Review:

High Sensitivity CRP (hsCRP) –Application In Pediatric Infecions

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C-reactive protein (CRP) was first discovered in 1930 by William Tillet and Thomas Francis.

CRP plays a role in the innate immune system by activating the complement pathway. Through the calcium dependent binding of ligands containing phosphocholine, as well to the phospholipids of damaged cells, CRP enhances the uptake of damaged cells by macrophages. Initially, CRP was considered a pathogenic secretion elevated in people suffering from one of many forms of tissue damage. However, and synthesis by hepatocytes demonstrates that CRP is a native protein. At the transcriptional level, CRP is regulated by the cytokine interleukin (IL)-6 and enhanced IL-1, with tumour necrosis factor (TNF)-± possibly playing a part in CRP synthesis through influencing IL-6 production.

Structure And Biochemistry Of C-Reactive Protein:

CRP is a protein of the pentraxin family. It consists of five non-covalently bound identical protomers arranged around a central pore. It has a relatively long half-life of 18 to 20 h, owing to its stable pentraxin structure. Each protomer subunit consists of a single polypeptide chain of 206 amino acids with a total molecular weight of approximately 23,000 Dalton (Da).

Each subunit has two calcium ions surrounding a hydrophobic pocket, thus making it capable of forming calcium-dependent bonds with ligands, usually phosphocholine (PCh). The above described Fraction C, present on the bacterial cell wall, contains phosphocholine. It is a non-glycosylated protein and the gene has been mapped to chromosome 1.

Production Of C-Reactive Protein:

CRP is produced in many sites within the human body (Figure 1). It is produced in the liver in response to IL-6. Products of activated monocytes in Hep 3B cells induce the production of human serum amyloid A (SAA) protein and CRP, but not by IL-1², TNF-±, or some hepatocyte-stimulating factor preparations. It is also produced in very limited concentration by non-hepatic cells like neurons, atherosclerotic plaques, monocytes, Kupffer cells and lymphocytes. Studies have shown that epithelial cells of both respiratory tract and renal epithelium can also produce CRP under certain circumstances. Recent studies have demonstrated that human coronary artery smooth muscle cells could also synthesize CRP upon stimulation by inflammatory cytokines. Cogent data have indicated that the protein is also produced by the atherosclerotic lesions (especially by smooth muscle cells and macrophages), kidneys, neurons, and alveolar macrophages. Additionally, there is evidence to suggest that lipid peroxidation and infection, such as cytomegalovirus may trigger a pro-inflammatory cytokine cascade resulting in CRP release. CRP may be secreted from active human peripheral blood monocytes, while generation from peripheral blood mononuclear cells (PBMC) is poorly established. Expression of CRP

by human respiratory epithelial cells and alveolar macrophages suggests contribution to bacterial clearance and direct involvement in pulmonary host defence and immune response. Biosynthetic labelling with S-met and immuno-precipitation with anti-CRP antibodies and Staphylococcus aureus indicate that cell surface CRP is produced by lymphocytes.

Physiology Of C-Reactive Protein:

C-reactive protein binds to several polysaccharides and peptido-polysaccharides present in bacteria, fungi and parasites in the presence of calcium. These complexes activate the classical complement pathway, acting as opsonins and promoting phagocytosis. Together with complement components, CRP is the only acute phase protein directly involved in the clearance of micro-organisms. In vitro, CRP stimulates cell-mediated cytotoxicity through activation of neutrophils, promoting platelet degranulation and enhancing NK cell activity. Under physiological conditions, CRP binds to small nuclear ribonucleoproteins, suggesting a direct role in the removal of necrotic tissue. The potential role of CRP in eliminating bacteria has been recently demonstrated. Transgenic mice that express high levels of human CRP in serum in response to endotoxin are partially protected against lethal infection by Streptococcus pneumoniae. This effect is probably mediated by CRP's ability to bind to phosphocoline moieties in the Streptococcus pneumoniae cell wall C-polysaccharide. CRP transgenic mice also exhibit increased resistance to lethal infection against the Gram-negative bacterium, Salmonella typhimurium.

Different Forms Of C-REACTIVE PROTEIN:

Studies have found different structural forms of CRP, such as, the pentameric ring, globulin and fibril structures, which are observed by combination of size-exclusion chromatography and electron microscopy. Denatured and aggregated forms of CRP (neo-CRP or modified CRP) have been also reported.

The pentameric ring-like CRP was observed mostly on ligand containing membrane in a calcium-dependent manner. The globulin-like monomers, found on negatively charged membrane in the absence of calcium, exhibit structural stability. The fibril-like structures were formed by face-to-face stacking of a number (several to hundreds) of pentameric CRP. The freshly purified CRP forms short single-strand fibrils, while that stored for more than several days form long and bundled fibrils.

In 1965, Gotschlich and Edelman reported for the first time that the CRPs purified from serum were mainly pentamers. CRP exists in two distinct forms: (i) native pentameric CRP (nCRP), detectable in serum with both pro-and anti-inflammatory effects, and (ii) the tissue-bound modified or monomeric CRP (mCRP), with predominantly pro-inflammatory effects.

Native CRP, which exists as a pentamer, dissociates to mCRP due to conformational rearrangement. There is growing evidence that mCRP may have novel pro-inflammatory and thrombotic properties. mCRP is found to be deposited in human aortic and carotid atherosclerotic plaques, but not in healthy vessels. In 1983, Potempa et al. reported another type of CRP, termed 'modified CRP', and produced on urea-EDTA or acid-EDTA treatment.

The modified CRP runs faster in gel electrophoresis and has lower solubility than native CRP.pCRP is formed by urea-chelation treatment and resembles the free subunit mCRP. mCRP has distinct physicochemical, antigenic and biologic activities compared to CRP. mCRP enhances platelet aggregation and secretion of serotonin, modulation of arachidonic acid metabolism, stimulation of interleukin-1 (IL 1) release, potentiation of the respiratory burst response of human neutrophils, and peripheral blood monocytes to heat modified IgG.

The different structural forms may convert to each other under certain conditions, suggesting structural basis of multiple functions of CRP. Native

CRP binds to CD32, whereas mCRP binds to CD16. It was suggested that native CRP dissociated into monomeric units on binding to plasma membrane, or in a denaturing or oxidative environment.

mCRP can inhibit as well as activate the classical complement pathway by binding to C1q, depending on its presence in a fluid phase or surface-bound state.48 Identification of suitable assays that allow direct testing of mCRP, instead of native CRP in serum or tissue, will further clarify its biological significance.

CRP Gene Regulation:

The CRP gene, located on the1q23.2 on chromosome 1, contains one intron separating the region encoding the signal peptide from that encoding the mature protein. The CRP gene sequence was determined in 1985 simultaneously by two different research teams. The first exon encodes a signal peptide and the first two amino acids of the mature protein. This is followed by a 278-nucleotide-long intron that includes a GT repeat sequence. The second exon encodes the remaining 204 amino acids, followed by a stop codon.[42] Goldman *et al*. has reported for the first time that the GT stretch in the intron is polymorphic in length. Two recent studies describe polymorphisms in the CRP intron gene and promoter that influences the normal expressionlevels.[43] Individuals with particular allele combinations exhibit two-fold lower baseline CRP levels, perhaps due to DNA structural changes that affect transcription. Within the promoter, several polymorphisms were discovered in transcription factor binding E-box sites, all of which resulted in different baseline circulating levels of CRP and response by other genes that encode cytokines affecting its synthesis, such as IL-6, IL-1 and TNF-±. Single nucleotide polymorphisms (SNPs) across the CRP gene have been associated with differences in basal CRP levels. CRP gene contains binding sites for STAT3 (transcription factors) and Rel proteins. It is well established that IL-6 stimulates the acute phase expression of CRP. Polymorphism in the human CRP gene resulting in a lower basal level of CRP has been associated with an increased risk of developing systemic lupus erythematosus.

Functions Of CRP:

Functions of CRP have been elicited in the following table.

TABLE 1: Functions of CRP

Sr no.	FUNCTION
1.	Binds to bacteria and interacts with natural killer cells and monocytes; may increase the tumoricidal activity of these cells.
2.	Activates endothelial cells to express adhesion molecules, chemokines and cytokines.
3.	Inhibits nitric oxide (NO) production and stimulation of nitric oxide release through down regulation of endothelial nitric oxide synthase.
4.	Upregulates angiotensin receptor-1 (AT 1-R) protein expression, increases AT 1-R number on vascular smooth muscles and promotes vascular smooth muscle migration and proliferation in vitro.
5.	Serves as chemoattractant for monocytes and incduces tissue factor expression in macrophages.
6.	Mediates uptake of native LDL into macrophages.
7.	Activates complement pathway upto C5 convertase and enhances phagocytes.
8.	Amplifies inflammatory responses.

Role of CRP in physiology and pathology:

CRP, mainly recognized as a biomarker of inflammation, is now viewed as a direct contributor in atherosclerosis as it functions both as 'pro-inflammatory' and 'anti-inflammatory' molecule. With the advent of high-sensitivity assays for determining CRP, the protein has emerged as one of the most powerful independent predictors of cardiovascular disease.

CRP level, which significantly increases in acute coronary syndromes, has a prognostic value in patients with cardiovascular complications and in apparently healthy individuals. The in vivo mechanisms of CRP as a mediator of the inflammatory state and thrombotic complications are continuing to be unravelled.

The capacity of human CRP to activate/regulate complement may be an important characteristic that links CRP and inflammation with atherosclerosis. Recent advances suggest that, in addition to classical pentameric CRP, mCRP may also play an active role in atherosclerosis. The capacity of mCRP to interact and activate the complement cascade is unknown.

A growing body of evidence implicates CRP as a direct mediator of endothelial dysfunction. Patients with elevated levels of CRP have been shown to elicit impaired endothelium-dependent vasodilatation, suggesting that CRP may be a useful clinical tool for endothelial vasomotion.

CRP and infection:

CRP is an important factor in determining the etiology of infection. The level of CRP can be significantly higher in bacterial infections. A value higher than 100mg/L strongly suggests bacterial infections, whereas that below 10 mg/L indicates viral infection. In tuberculosis, it is often found to be between 10 to 100 mg/L.

Additional determination of procalcitonin can add specificity in the case of bacterial infections. The above information is also helpful to distinguish infection from an autoimmune flare. Similarly, the rate of change in CRP levels can differentiate tuberculosis from bacterial pneumonia.

Table 2: CRP levels in various Bacterial & viral infections

Sr.no.	CRP Levels (mg/L)	Advantages	Disadvantages
Human immunodeficiency Virus (HIV)			
1.	Baseline conc. <10	High systemic levels of IL-10, CRP and IL-22 in HIV infected patients are associated with low viral replication in vitro	Lacks immune modifying actions
2.	<1 low,1-3 median>3 high risk Median CRPHCV- = 1.86HCV	Demonstrated an association of lower serum lipid and CRP levels with hepatitis C virus (HCV) infection.HCV status should be assessed as an important correlate of CV risk factors in older men with or at risk for HIV	

Sr.no.	CRP Levels (mg/L)	Advantages	Disadvantages
3.	hsCRPLow risk <1 Average risk 1-3 HIGH RISK >3MEDIAN (IQR)2.94 (0.83,5.53)	hsCRP was elevated and independently associated with body mass index and lipid changes.	
		Infections	
1.	Bacterial infections with high CRP>100Viral infections with lower CRP <10 Tuberculosis <100 varies with severity of disease	Aids in the diagnosis of infection, especially using radioimmunoassay kits	
		Bacterial Infections	
1.	The best cut off value	CRP and serum procalcitonin (PC1) levels to be significantly higher in the infection group than in the autoimmune disease flare group	PCT level had and sensitivity and specificity compared to CRP in distinguishing between bacterial infections and autoimmune disease flares.
2.	Cut off 12.5	High sensitivity and negative predictive value for differentiating pulmonary TB from bacterial community-acquired pneumonia (CAP) Plays a supplementary role in the exclusion of pulmonary TB from Bacterial CAP	
3.	Febrile bacterial infections: mean 63.77 mean ratio: 3.61 mg/L/hourNon-bacterial febrile illnessesMean 23.2 mean rate: 0.41mg/L/hour	CRP and rate of increase in CRP can differentiate between acute bacterial and non-bacterial febrile illness, better than CRP alone	
		Viral Infections	
1.	>100		High plasma CRP not reflect the severity of the nephropathiaepidemica
2.	Serum CRP<28 (lower tertile) >70 (upper tertile) Median (range) ICU 123 (69-184) Non-ICU40 (20-82)	Serum CRP at early emergency department admission of patients presenting with pandemic H1N1 influenza A infection were found to serve as a useful gauge for predicting disease course and assisting in patient management	

Laboratory Methods to Measure CRP:

The 3 clinical laboratory methods used to measure serum CRP levels are as follows:

- Qualitative
- Semi-quantitative
- Quantitative

All 3 tests are based on the ability of CRP to bind to a variety of biologic ligands forming CRP-ligand complexes. When a reagent containing anti-human CRP antibodies is added to a serum sample containing CRP, the CRP binds to the antibodies forming insoluble CRP-ligand complexes that clump and precipitate which can then be visualized and measured.

The qualitative latex agglutination test is the first laboratory method developed to measure CRP. This method measures the presence or absence of agglutination and precipitation, indicating only whether CRP is present or absent in the serum sample. A positive test result indicates the presence of CRP-ligand complexes formed when CRP binds to cause agglutination and precipitation, whereas a negative test result occurs when no agglutination is present. Positive test results indicate a CRP level greater than 6 mg/L or more than 10 mg/L, depending on the specific testing kit and reagent being used. Qualitative tests can be performed rapidly at the bedside within 10 to 15 minutes.

Purely qualitative CRP testing methods typically are not used to measure CRP levels because they have a low sensitivity, with positive results occurring with any condition involving inflammation or tissue destruction. A positive qualitative CRP test should always be followed by a semi-quantitative test, which is a more sensitive measuring method to quantify the concentration of CRP.

Laser and rate nephelometry quantitative immunoassays use infrared light-emitting diodes and detectors passed through test tubes containing fixed amounts of anti-CRP monoclonal antibody mixed with human serum, resulting in the formation of CRP-ligand complexes. As the antibody concentration remains constant, the extent of light scatter is determined from the amount of CRP-ligand complexes.

This method can be performed within 30 to 60 minutes. When using a fully automated analyzer, however, it takes 10 minutes. Qualitative and semi-quantitative methods of measuring CRP, although less expensive, are not accurate enough to be used for infants. The limited detection level of 6 mg/L in some kits could easily miss infants with true sepsis and result in longer antibiotic therapy. It is imperative that practitioners be aware of which CRP testing method is used in their own institution and the sensitivity and specificity of that test when evaluating measured CRP levels in the clinical setting.

Pattern Of Rise Of CRP In Infections:

CRP plays a role in the innate immune system by activating the classical complement pathway through the calcium dependent binding of ligands containing phosphocholine. As well as through the phospholipids of damaged cells, CRP enhances the uptake of damaged cells by macrophages. Initially, CRP was considered a pathogenic secretion elevated in people suffering from one of the many forms of tissue damage. However, synthesis by hepatocytes demonstrates that CRP is a native protein. At the transcriptional level, CRP is regulated by the cytokine interleukin (IL)-6 and enhanced IL-1, with tumour necrosis factor (TNF)-± possibly playing a part in CRP synthesis through influencing IL-6 production.

Starts rising 6–12 hours after an infection, Peaks around 48 hours and Returns to normal in 2 – 7 days. Peak attainable levels of hsCRP are less for coagulase negative staphylococcus as compared to E coli and few other microbes and also more in preterms as compared to term babies. Normal levels of CRP have been considered as indications for antibiotic discontinuation. Combining with other

parameters/ biomarkers like PCT, IL-6, etc. serial levels after 24 – 48 hours increases NPV of the test.

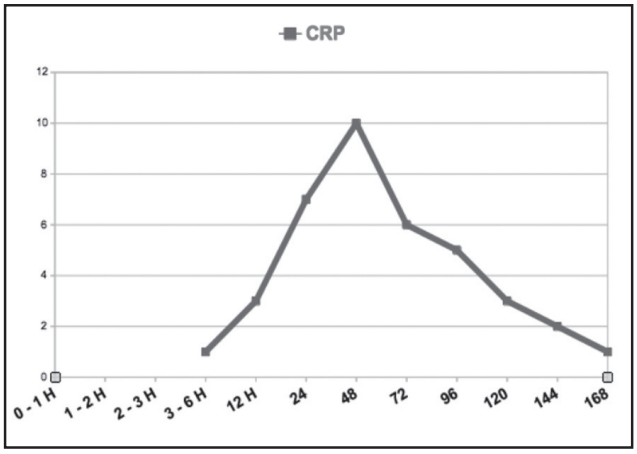

Fig. 4: Pattern of CRP rise and fall

High Sensitivity C-Reactive Protin

In conditions like infections, inflammation and trauma the serum CRP levels rise rapidly generally beyond 10 mg/l with a concomitant elevation of erythrocyte sedimentation rates (ESR). In the past decade, high-sensitivity assays with rapid turnaround times for measurement have become available. High-sensitivity assay techniques such as immunonephelometry, immunoturbidimetry, high-sensitivity enzyme-linked immunosorbent assay (ELISA) and resonant acoustic profiling (RAP) can detect CRP with a sensitivity range of 0.01 to 10 mg/ l.

These high-sensitivity assays help quantify low grades of systemic inflammation, in the absence of overt systemic inflammatory or immunologic disorders. The hsCRP assays have been standardized across several commercial platforms and can be accurately measured from fresh or frozen plasma.

Conclusion:

hs CRP is commonly used in cardiology as marker of inflammation in ischemic heart disease. The role of CRP, in neonatal infection is well known. hs CRP is a new and quantitative method of estimation of CRP. It will be definitely better than relatively crude qualitative and semiquantitative methods. Few studies are underway as its role in paediatric infection and nephrotic syndrome.

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