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EDITORIAL

C-reactive protein predicts outcome in COVID-19: is it also a therapeutic target?

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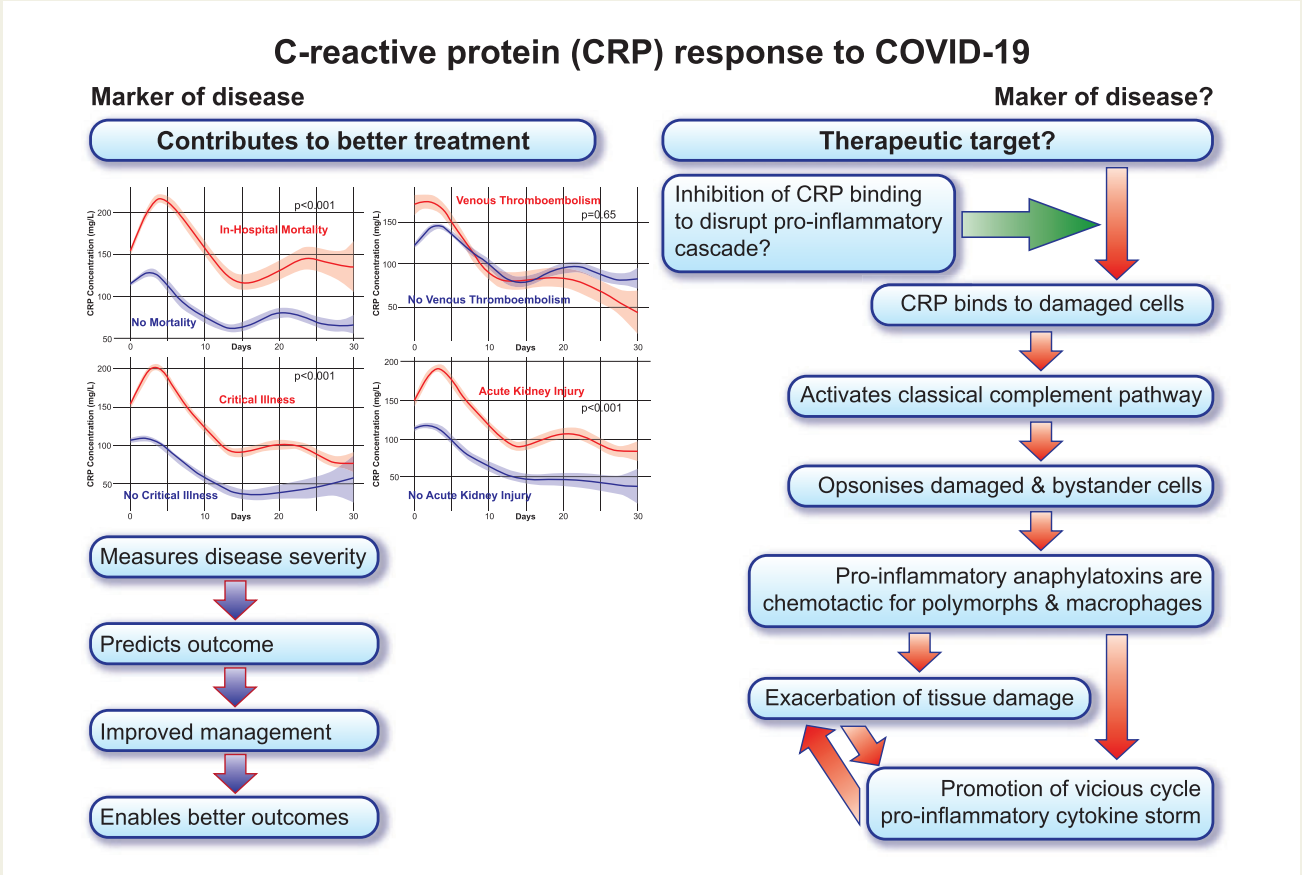
This editorial refers to 'C-reactive protein and clinical outcomes in patients with COVID-19', by N.R. Smilowitz *et al.*, doi:10.1093/eurheartj/ehaa1103.



Graphical Abstract C-reactive protein (CRP) response to COVID-19.

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C-reactive protein (CRP) has an illustrious scientific 'parentage'. The laboratory of Oswald Avery, who first demonstrated unequivocally that DNA is the genetic material, also characterized CRP as a protein with calcium-dependent binding to pneumococcal somatic C-polysaccharide. In addition, he introduced the term 'acute phase' for serum containing CRP from patients acutely ill with infectious disease.[1,2] The early, insensitive, semi-quantitative tests for CRP established it as a marker of infections, inflammatory, ischaemic, and traumatic tissue injuries, and malignancy, whilst the advent of sensitive quantitative immunoassays in the 1970s greatly enhanced its clinical utility.

C-polysaccharide is a phosphocholine-substituted ribitol teichoic acid and, in 1971, Volanakis and Kaplan identified phosphocholine as the structure to which CRP binds.[3] Indeed, phosphocholine residues are the natural ligand bound with highest affinity by CRP. In 1974, Kaplan and Volanakis[4] and Siegel *et al.*[5] independently reported that CRP bound to C-polysaccharide and other ligands, and activated the classical complement pathway, and showed that CRP was thereby capable of mediating inflammation. In fact, Francis and Abernethy[6] had already shown in 1934 that intracutaneous injection of C-polysaccharide in acutely ill patients elicited a characteristic immediate weal-and-flare reaction, followed by a more extensive oedematous erythema maximal at 6–10 h. These reactions only occurred when CRP was detectable in the patient's serum.

Reviewing the CRP field in 1981[7] and again in 2003,[8] I was able to show the range of clinical applications of CRP measurements and to hypothesize about a pathophysiological role for CRP (*Graphical Abstract*). Sensitive quantitative clinical measurement of CRP has three different roles: (i) as a sensitive screening test for an active inflammatory or tissue-damaging process; (ii) to monitor the activity, extent, and response to treatment of any disease process that triggers an acute phase response; and (iii) among the small number of disease processes, such as systemic lupus erythematosus and leukaemia, that themselves stimulate little or no acute phase response, CRP is a very sensitive and useful marker of intercurrent microbial infection. The exemplary study of CRP in coronavirus disease 2019 (COVID-19) by Smilowitz *et al.*[9] in this issue of the *European Heart Journal* illustrates the value of CRP for monitoring and prognosis in a most challenging clinical situation (*Graphical Abstract*).

The universal application of their actual CRP values is underpinned by the robust standardization of clinical CRP assays. The automation of clinical chemistry CRP immunoassays in the early 1980s, and resulting major expansion of CRP testing, had mandated robust reference standards. The World Health Organization therefore invited me to create and validate the International Standard for Human C-reactive protein, 85/506, on which gold standard all commercial clinical CRP assays have subsequently been based. The International Federation of Clinical Chemistry and Laboratory Medicine and the European Union secondary serum standards were also constructed using CRP that I isolated.

The major acute phase response of CRP in COVID-19 was predictable, based on the known behaviour of CRP in general and particularly in severe viral respiratory infections. From the outset, CRP values were found to reflect the severity of COVID-19 and to predict outcome, as reported in many, mostly small, series published worldwide. However, the present report by Smilowitz *et al.*[9] is distinguished by its large scale, its analysis in relation to more of the major clinical consequences of severe COVID-19, its highlighting of the additive value with D-dimer measurements, and its robust demonstration of prognostic significance. All of these crucial insights will increase in importance as new and effective therapeutic interventions become available, whether directly antiviral or targeting host responses.

What more can be done to exploit for patient benefit the remarkable properties of CRP as a marker of COVID-19 disease activity, extent, and severity? The most obvious is to measure its concentration serially and very frequently. The plasma half-life of CRP is just ~19 h and is constant, regardless of any pathological processes present.[10] Circulating CRP concentration is determined only by its hepatic synthesis rate and, in most conditions, the plasma CRP value very closely reflects the prevailing disease severity. Routine, frequent, serial CRP values, rather than just the admission value and a few other sporadic measurements, combined with critical measures of disease activity and therapeutic interventions, should provide for more granular CRP-based prognostic modelling, potentially leading to more effective management.

Progress on CRP as a potential therapeutic target in human disease has lagged behind universal routine clinical CRP measurement. The compelling association of higher CRP values with disease severity in many different conditions, and the presence of CRP co-localized with activated complement in damaged tissues, stimulated years of speculation about the pathogenicity of CRP; see, for example, Francis and Abernethy[7] and Griselli *et al.*[11] However, the first direct experimental evidence of exacerbation of tissue damage by CRP came from the rat acute myocardial infarction model.[11] Administration of human CRP after ligating the coronary artery substantially increased infarct size; human CRP was bound to the injured myocardium, activating rat complement, and the adverse effect was completely inhibited by depletion of circulating rat C3, i.e. the pathogenicity of the human CRP was absolutely complement dependent. We then rationally designed bis(phosphocholine)hexane, a new chemical entity, as the first small molecule inhibitor of CRP binding to ligands specifically exposed on damaged but not on healthy cells *in vivo*.[12] Administration of the inhibitor completely abolished the increased infarct size produced by human CRP in the rat model.[12] The pathogenicity of CRP has been independently confirmed in different models and inhibited by using antisense oligonucleotide to reduce CRP synthesis[13] or apheresis to remove CRP from the circulation.[14] Apheresis, pioneered by Pentracor GmbH (https://en.pentracor.de/) using an extracorporeal column of immobilized phosphocholine to absorb CRP from the circulation, is approved for routine clinical use in Germany. The procedure reduces circulating CRP by ~60% over a couple of days, and unpublished results apparently show significantly reduced infarct size in ST-elevation myocardial infarction patients compared with matched controls. This clinical evidence that CRP contributes to infarct size in humans is fully consistent with the effects of CRP inhibition we originally demonstrated in the rat model.

Following our original report of the predictive significance of CRP and serum amyloid A protein measurement in acute coronary syndrome,[15] there was intense cardiological interest in CRP, producing much controversial, confusing, and misleading literature. The early observational epidemiology studies, based on a limited number of events despite large population sizes, exaggerated the significance of the association between increased baseline CRP values and

cardiovascular disease risk. The conflation of association with causality popularized the idea that CRP was a risk factor for cardiovascular disease, not just a possible risk marker, and this misconception became widespread despite the general agreement that, for example, the cock crowing before dawn is not actually what causes the sun to rise. The misapprehension was strengthened by poorly conducted and uncontrolled experimental work purporting, falsely, to show that CRP is an inherently proinflammatory mediator of atherosclerosis; see, for example, Pepys.[16]

In fact, the predictive association between baseline CRP and cardiovascular disease risk in general populations is modest.[17,18] It is shared equally by other markers of inflammation such as fibrinogen, plasma viscosity, erythrocyte sedimentation rate (ESR), albumin, and white cell count, and reflects the fact that most of the proven causal risk factors for cardiovascular disease, including obesity, metabolic syndrome and type 2 diabetes, hypertension, smoking, lack of exercise, low socioeconomic status, and chronic low grade inflammatory conditions, are all associated with low grade systemic inflammation and modestly increased baseline CRP values. Even the very limited clinical utility of CRP measurement for cardiovascular disease risk is likely to be outweighed if increased CRP values are used by cardiologists exclusively for this purpose and not considered in the whole-patient context. This risks missing serious underlying diseases. On the other hand, full investigation before CRP testing has been repeated, to ensure that a raised value is persistent, will often reveal nothing but will incur large costs and provoke much spurious anxiety. Clinical CRP testing must be done and interpreted properly.[8]

Similarly, amidst the plethora of claims and assertions about proinflammatory effects attributed to CRP but actually caused by contaminants in the CRP preparations, it is crucial to focus on the overwhelming negative observations made when rigorously characterized, pure, isolated human CRP is tested. Such pure CRP is not proinflammatory for human cells *in vitro*, in mice *in vivo*, or when infused into normal healthy young adult humans.[16,19–21] Extensive transgenic animal studies also show that human CRP is either atheroprotective *in vivo* or has no effect. Most importantly, the compellingly negative large-scale Mendelian randomization studies confirm that CRP does not cause clinical cardiovascular disease events.[22] Critically, this must be distinguished from the adverse effects of CRP once an atherothrombotic, or other tissue-damaging, event has occurred, engendering a very different pathobiological environment.

Finally, what about CRP as a therapeutic target in COVID-19? Tissue damage by CRP involves binding of CRP to phosphocholine residues, and potentially other cellular and tissue structures, that are abnormally exposed only on damaged and dead cells. The phosphocholine head group of cellular membrane phospholipids is not accessible in the plasma membrane of normal healthy cells but, when cells are damaged and die, increased abundance of lysophosphatidylcholine and disruption of the lipid bilayer expose phosphocholine residues to which CRP avidly binds.[23] Bound CRP then activates complement via C1q and the classical complement pathway, leading to activation of C3, the central and most abundant complement component. Release of the chemotactic anaphylatoxic C3a fragment and fixation of the C3b opsonin are proinflammatory and engage potentially destructive polymorphs and monocyte/macrophages. Complement is necessary for CRP to exert its pathogenic effects in all experimental models in which it has been tested, and there is abundant evidence that this mechanism operates in human diseases. CRP and co-localized activated complement are universally present in the lesions of such disparate conditions as, for example, acute myocardial infarction, rheumatoid arthritis, and influenza virus infection. However, presence at the scene of a crime does not establish guilt. Indeed, CRP and complement might just be facilitating clearance of debris by phagocytic cells. On the other hand, the close positive association between CRP values and severity of tissue damage in many different pathologies, notably including COVID-19 as illustrated by Smilowitz *et al.*, is equally consistent with a pathogenic role for CRP.

Nevertheless, even the strongest associations do not establish causality, and robust validation of CRP as a therapeutic target requires a specific intervention, selectively targeting the proposed pathogenic mechanism. This has been achieved in the rat acute myocardial infarction model, where either C3 depletion[11] or bis(phosphocholine)hexane[12] completely abrogated the adverse effects of human CRP. Similarly, bis(phosphocholine)hexane treatment of mice infected with virulent avian influenza A markedly reduced morbidity and mortality.[24] The presence of CRP in the lesions of COVID-19 has not yet been reported but, given the extensive cell damage and the abundance of circulating CRP, it must be present. CRP bound to damaged tissues, injured by the virus and/or by the host response, will activate complement locally, potentially exacerbating the injury as well as contributing to systemic complement activation. Development of a novel small molecule drug that inhibits CRP binding *in vivo* is currently in progress to test whether this CRP–complement mechanism contributes significantly to severity of COVID-19 and other diseases (https://apollotherapeutics.com).

Conflict of interest: none declared.

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