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Research review paper

Bioanalytical advances in assays for C-reactive protein

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ABSTRACT

This review presents advances in assays for human C-reactive protein (CRP), the most important biomarker of infection and inflammation for a plethora of diseases and pathophysiological conditions. Routine assays in clinical settings are based on analyzers, enzyme-linked immunosorbent assays and lateral flow assays. However, assays encompassing novel sensing schemes, improved chemistry, signal enhancement, lab-on-a-chip, microfluidics and smartphone detection, have emerged in recent years. The incorporation of immune-transducing chips or sensing interfaces with nanomaterials enables multiplexing analysis of CRP with co-existing biomarkers. However, there are still considerable challenges in the development of rapid diagnostics for both pentameric and monomeric CRP forms.

Contents

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1. Introduction

C reactive protein (CRP) was identified from patients with acute pneumococcal pneumonia (Tillett and Francis, 1930), a name that reflects its reaction with the C-polysaccharide of *Pneumococcus*. This native protein with MW 118 kDa has five non-covalently bonded and non-glycosylated identical subunits of 206 amino acids each (monomeric) to form a disk-shaped pentagon, or pentameric form (Fig. 1). CRP has been regarded as an early indicator of infectious or inflammatory conditions as well as a universal biomarker for numerous diseases and disorders. As such diseases often commence at very low CRP levels, nM or even pM, analytical methods must be highly sensitive, selective, fast and reliable using minimum sample volume. Hence, sensitive analysis of CRP levels in biological samples with these requirements is of critical importance for diagnosis and monitoring the progress of chemotherapy/personalized healthcare intervention.

In brief, most advanced formats have just emerged in the last three years and originated with turbidimetry based assays to detect CRP in μg mL^{-1}, followed by more sensitive enzyme-linked immunosorbent assay (ELISA), chemiluminescent, fluorescent and electrochemical assays with detection sensitivity down to fg mL^{-1}. In concert with significant advances in microfluidics, lab-on-a-chip (LOC) and fully integrated/automated bioanalytical platforms, innovative assay formats have emerged, e.g., lateral flow assay (LFA) formats with a wide dynamic range and enhanced sensitivity. Recently, nanomaterials-based signal enhancement, multi-labeling, novel biosensor concepts and smartphone (SP)-based point-of-care (POC) detection provide an impetus to the advancement of next-generation assays including the platform based on SP readout. The current trend also unravels more applicability of robust biorecognition elements beyond conventional antibodies (Ab) such as aptamers, affimers, etc. to enhance bioanalytical performance.

This review highlights clinical significance of CRP in various diseases, analytical methods for CRP including commercial analyzers, and diversified emerging assay formats for personalized CRP monitoring at POC and decentralized settings. Several technical pending issues, challenges, and future possibilities are also discussed including a need for simultaneous detection of pentameric and monomeric CRP together with other biomarkers.

2. CRP: Pathophysiological role and clinical significance

As a member of the pentraxin family of proteins, CRP is a class of acute-phase reactants and the first pattern recognition receptor ever described that coordinates the host's immune response against pathogens and damaged tissues. CRP is one of the most prominent acute phase liver proteins as its concentration in serum can increase a 1000-fold after the first inflammatory event. Inflammation extends beyond microbial infection to encompass all kinds of stress situations, summarized as “sterile inflammation” related to surgery, trauma, psychic stress, myocardial infarction, and neoplastic diseases. CRP functions as a pattern recognition receptor to guide the activation of humoral and cellular effector pathways. It plays an important physiological role in the host defense by binding to phosphocholine and related molecules expressed on the surface of dead/dying cells and microorganisms. CRP binds C1q complex to elicit activation of the C1-C9 complex (Thompson et al., 1999) and, more importantly, the lectin-dependent complement pathway. To date, CRP serves as an early indicator of infectious or inflammatory conditions, related to diversified diseases, disorders and pathological conditions (Supplementary Information, Table S1). In the context of pathogen-independent inflammation, CRP binds deoxyribonucleic acid (DNA) and histones, and scavenges the nuclear materials released from damaged circulating cells to activate innate immune cells via DNA binding receptors (Mold and Clos, 2013). Hepatocytes release CRP in response to inflammatory cytokines including interleukin (IL)-6, IL-1 and tumor necrosis factor alpha (TNFα), which are released by macrophages during infection or tissue inflammation, together with other factors released by adipocytes.

A persistently high CRP level indicates a risk for continued joint deterioration (Jansen et al., 2001), whereas significantly elevated serum CRP levels are linked to very high 30-day mortality rates in hospitalized patients (Chundadze et al., 2010). CRP increases the clearance of apoptotic cells and prevents autoimmunity by binding to autoantigens such as immunoglobulin and fragment crystallizable (F_c) receptors (Du Clos, 2003). Besides its role as a response molecule, CRP also promotes both inflammation and anti-inflammation per se (Blaschke et al., 2006; Filep, 2008; Hanriot et al., 2008), purportedly via a feedback mechanism that is at least partly related to CRP-derived peptides (El Kebir et al., 2011).

The serial measurements of CRP, serum amyloid A and procalcitonin are the accurate and reliable markers to diagnose necrotizing enterocolitis in premature infants (Cetinkaya et al., 2011). In middle-aged subjects, the serum CRP level decreases continuously with increasing physical fitness (Aronson et al., 2004) to reflect wellness assessment (Du Clos, 2013; Kao et al., 2006; Leshem-Rubinow et al., 2015). Repeated CRP analysis in an acutely infected patient provides valuable information to ascertain the diagnosis and avoid unnecessary antibiotics therapy. In renal colic patients, CRP plays a critical decision role whether an implanted urinary stent should be replaced (Angulo et al., 2010).

CRP is the best indicator of neonatal sepsis (Dollner et al., 2001) in comparison to IL-6, soluble TNF receptors (p55 and p75) and intercellular adhesion molecule 1 (ICAM-1) and E-selectin). Bacterial sepsis is one of the major causes of neonatal morbidity and mortality affecting 1–10 per 1000 live births, due to bacterial exposure during their intrauterine life or at the delivery. A serial measurement on 2nd and 3rd days is more informative than a single CRP measurement owing to physiological changes in CRP levels in neonates during their initial life. As the CRP levels above 5 μg mL^{-1} are indicative of neonatal sepsis, a high sensitivity assay (0.08–80 μg mL^{-1}) is first performed; followed by a normal CRP assay (0.2–480 μg mL^{-1}) if the CRP level is >80 μg mL^{-1}. The clinically relevant CRP concentration (CRPC) ranging from 5 to 10 μg mL^{-1} is significant for an inflammatory process as the onset starts between 6 and 12 h and peaks after 48–60 h. However, as the increase of CRP is highly nonspecific, it can only be applied for inflammation screening and disease appraisal.

Elevated levels of high-sensitivity CRP (hsCRP) are directly associated with cardiovascular risk (Ridker, 2003, 2004), atherothrombotic disease (Ridker, 2003; Sung et al., 2014), acute myocardial infarction (Tanaka et al., 2005) and acute coronary syndrome (De Servi et al., 2005). After a landmark report that confirms the central role of hsCRP in predicting future myocardial infarction (Ridker et al., 1997), hsCRP is widely accepted as an important biomarker of atherosclerosis and its sequelae. Although the role of hsCRP in atherogenesis remains unclear, a topic of future endeavor, CRP is one of a few biomarkers that has generated considerable interest, promise, and intensive research.

3. Routine CRP assays

The bioanalytical performances and features of assorted routine CRP assays with high sensitivity, usually in pg mL^{-1} range, are provided in