CRP: an important biomarker for infectious disease and its role in POC

C-Reactive Protein (CRP) has proven, over time, to be a reliable marker for infectious and chronic inflammatory diseases. The availability of accurate and quantitative CRP tests within point of care (POC) enhances the clinical decision-making of physicians in the diagnosis and management of difficult inflammatory and infectious disorders by reducing their dependence on secondary health services and by providing a quality assurance of treatment and referral choices. Investment in rapid CRP tests for POC has been shown not only to reduce the turnaround time required for disease treatment and the use of unnecessary antibiotic therapy, but also to reduce overall healthcare costs associated with disease management and treatment.

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Measurement of C-Reactive Protein (CRP) is routinely used in clinical practice for the diagnosis and monitoring of a wide variety of infectious diseases, including pneumonia, sepsis, meningitis, bronchitis and soft-tissue infections, among others [1]. Within primary care settings, CRP has been found to be a valuable measure of bacterial diseases and, more importantly, an effective means to differentiate between bacterial and viral infections. CRP has also proven to be an important diagnostic tool for chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases.

Acute phase response

CRP is an acute-phase protein produced by hepatocytes as part of the non-specific acute phase response to inflammatory conditions, e.g. different types of infections, but also other types of inflammatory related diseases. The CRP concentration in the blood reflects the rate of hepatic synthesis of CRP. The transcription of the CRP gene in hepatocytes is up-regulated by interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor (TNFα and TNFγ) secreted by monocytes/macrophages [2]. Normally, CRP is present in the blood in very low concentrations (<1 mg/L), but during the inflammatory process this concentration increases significantly (up to several hundredfold). This rapid increase in the CRP serum concentration can already be detected six hours after the onset of inflammation and reaches a maximum level by 48 hours [3]. A rapid decrease (approximately 50% in 72 hours) of the CRP concentration is also observed once the stimulus ceases, for example, during effective antibiotic treatment of a bacterial infection. This quick and wide variation in the CRP response makes it a sensitive marker of inflammation and a useful tool for screening and monitoring infectious and inflammatory diseases. CRP often reflects changes in inflammatory activity faster and more specifically than other acute phase biomarkers, such as white blood cell count (WBC), erythrocyte sedimentation rate (ESR), fibrinogen or haptoglobin.

Structure and function

CRP is a cyclic pentameric plasma protein consisting of five identical non-covalently bound subunits with a combined molecular mass of about 120 kDa. It is a member of the pentraxin protein family of oligomeric calcium-binding proteins. CRP undergoes Ca^{2+}-dependent binding to different target molecules of microbes and cell membrane residues, as well as to cell nuclear materials. This facilitates their clearance from the blood stream. CRP functions as an opsonin and activates the classical complement pathway, as well as leukocyte phagocytosis, lymphocyte stimulation and monocyte/macrophage functionsn [4]. In addition, studies have shown that CRP binds low-density lipoprotein (LDL) and it has been detected in atherosclerotic plaque. CRP may also increase the production of cell membrane-bound tissue factor (TF) by macrophages.

CRP in hospital settings

In hospital settings, CRP analysis is among the most common tests performed in central laboratories using automated clinical analysers. Measurement is performed in plasma or serum using immunoturbidimetric or nephelometric immunoassay techniques. These immunoassays are sensitive (detection limit approximately 0.1 mg/L), accurate (total CV <5%) and require very little sample volume (a few micolitres per analysis). The introduction of the international standard IFCC CRM 470 has ensured that the currently available assays are extremely reliable. It is generally accepted that serum levels of CRP below 10 mg/L suggest minor viral infections such as the common cold, whereas levels between 10-20 mg/L are expected in more serious viral infections such as viral pneumonias, viral meningitis or influenza [5]. CRP serum levels above 50 mg/L are usually observed with bacterial infections in adults; in children this level is above 20-30 mg/L. However, there are many factors that 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. When interpreting a CRP result, the overall health of the patient, the suspected disease and the time of sampling must also be taken into consideration.

Point-of-care testing

Measurement of CRP has since long been regarded as an acute test in clinical practice, and has thus been considered a strong candidate for POC testing where a short turnaround time is absolutely crucial [6]. In POC settings, the patient is sampled, the sample is analysed and the result is presented and discussed before the patient ever leaves the clinical setting. This allows for faster diagnosis and consequently leads to better disease management and treatment. Although several semi-qualitative rapid tests for CRP are commercially available, only a few quantitative POC tests exist for the measurement of CRP in plasma and whole blood. Several studies [7] have been published on the performance of NycoCard from Axis Shield and Quick Read from Orion Diagnostica in POC settings. Both technologies have been shown to be useful in distinguishing between bacterial and viral infections, as well as monitoring the course of an illness or ongoing treatment with antibiotics. The availability of trained personnel to perform the CRP measurement, the total analysis time (approximately six minutes including sampling and multi-step handling procedures) and assay sensitivity (5-8 mg/L) were some of the main drawbacks of these commercially available POC assays. With regards to infectious disease and chronic inflammation, in cases where diagnosis can normally be made by clinical examination alone, the CRP measurement in the POC setting provides a quality-assurance step, confirming diagnosis and subsequent referral or treatment choices made by the physician.

The magnetic particle-based point-of-care test for CRP

Two separate magnetic particle-based tests, one for high-sensitive CRP (hsCRP) and one for normal-range CRP, have been developed for POC use. Based on a sandwich immunometric immunoassay principle, the test system uses magnetic nanoparticles to label CRP [8]. A reader, which is an electromagnetic detector, measures the quantity of magnetic nanoparticles bound to CRP. To perform a measurement [Figure 1] a single blood drop (20 or 4µL from a fingerprick specimen is collected using a sampling device that fits into a specially designed reagent vial. The reagent vial is then placed into the reader. The quantitative CRP result is automatically displayed within minutes using a disposable algorithm chip. Lastly, the reagent vial is removed for safe disposal. The measuring ranges are 0.5 – 30 mg/L and 5 – 150 mg/L for hsCRP and normal-range CRP, respectively.

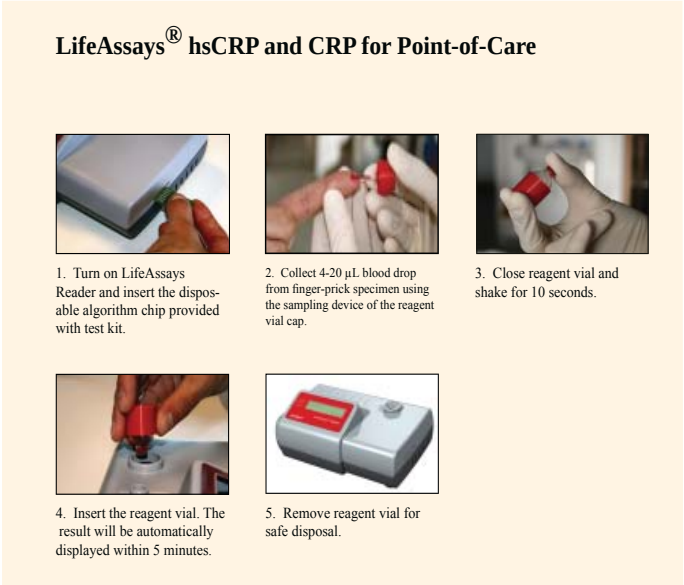

Figure 1. Methodology for the LifeAssays hsCRP and CRP for Point-of-Care test.

The disposable algorithm chip is the key to this magnetic particle-based POC system, which ensures assay performance through a secure encryption communication with the reader. It contains reagent batch-specific identification parameters, as well as a self-executable algorithm. The chip is inserted into the reader when a new reagent kit is opened and remains in the reader until the last reagent vial in the kit is used. Instrument and reagent upgrades are easily provided with each new reagent kit. The main advantages of the system are the one-step measuring procedure and automatic result calculation and presentation.

Cardiovascular risk indicator

Low-grade systemic inflammation has been associated with an increased risk of future coronary events, suggesting that repetitive CRP measurements (risk levels between 1-3 mg/L) may also be used to indicate risk of cardiovascular disease [9]. Although current POC assays are well suited for differentiation between bacterial and viral infections or for follow-up of anti-inflammatory therapy, they are inadequate for cardiovascular risk assessment due to poor sensitivity (detection limit between 5-8 mg/L). LifeAssays hsCRP test kit can measure whole blood CRP levels between 0.5 – 30 mg/L providing screening possibilities in POC for low-levels of inflammation.

Conclusions

CRP is an important biomarker for infectious and chronic inflammatory diseases. Its measurement in POC has proven vital for bacterial infection diagnosis and follow-up disease management and antibiotic therapy. It has also provided practitioners with a quality-assurance tool for confirming clinical choices made at initial patient consultation. The advantages of measuring baseline or low levels of CRP in POC at regular intervals will require further assessment before the clinical significance can be determined.

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