subunits each comprised of single polypeptide chain of 206 amino acid residues with a molecular mass of 23.017 daltons. This characteristic structure places CRP in the family of pentraxins-calcium binding proteins with immune defence properties found in all vertebrates and most invertebrates (3). CRP is synthesized in the liver as a result of induction by the interleukin-6 family of cytokines. At the peak of an acute phase response as much as 20 % of the liver protein synthetics maybe directed towards this process. Extra-hepatic synthesis makes no contribution to the serum levels. The normal synthetic rate is 1-10 mg/day, increasing to more than 1 gram/day in acute inflammation (4). The functions of CRP include the detection, clearing and elimination of apoptotic tissue cells and their products such as DNA, which can be toxic or allergenic. At the same time, CRP acts as a non-adaptive defense mechanism by opsonizing microorganisms for phagocytosis. CRP bind-

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ing occurs only during targeting of affected cells when the normal structure of the lipid dual layer has been disrupted, leading to exposure of internal phospholipids of the cell membrane (5). Many disorders of the central nervous system (CNS) are accompanied by increased CRP concentration in the cerebrospinal fluid (CSF) (6). Examination of CSF specific proteins used mainly to detect increased permeability of the blood-brain barrier. Several disorders of the CNS such as bacterial meningitis, multiple sclerosis and other CNS inflammatory diseases are associated with an increase in CRP concentration in CFS (7). Patients with symptoms of meningitis usually undergo lumbar puncture and in most cases of bacterial infections this provides a typical image. However in the selected group with negative microscopic evidence of infection the CSF-CRP is a useful diagnostic adjunct (8). Several studies in adults and children of all ages show that an increased CRP level is highly suggestive of a bacterial etiology (9). The subject of our investigation was the determination of C- reactive protein in cerebrospinal fluid (CSF) during acute bacterial meningitis.

Methods

Twenty patients in the age group of 1 to 50 years with clinical diagnosis of bacterial meningitis were included in the study. The control group included 25 non-infected subjects. A sample of cerebrospinal fluid is collected during a procedure called lumbar puncture. All specimens for investigations were collected before introduction of antibiotics. HsCRP was analyzed in unconcentrated CSF by a sensitive immunoturbidimetric assay using the Dimension RxL analyzer (Siemens) with calibrators and internal controls provided by Simens and according to manufacturer's recommendations. This is a latex immunoassay developed to accurately and reproducibly measure hs CRP. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination occurs. This agglutination is detected as an absorbance change(572 nm), with the rate of change being proportional to the quantity of hs CRP in the sample. The procedure of the Siemens assay accuracy evaluation included duplicate calibrators determining as samples (Calibrators levels 0, 5, 10, 20, 40 mg/

L),while the accuracy of the method was calculated by linear and regression analyses. Three quality control materials were used for quality control. Precision was calculated by measuring quality control materials in 20 duplicate with a single analytical run. Statistical analyses were performed using Microsoft Office Excel program package 2003, for the function of arithmetic mean and standard deviation. The correlation was analyzed by linear regression test. Values of $p < 0.05$ were considered as statistically significant.

Results

The results of the hsCRP assay in cerebrospinal fluid assay precision analyses are showed in table 1.

TABLE 1. Precision of hsCRP

Sample	Sample value (n)	Mean value (mg/L)	Sd (mg/L)	CV (%)
Control 1	20	0.46	0.02	4.3
Control 2	20	4.9	0.13	2.6
Control 3	20	11.3	0.24	2.1

The coefficients of variation (CV%) values of the precision were 2.1-4.3 %. The results variation was greater at lower concentrations. The hsCRP assay accuracy results were presented in table 2.

TABLE 2. Accuracy of hsCRP assay on Dimension RxL

Calibrators (mg/L)	0	5.0	10.0	20.0	40.0
Measured values* (mg/L)	0.1	4.9	9.8	19.7	38.2

*Mean of two measurements of calibrators as sample.

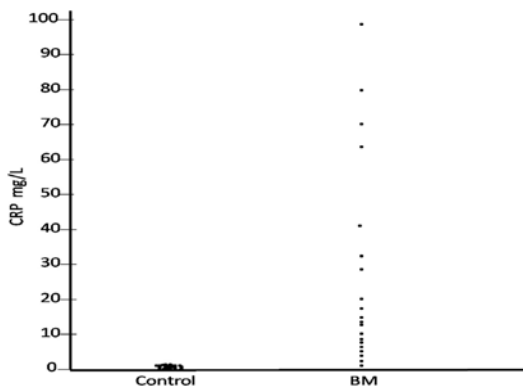
The statistically significant correlation between labeled and measured hsCRP values was obtained ($r=0.99$; $p<0.001$), presented by the following equation: $y=0.98x + 0.23$ were y represented the measured hsCRP levels, and x labeled hsCRP levels. An intercept (0.23) presented the systemic error of the method, which was not statistically significant ($p>0.05$) and slope (0.97) was a percentage deviation of -3% ($100\% - 97\% = 3\%$) and was non-significant ($p>0.05$).

TABLE 3. Values of the hsCRP in patients with acute infectious meningitis

Examines	N	Minimum value (mg/L)	Maximum value (mg/L)	Mean value (mg/L)
Patients with clinical diagnosis of meningitis	20	0.40	100.0	21.4
Control group	25	0.10	0.55	0.25

We determined the minimum, maximum and mean value(s) hsCRP in cerebrospinal fluid and the results are showed in table 3.

Comparasion data for the group patients with bacterial meningitis (BM) and control subjects are presented in Figure 1.

**FIGURE 1.** CRP distribution in the examined groups

The mean CRP value in 25 uninfected control group was 0.25 mg/L (range 0.10-0.55). The mean CRP for patients with bacterial meningitis was 21.4 mg/L (range 0.40-100). Elevated levels of hsCRP were found in 95 % patients with bacterial meningitis.

Discussion

Biochemical markers for diseases of central nervous system are glucose, lactate, total proteins and C-reactive protein. CRP is an acute phase reactant synthesized by the liver upon stimulation by pro-inflammatory cytokines reflecting both the acute and chronic inflammatory states (10). Acute phase reactant changes reflect the presence and intensity of inflammation, and have been used as a clinical guide to diagnosis and therapeutic management.

CRP has many pathophysiologic roles in the inflammatory process (11). A major function of CRP is its ability to bind phosphocholine and thus recognize some foreign pathogens as well as phospholipid constituents of damaged cells. In bacterial meningitis the changes in CRP concentrations are not induced by living bacteria and leukocytes. The anaerobic brain metabolism contributes to the development of increased CSF- CRP concentrations. The cytokinine-endothelium-leukocyte interaction is maybe responsible for the disruption of the blood-brain barrier by opening intercellular junctions and permitting the passage of C-reactive proteins into the subarachnoidal space (12). CSF-CRP has been reported to be one of the most reliable and early indices to differentiate bacterial from non-bacterial meningitis. It is also useful in monitoring the clinical course of the meningitis (13,14). The analysis of CSF-CRP by latex agglutination is rapid and easy to perform. The limit of quantification for hs CRP assay is 0.1 mg/L, which is acceptable for routine clinical use. The CV for the imprecision in this assay is not greater than 5 % at the lowest measurable concentration. The obtained CV% values for precision were 2.1 - 4.3. The hsCRP assay in cerebrospinal fluid showed good accuracy. The obtained CV% values was in accordance with the manufactures recommendation. Linearity was confirmed with calibration curve in 5 points in concentration range from 0 to 40 mg/L. The CSF hsCRP concentration was significantly increased in patients with disease. During our investigation it was noticed that the minimum concentration for hsCRP in patients with clinical diagnosis of bacterial meningitis was 0.40 mg/L. Maximum concentrations of 100 mg/L and mean values of 21.4 mg/L was established during the study. The mean CRP value in 25 uninfected control group was 0.25 mg/L (range 0.10-0.55). In the present study, CSF-hsCRP was positive in 19 of 20 cases of bacterial meningitis giving it a sensitivity rate of 95 %.

Conclusion

The hs CRP assay on the Dimensin RxL analyser demonstrates adequate performance characteristics for routine clinical use. The elevated hs CRP concentration of CSF during bacterial meningitis is caused by an increased permeability of the

blood-brain barrier. The sensitivity determination of hs CRP in cerebrospinal fluid is 95% in case of infective bacterial meningitis. It is concluded that C-reactive protein in CSF is a useful addi-

tional test for diagnosis of bacterial meningitis.

Competing interests

Authors have no conflict of interest to report.

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