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Role of C-Reactive Protein in Unstable Angina

Kulkarni Sweta[1*], Wilma Delphine Silvia CR[2], Bidkar Prasanna Udupi[3], Gandham Rajeev [4]

Abstract
Unstable angina is frequently encountered by general practitioners and cardiovascular specialists. In the United States, of the 2.5 million patients admitted to hospital every year with suspected acute coronary syndromes, 1.5 million have unstable angina. The rest have myocardial infarction with or without ST elevation.
Braunwald described unstable angina as a syndrome with five mutually non-exclusive causes; thrombosis, mechanical obstruction, dynamic obstruction (spasm of microvasculature and macrovasculature), inflammation or infection, and increased oxygen demand. The inflammatory etiology of atherosclerosis has prompted a search for biomarkers of inflammation that predict risk for coronary artery disease and its sequelae. Within the acute coronary syndromes (ACS) inflammatory biomarkers may provide independent information regarding pathophysiology, prognosis and optimal therapeutic strategies.
CRP (C-reactive protein) an acute phase protein, an inflammatory biomarker produced by the hepatocytes in response to IL-6 accurately reflects ongoing inflammation than other inflammatory biomarkers because the plasma half-life of CRP is the same (about 19 h) under all conditions and the sole determinant of the plasma concentration is therefore the synthesis rate, which, in turn, reflects the intensity of the pathological process(es) stimulating CRP production.

Author Affiliations: [1]Department of Biochemistry, Mahatma Gandhi Medical College and Research Center, Puducherry, [2,4] Department of Biochemistry, Akash Institute of Medical Sciences & Research Centre, Devanahalli, Bangalore, [3]Department of Anesthesiology and Critical Care, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry.

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*** Corresponding Author:** Dr Kulkarni Sweta. MBBS., MD., Assistant professor, Department of Biochemistry, Mahatma Gandhi Medical College and Research Center, Puducherry, Contact Number: 09442232578, Email ID: widel2008@gmail.com.

1. INTRODUCTION

Unstable angina is frequently encountered by general practitioners and cardiovascular specialists. In the United States, of the 2.5 million patients admitted to hospital every year with suspected acute coronary syndromes, 1.5 million have unstable angina. The rest have myocardial infarction with or without ST elevation [1].

Braunwald described unstable angina as a syndrome with five mutually non-exclusive causes; thrombosis, mechanical obstruction, dynamic obstruction (spasm of microvasculature and macrovasculature), inflammation or infection, and increased oxygen demand [2]. Unstable angina occurs from the interplay of these factors with thrombosis and mechanical obstruction usually dominating. Transient or subtotal obstruction due to a platelet rich "white clot" over a fissured atherosclerotic plaque is considered causal in most episodes of unstable angina. This differs from the fibrin rich "red clot" associated with total coronary occlusion in infarction with ST elevation. In contrast to the Braunwald model, European investigators have advocated a central role for inflammation in unstable angina. The inflammatory etiology of atherosclerosis has prompted a search for biomarkers of inflammation that predict risk for coronary artery disease and its sequelae. Within the acute coronary syndromes (ACS) inflammatory biomarkers may provide independent information regarding pathophysiology, prognosis and optimal therapeutic strategies [3].

C-reactive protein (CRP) is a pentraxin acute-phase protein, members of which are evolutionarily conserved in most vertebrates. Hepatocytes and possibly smooth muscle cells and macrophages transcriptionally activate production of CRP in response to inflammatory cytokines, including interleukin-1 and interleukin-6 [4]. In healthy volunteer blood donors, the median concentration of CRP is 0.8 mg/l but following an acute-phase stimulus values may increase by as much as 10, 000-fold with de novo hepatic synthesis starting very rapidly serum concentrations beginning to rise by about 6 h and peaking around 48 h after a single stimulus [5]. In the general, ostensibly healthy population, the median baseline value is slightly higher and tends to increase with age, females having slightly higher circulating concentrations. In most, but not all diseases, the circulating value of CRP much more accurately reflects on-going inflammation than do other biochemical parameters of inflammation, such as plasma viscosity or the erythrocyte sedimentation

rate. This is because the plasma half-life of CRP is the same (about 19 h) under all conditions and the sole determinant of the plasma concentration is therefore the synthesis rate, which, in turn, reflects the intensity of the pathological process(es) stimulating CRP production [6]. In vivo turnover studies of human CRP in man did not demonstrate any detectable tissue deposition of CRP, even in inflamed or infected foci and in animal studies the only significant cellular site of CRP clearance and catabolism was the hepatocyte [7].

Liver failure impairs CRP production, but no other intercurrent pathologies and very few drugs reduce CRP values, unless they also affect the underlying acute-phase stimulus. The CRP value is thus a very useful non-specific biochemical marker of inflammation, measurement of which contributes importantly to: (i) screening for organic disease; (ii) monitoring the response to treatment of inflammation and infection; and (iii) detecting intercurrent infection in the few specific diseases characterized by modest or absent acute-phase responses to those diseases themselves [8].

2. Structure and function of CRP

The pentraxin family, named for its electron micrographic appearance, from the Greek penta (five) and ragos (berries) comprises CRP and serum amyloid P component (SAP) in man and is highly conserved in evolution with homologous proteins throughout the vertebrates [9].

Human CRP is a calcium-dependent ligand binding protein, which binds with highest affinity to phosphocholine (PC) residues, as well as a variety of other autologous and extrinsic ligands and aggregates or precipitates the cellular, particulate or molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles and apoptotic cells [10]. Extrinsic ligands include many glycan, phospholipid and other components of micro-organisms, such as capsular and somatic components of bacteria, fungi and parasites, as well as plant products [11]. When human CRP is ligand-bound, it is recognized by C1q and potently activates the classical complement pathway, engaging C3, the main adhesion molecule of the complement system, and the terminal membrane attack complex, C5-C9. Bound CRP may also provide secondary binding sites for factor H and thereby regulate alternative pathway amplification and C5 convertases [12]. The secondary effects of

CRP that follow ligand binding resemble some of the key properties of antibodies, suggesting that under various circumstances CRP may contribute to host defence against infection, function as a pro-inflammatory mediator and participate in physiological and pathophysiological handling of autologous constituents. The conservation of the structure of CRP and of its calcium-dependent specific binding of ligands containing PC and related substances, together with the lack of any known deficiency or protein polymorphism, suggest that this protein must have had survival value [13]. Microbial infection is a major driving force of change during evolution, and CRP has many features compatible with a role in innate immunity.

CRP an acute phase protein produced mainly by the liver in response to interleukin 6, is a marker of inflammatory processes that contribute importantly to atherogenesis, plaque disruption and thrombosis. Indeed products of thrombosis, including thrombin and platelet derived growth factor, themselves cause vascular smooth muscle cells within the ruptured plaque to augment production of interleukin 6 (IL-6), amplifying hepatic CRP release and completing a vicious cycle of thrombosis and inflammation in acute coronary syndromes [14]. CRP may have additional pathogenic effects - specifically by activating the complement system and promoting tissue factor release from monocytes. Thus CRP is intimately involved with the pathogenic mechanisms that drive acute coronary syndromes and is predictive not only of cardiovascular events in apparently healthy middle aged men and women but also of outcomes following presentation with unstable angina and myocardial infarction. The pathophysiological mechanisms responsible for unstable angina (UA) and non–Q wave myocardial infarction (NQMI) are believed to involve an acute inflammatory stimulus that contributes to coronary plaque disruption and subsequent platelet aggregation and vessel thrombosis [15]. The hypothesis of an acute inflammatory reaction playing a role in destabilizing the fibrous tissue cap of the atherosclerotic plaque is supported by the finding that inflammatory cells, predominantly macrophages, are present at the site of plaque erosion or rupture. Further evidence in support of the inflammatory hypothesis comes from the finding of activated leukocytes in the coronary and systemic circulation, the release of thromboxanes and leukotrienes related to the occurrence of ischemic chest pain and elevated levels of the acute phase reactants

C-reactive protein (CRP) and serum amyloid A protein (SSA) in patients with acute coronary syndromes [16]. The rise in CRP in patients with UA occurs independently of myocardial cell injury or death.

The inflammatory response is associated with the generation of cytokines and other inflammatory mediators that induce increased expression of cellular adhesion trans endothelial migration of leukocytes from the blood to the arterial intima. Intercellular adhesion molecule-1 (ICAM-1) is expressed on both resting and activated endothelial cells and promotes adhesion of neutrophils, monocytes and lymphocytes [17]. Vascular cell adhesion molecule-1 (VCAM-1) binds monocytes and T-lymphocytes to activated endothelial cells. Endothelial selectin (E-selectin) is expressed by activated endothelial cells and binds to mucin like cell adhesion molecules on the neutrophil surface membrane, thus initiating neutrophil extravasation [18]. Platelet selectin (P-selectin) is similarly expressed by activated endothelial cells and platelets and mediates leukocyte adhesion to platelets and endothelial cells during inflammation, thrombosis and atherosclerosis [19].

The extracellular portion of these adhesion proteins can be cleaved by proteolytic enzymes to form a circulating molecules that can be detected in serum and are referred to as the soluble cell adhesion molecules (sCAMs). Alternative splicing of the messenger RNA encoding for P-selectin can generate a soluble form of the protein that can be detected in serum [20]. Levels of sICAM-1, sVCAM-1 and sE-selectin are elevated in patients with UA and NQMI throughout the first 72 h of acute presentation [21]. Soluble P-selectin levels are elevated in patients with UA in comparison with patients with stable angina and controls. The pathological significance of these soluble forms may be underestimated as they can interfere with leukocyte-endothelial interactions in vivo as has already been demonstrated in vitro. Alteration in concentrations of these soluble adhesion molecules may relate to activation or damage of various cell types including platelets and endothelial cells. There is recent evidence that platelet activation and inflammation continues for many weeks after the acute ischemic event in patients with acute coronary syndromes [22]. The activation of neutrophils as they traverse the coronary circulation of patients with unstable angina is a marker of a widespread inflammatory process occurring in the coronary vasculature.

3. CRP and Cardiovascular disease

The commercial availability of routine high-sensitivity assays for CRP has enabled a flood of studies demonstrating a powerful predictive relationship between increased CRP production, even within the range previously considered to be normal, and atherothrombotic events [23]. Circulating CRP values correlate closely with other markers of inflammation, some of which show similar, albeit generally less significant, predictive associations. However CRP itself is particularly interesting with respect to cardiovascular biology and pathology, because not only does it bind selectively to LDL especially oxidized and enzyme-modified LDL as found in atheromatous plaques but it is actually deposited in the majority of such plaques and it has a range of proinflammatory properties that could potentially contribute to the pathogenesis, progression and complications of atheroma [24].

Tissue necrosis is a potent acute-phase stimulus and following myocardial infarction, there is a major CRP response, the magnitude of which reflects the extent of myocardial necrosis. Furthermore, the peak CRP values at around 48 h after the onset, powerfully predict outcome after myocardial infarction [25]. Importantly, CRP is deposited within all acute myocardial infarcts and compelling experimental evidence now suggests that the CRP response not only reflects tissue damage in this context, but may also contribute significantly to the severity of ischaemic myocardial injury [26].

The production of CRP following myocardial necrosis is the typical acute phase response to cell death and inflammation, mediated by the action on the liver of the cytokine cascade, especially IL-6 triggered by such events. However, the stimuli that trigger the low-grade up-regulation of CRP production that predicts coronary events in general populations or the more substantial CRP values associated with poor prognosis in severe unstable angina [27] or after angioplasty are not clear.

CRP is a robust clinical marker because of its stability, reproducible results, and ease of assay. Although it was originally proposed as a nonspecific marker of inflammation, several reports suggest that CRP may play a direct pathophysiological role in the development and progression of atherosclerosis. Proposed mechanisms include induction of endothelial dysfunction, promotion of foam cell formation, 4inhibition of endothelial progenitor cell survival and differentiation, activation of complement in

atherosclerotic plaque intima and ischemic myocardium [28].

Patients with ACS have elevations in CRP in association with their presenting symptoms. There appears to be a bimodal CRP response among patients with ACS. In some patients, CRP may remain elevated for up to 3months, whereas in others, CRP slowly declines during the hospital admission. A correlation exists between troponin elevation and CRP levels, although a significant percentage of patients without troponin elevation have elevated levels of CRP. In patients presenting with acute myocardial infarction, CRP levels correlate with the presence of plaque rupture, as assessed by intravascular ultrasound [29]. The cause of CRP elevations in the absence of overt myocardial necrosis is uncertain but may be related to plaque instability or myocyte necrosis below the limit of detection of standard assays. An early study examining CRP in ACS found that CRP identified a subset of patients with severe unstable angina at increased risk for death and MI. Studies of CRP elevation and short-term risk after ACS have found that CRP elevation may predict 14-days mortality [30]. A meta-analysis through 2001 found that elevated CRP at admission in patients with non–ST elevation MI (NSTEMI) or unstable angina conferred a 1.5-fold increased risk of death or nonfatal MI at 30days.

CRP elevations during admission for ACS also predict long-term risk of mortality. Patients with unstable angina and CRP 3 mg/L at discharge are more likely to be readmitted for recurrent cardiovascular instability or MI within 1 year. In a prospective study of patients who underwent early invasive therapy for non–ST-elevation ACS (NSTE ACS), CRP 10 mg/L during admission remained associated with increased risk of death over a mean follow-up of 20 months. Similar results were observed in the FRISC (FRagmin during InStability in Coronary artery disease) trial, in which CRP remained an independent predictor of mortality after an average of 30 months [31].

4. Raised CRP and Adverse Outcomes

Recent data confirm that the association of raised CRP concentrations with adverse outcomes in acute coronary syndromes is independent of commonly used markers of risk, including ECG characteristics and troponin release [32]. These studies-many of which were primarily designed to assess various therapeutic strategies-represent the evidence base supporting the routine measurement of CRP concentrations for risk stratification in acute coronary syndromes.

Continued elevations in CRP portend increased risk of mortality despite currently available therapeutic strategies. Patients with ACS have elevations in CRP in association with their presenting symptoms. There appears to be an increased concentration of C reactive protein at admission among patients with unstable angina has been correlated with worse outcomes both in hospital and after one year [33].

Several authors have shown varying associations of different subpopulations of T lymphocytes, granulocytes, macrophages, and cytokines with unstable angina. Although the role of inflammation or other mechanisms in unstable angina is not fully understood, it seems that inflammation in a coronary arterial plaque, leading to fissuring, rupture or erosions, and subsequent thrombosis is involved in the final step of most episodes of unstable angina [34]. Mulvihill and colleagues found that a CRP concentration of < 3 mg/l had a negative predictive value for major adverse cardiovascular events within six months of 97%. Conversely a CRP concentration of > 3 mg/l had a sensitivity of 96% for predicting adverse cardiovascular events, albeit with a specificity of 52%. CRP was as sensitive as soluble VCAM-1 for predicting major adverse events and measuring VCAM-1 did not appear to add to CRP's predictive accuracy. Whether measuring cytokines such as IL-6 or surface expression of leucocyte activation antigens (for example, CD11b/CD18) provides further information remains to be determined, but arguably measurement of CRP, which is readily available and relatively cheap, may be sufficient for evaluating ongoing inflammation as a risk for poor outcome, particularly if measured at discharge rather than admission [35].

5. A target for therapy in cardiovascular disease?

There is compelling epidemiological and laboratory evidence that CRP is a sensitive marker of the inflammation and/or metabolic processes associated with atherothrombotic events, and some observations suggest that CRP may contribute to their pathogenesis. Availability of drugs to block CRP binding and its effects in vivo would provide a powerful tool for determining whether CRP is just a marker or does indeed participate in pathogenesis of atheroma and/or its complications. Such agents may also have cardioprotective effects in acute myocardial infarction. Existing knowledge of the structure and function of CRP, including its three dimensional structure alone and complexed with ligands, coupled with experience in developing an

inhibitor of the related protein, SAP, establishes an excellent platform for drug design [36].

CONCLUSION

CRP is intimately involved with the pathogenic mechanisms that drive acute coronary syndromes and is predictive not only of cardiovascular events in apparently healthy middle aged men and women but also of outcomes following presentation with unstable angina and myocardial infarction.

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