

# High-Sensitive C-Reactive Protein: a marker to assess risk of cardiovascular disease

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C-reactive protein plays a key role in the acute phase inflammatory response. Measurement of CRP concentration in the blood has been routinely used in clinical practice as a measure of inflammation, for example, to distinguish between bacterial and viral infections, to monitor the course of an illness or post-operative infection and to monitor treatment with antibiotics. Low-grade systemic inflammation has been associated with an increased risk of future coronary events, suggesting that CRP measurement can also be used to indicate risk of cardiovascular disease.

Cardiovascular disease (CVD) is a leading cause of death in the western world. In the United States, more than 70 million Americans have one or more types of CVD, and a recent report states that CVD is the cause of approximately as many deaths each year as the next five leading causes of death combined (cancer, chronic lower respiratory diseases, accidents, diabetes mellitus and influenza and pneumonia) [1]. The healthcare costs associated with CVD are enormous. In 2005 the estimated direct and indirect cost of CVD in the United States alone was \$393.5 billion.

Currently various tests are carried out to identify individuals with the metabolic syndrome, who are at high risk of developing CVD and diabetes. These tests include routine screening of blood cholesterol, blood pressure and fasting glucose levels. Half of all heart attacks occur however in individuals who have normal blood cholesterol levels. More than 20 prospective epidemiological studies have demonstrated that low concentrations of CRP can be a marker of low-levels of inflammation. The high sensitivity measurement of CRP (hsCRP) can thus independently predict vascular risk in seemingly healthy individuals [2]. Furthermore, inflammation and its links to CVD have initiated a debate about the clinical utility of hsCRP measurement to assess cardiovascular disease risk, and whether a test for hsCRP should be included when identifying individuals with the metabolic syndrome.

## Inflammation and the immune response

CRP is an acute phase inflammatory response protein and a major component of the body's innate immune defence mechanism which reacts promptly without specificity or memory upon exposure to different types of inflammatory stimuli. The acute phase response refers to initiation of the cellular immune response and the changes in the serum protein profile

which result during an ongoing inflammation process. Normally CRP is present in the blood in very low concentrations ( $<1$  mg/L), but during the inflammatory process this concentration increases significantly. CRP undergoes  $\text{Ca}^{2+}$ -dependent binding to different target molecules of microbes and cell membrane residues as well as to cell nuclear material; this facilitates their clearance from the blood stream. CRP also functions as an opsonin and activates the classical complement pathway, as well as leukocyte phagocytosis, lymphocyte stimulation and monocyte/macrophage functions. In addition, studies have shown that CRP binds low density lipoprotein (LDL), and it has been detected in atherosclerotic plaques [3]. CRP may also increase the production of cell membrane-bound tissue factor (TF) by macrophages.

The CRP concentration in the blood reflects the rate of synthesis of the protein in the liver. The transcription of the CRP gene in hepatocytes is upregulated by interleukin-6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor ( $\text{TNF}\alpha$  and  $\text{TNF}\gamma$ ) secreted by

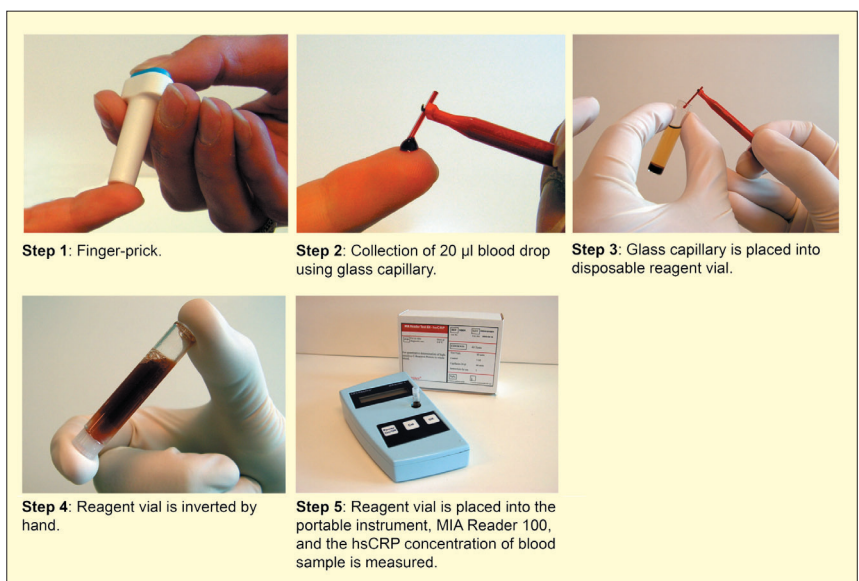


Figure 1. hsCRP immunoassay for near patient testing.

Sample	Mean (mg/L)	Total CV (%)	n
CRP standard 1	1.21	5.7	15
CRP standard 2	10.1	3.6	15
Whole blood (low)	2.94	5.8	20
Whole blood (high)	14.6	5.3	20

Table 1. Precision of the hsCRP immunoassay performed with LifeAssays portable MIA Reader 100 instrument and hsCRP test kit.

monocytes/macrophages. The elevation of CRP concentration can be detected as early as 4-7 hours after stimulation, and during the acute response phase the concentration of CRP in the blood can increase a thousand-fold. Once the relevant inflammatory stimuli have subsided, the CRP concentration decreases quite rapidly. The plasma half-life for  $^{125}\text{I}$ -CRP is about 19 hours [4].

### C-Reactive Protein

CRP was first described by Tillet and Francis in 1930. They observed that during the acute response phase an unknown serum factor, in the presence of calcium, bound to the C-polysaccharide of *Streptococcus pneumoniae* and caused its precipitation. This serum factor was later identified as C-reactive protein. CRP is a cyclic pentameric plasma protein consisting of five identical non-covalently bound subunits with a combined molecular mass of 120 kDa [5]. It is a member of the pentraxin protein family of oligomeric calcium-binding proteins.

### Clinical utility of CRP

CRP assays are now commonly performed in hospital central laboratories and in point-of-care settings. Immunoturbidimetric or nephelometric immunoassays are mainly used for routine CRP analysis. The introduction of the international standard IFCC CRM 470 has ensured that the currently available assays are extremely reliable.

CRP detection is regarded as an important diagnostic tool, especially for differentiating bacterial and viral infections. CRP concentrations are usually markedly increased in patients with bacterial infections (>100 mg/L), whereas in the case of viral infections, only a moderate increase of CRP level occurs. [6]. CRP detection is also carried out to diagnose and monitor other inflammatory processes including post-operative complications. The severity of inflammatory diseases can be assessed with the help of CRP measurement, which can also help to determine suitable therapeutic approaches. Since the CRP concentration in the blood increases and decreases rapidly, daily monitoring of the CRP concentration can be used to assess the efficacy of antibiotic therapy. One of the key requirements for using CRP analysis as a diagnostic tool is that the CRP test result should be available quickly, since a significantly increased concentration requires prompt action. The

development of rapid CRP assays has provided faster diagnosis and more effective treatment in point-of-care settings, especially in the case of children and elderly patients, who are more susceptible to infection.

There are many factors which influence the CRP serum concentration including age, gender, ethnicity, body mass index, smoking status, pregnancy, level of physical activity, stress level, duration of disease, type of infection and tissue involvement [7]. When carrying out CRP analysis, the overall health of the patient, the suspected disease and the sampling procedure must also be taken into consideration when interpreting the test results.

### Low-grade inflammation and increased cardiovascular risk

Studies have shown that low-grade systemic inflammation plays a pivotal role in the development and progression of cardiovascular disease. The current reference range for CRP in healthy individuals is <1 mg/L. However, many laboratories currently still use an upper reference limit of approximately 6 mg/L for healthy individuals [8]. Epidemiological and clinical studies have shown strong and consistent relationships between elevated levels of markers of inflammation and the risk of future cardiovascular events. The most promising of these markers is high-sensitive CRP, since its analysis is inexpensive, standardised hsCRP assays are widely available, and the analyte has a decade-to-decade variation similar to that of cholesterol. The following levels of hsCRP: <1 mg/L, 1-3 mg/L and >3 mg/L have corresponded respectively to low, moderate and high risk of future coronary events in individuals with metabolic syndrome [9]. Measures for lowering the blood hsCRP level include adoption of a healthy diet, exercise, smoking cessation, statin therapy and improved glycaemic control. Additionally, hsCRP is also used as a prognostic marker in acute coronary syndrome, after angioplasty, and in the long-term management of post-infarction patients. The American Heart Association and Centers for Disease Control and Prevention have recommended hsCRP screening in addition to routine cholesterol screening for metabolic syndrome patients in the moderate to high risk groups. However, the use of hsCRP as a global cardiovascular risk indicator has not been recommended due to the variability in clear cut-off points for low-moderate-high risk levels, as well as the lack of an absolute predictive value.

### High-sensitive CRP analysis in primary care

Currently hsCRP analysis is mostly performed at central hospital clinical laboratories using immunoassay technologies that have sensitivities of 0.1-0.2 mg/L CRP. A key requirement for using hsCRP as a cardiovascular risk indicator for preventive intervention is rapid hsCRP analysis in point-of-care settings, including physicians' offices and community health centres, where measurement can take place directly using whole blood. Current POC CRP assays have detection limits of 8 mg/L from whole blood and therefore cannot be used for hsCRP analysis.

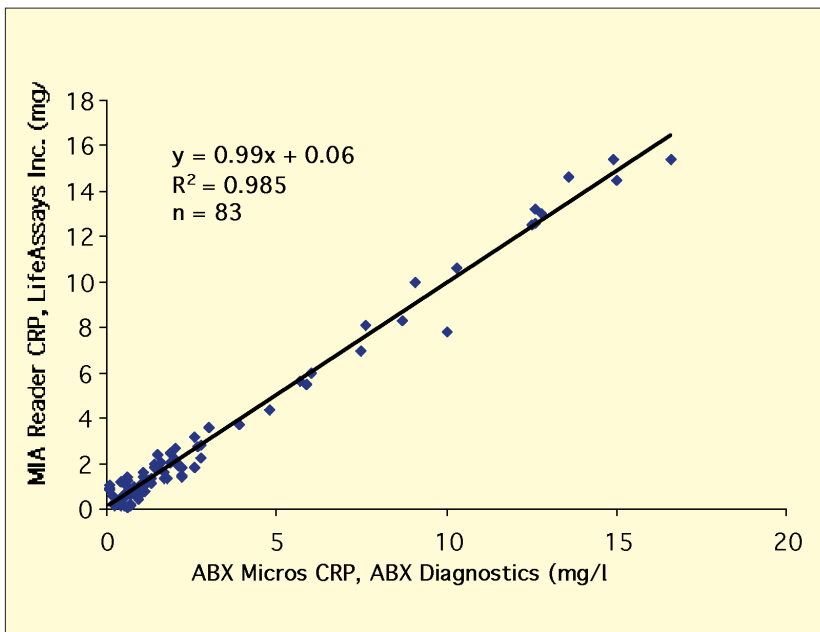


Figure 2. Correlation between results with the LifeAssays hsCRP immunoassay and the commercially available ABX Micros CRP immunoassay for 83 unidentified whole blood samples.

### Magneto ImmunoAssays (MIA) for hsCRP analysis

LifeAssays AB has developed a new immunoassay technology platform, referred to as Magneto Immunoassays (MIA) [10], for the point-of-care market. The hsCRP immunoassay is recommended for use in physicians' offices and community health centres, and has been developed for the quantitative determination of CRP in whole blood. Based on a sandwich immunometric immunoassay principle, the test system uses magnetic nano-particles to label CRP. The kit consists of reagent vials designed for one-time use applications, and a hand-held portable in-instrument, the MIA Reader 100, for the quantification of the analyte. The reader is a magnetic induction-based detector which allows measurement of the quantity of magnetic nanoparticles bound to CRP. The measuring range is optimised to 0.4-30 mg/L and the assay can be performed using only 20 mL of capillary blood. The procedure involves one step, as illustrated in Figure 1, and the total analysis time is approximately 5 minutes.

### Assay performance

The hsCRP Immunoassay has a good analytical performance; relevant data are shown in Table 1. The correlation between the results obtained with the hsCRP assay and those obtained with a commercially available instrument, ABX Micros CRP (ABX Diagnostics, Montpellier, France), for whole blood measurements is shown in Figure 2. The assay is very precise, with coefficients of variation (CVs) below 10% within the measured range. The linearity of the assay was found to be acceptable and the detection limit was estimated to be 0.2 mg/L.

### Conclusions

CRP analysis has long been established as an important diagnostic tool for monitoring inflammatory processes. Its link to CVD should provide new insights into the prevention and treatment of cardiovascular disease. The development of a rapid point-of-care hsCRP assay represents a powerful tool to facilitate assessment of cardiovascular disease risk.

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